

Comparative study of Streptococcus mutans in pregnant women's saliva in the first and third trimesters

Edon Behluli¹, Fatmir Dragidella¹, Donika Bajrami-Shabani², Anila Kamberi^{3*},
Mirsada Behluli⁴, Blerim Kamberi²

1. University of Prishtina, Medical Faculty, Department of Periodontology and Oral Medicine, Prishtina, Kosovo.
2. University of Prishtina, Medical Faculty, Department of Dental Pathology and Endodontics, Prishtina, Kosovo.
3. University Dentistry Clinical Center of Kosovo, Department of Dental Pathology and Endodontics, Prishtina, Kosovo.
4. University Clinical Center of Kosovo, Clinic of Gynecology and Obstetrics, Prishtina, Kosovo.

Abstract

For the objectives of this study 66 primiparous women were included. Clinical evaluation included the oral hygiene index (OHI), the plaque index (PI), the gingival index (GI), and interdental gingival bleeding index (PBI) in the first and third trimesters. The prevalence of Streptococcus mutans in saliva was determined by a CRT-bacteria diagnostic test.

The OHI value in the third trimester of pregnancy was significantly higher than the value in the first trimester; the GI and the PBI values in third trimester were significantly higher than the first trimester value. The relationship between the OHI index and the prevalence of S. mutans in pregnant women in the first and third trimesters was found to have a weak positive correlation ($p > 0.05$). The relationship between PBI and the value of S. mutans in pregnant women in the first and second trimesters showed a moderately weak negative correlation ($p > 0.05$).

The results from this study showed differences in the examined parameters, which are important for the early detection and timely treatment of S. mutans infection.

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Introduction

Pregnancy is a physiological condition during which important physical, hormonal, physiological, emotional, and metabolic changes occur. Pregnancy causes various changes in the oral cavity¹, the most common of which are gingival hyperplasia, gingivitis, pyogenic granulomas, dental caries and erosions, and qualitative and quantitative changes in saliva².

The role of high circulating estrogen levels is well established and is associated with a higher prevalence of gingivitis and gingival hyperplasia³. The most common problem during pregnancy is gingivitis. It is estimated that 30% to 75% of pregnant women experience gingivitis to a greater or lesser extent during pregnancy.

There are numerous reasons for this. Unfortunately, many women who had appropriate oral hygiene before pregnancy, may neglect this part of their self-care, usually unconsciously, during pregnancy. Reasons for this may include nausea, vomiting, weakness, chronic fatigue, and insomnia. These conditions may divert attention from usual hygiene habits. However, when combined with the hormonal changes that accompany pregnancy, dramatic changes in the oral cavity may occur^{4,5}. Saliva analysis has become an important resource in determining the physiological and pathological roles of saliva and is a useful tool for disease diagnostics, offering insights based on its origin, composition, function, and interaction with other organic systems. Although harmful processes that accompany periodontal disease (such as bone destruction and periodontal ligament destruction) are associated with bacterial plaque, in general they occur as a result of the host's response to microbial invasion⁶. Elevated levels of *Streptococcus mutans* in the saliva of women during late pregnancy were detected by Pirie *et al.*⁷. The increased prevalence of this bacterium

*Corresponding author:

Anila Kamberi
ALMA MATER EUROPAEEA
CAMPUS COLLEGE "REZONANCA"
Department of Dentistry
Glloku te Shelgjet " Veternik " 10000-Prishtinë, Kosovo.
E-mail: anilakamberi95@gmail.com

may be the reason for the higher incidence of dental diseases during the late stage of pregnancy.

Streptococcus mutans is considered to be the most prominent dental caries bacteria due to their ability to form biofilms known as plaque on the tooth surfaces⁸.

Dietary changes in early pregnancy, such as the regular consumption of sugar between meals and sugary beverages to satisfy cravings or prevent nausea, can cause a drop in the pH of saliva and changes to the teeth and soft tissues in the oral cavity. Numerous studies have described dental and oral changes in pregnant women compared with non-pregnant women^{9,10}. Periodontal diseases are closely connected to the general health of an individual^{11,12}. Dental plaque microorganisms (which include more than 700 different types of microorganisms) play a vital role in the development and progression of periodontitis¹¹.

The aim of our current research was to assess the impact of *S. mutans* in saliva on the oral hygiene and degree of gingival inflammation in pregnant women in the first and third trimesters of pregnancy.

Materials and methods

Subjects

This study included a sample of 66 pregnant women who were undergoing their first pregnancy and lived in or around Pristina (Kosovo). All subjects were treated in the Department of Gynecology and Obstetrics at the University Clinical Center, Prishtina, Kosovo. Women classified as undergoing a high-risk pregnancy and pregnant women with soft tissue changes in their oral cavity were excluded from the study.

Design of the study

Clinical examinations

Clinical examinations consisted of the following clinical procedures: recording a detailed clinical history of the patient and determining their condition and gestational age (weeks/months of pregnancy), determining their oral hygiene, and noting the basic condition of the periodont.

Clinical history of the patient

A detailed clinical history was obtained for each patient according to previously established protocols for guiding pregnancy.

Indexes to measure oral health

The soft deposits index of the teeth (the oral hygiene index, OHI) was determined according to the Green–Vermillion scoring system¹¹, as follows:

0 - no soft deposits (plaque);

1 - soft deposits (plaque) localized only in the gingival third of the tooth crown;

2 - soft deposits (plaque) cover more than one-third but less than two-thirds of the tooth crown surface;

3 - very poor oral hygiene (soft deposits covering more than two-thirds of the crown surface).

The gingival index (GI) was determined according to Silness-Löe¹³ based on the following scoring system:

0 - normal gingiva;

1 - mild inflammation;

2 - moderate inflammation;

3 - severe inflammation.

The index of interdental gingival bleeding (papilla bleeding index, PBI) was determined according to the guidelines of Ainamo¹⁴ based on the following scoring system:

0 - no evidence of hemorrhages on examination;

1 - hemorrhage evident only on one place after probing;

2 - linear or multiple points of bleeding from the papilla;

3 - interdental space filled with blood immediately after probing;

4 - excessive bleeding after probing.

Collection of saliva samples

Saliva was collected in sterile tubes, early in the morning, between 8 and 10 a.m., at least two hours after a meal, after teeth brushing, and without the use of rinse aid.

Assessment of Streptococcus mutans in saliva

The abundance of *S. mutans* in saliva was determined by a diagnostic test, CRT-bacteria (Vivadent, Schaan, Lihtenstein), strictly following the manufacturer's instructions. We also used the Dentocult SM system. This method is based on the use of a selected liquid culture and the application of *S. mutans* onto a test strip. The SM test strip discriminates the ability to develop on a solid surface in combination with a selected liquid culture (high sucrose

concentration with bacitracin). Dentocult LB media included a paraffin tablet to stimulate saliva. Bacitracin, which prevents bacterial growth, was added to the saliva at least 15 minutes before use. Strips were divided into four test groups and a control in sterile tubes containing selective LB media, and the number of *S. mutans* colonies / ml saliva was determined.

The density of SM colonies (CFU / ml) in saliva was determined according to the following scoring system: 0, <10³ CFU / ml saliva, only about 5% of the crown surface is colonized by bacteria; 1, 10⁴ CFU / ml saliva (low SM level), about 20% of the surface of the crown is colonized with bacteria; 2, 10⁵ CFU / ml in saliva (high presence of CMU in saliva), about 60% of the surface of the crown is colonized by bacteria; 3, 10⁶ CFU / ml in saliva (very large amount of MS in saliva), about 80% of the surface of the crown is colonized by bacteria. All microbiological analyses were performed in the Department of Microbiology and Parasitology at the University Clinical Center, Prishtina, Kosovo.

Statistical analysis

Statistical analysis of the data was performed using a variety of methods, as detailed below, and the software Statistica 7.1 for Windows and SPSS Statistics 23.0. For the analysis of series with characteristics attributable to *S. mutans*, the percentages of the structure that were affected were determined (%). Differences in series with characteristics attributable to the first and third trimesters were tested using Fisher's Exact test / Monte Carlo significance test (two-sided) (p values). Differences between the first and third trimesters were analyzed using the Wilcoxon matched pairs test (Z / p). A p value <0.05 was considered to indicate statistical significance. The data are presented in tabular and graphical form.

Results

The values of OHI, GI, and PBI were determined for pregnant women in their first trimester of pregnancy. The OHI ranged from 1.02 ± 0.54; ± 95.00% CI: 0.88–1.15; the GI value ranged from 0.33 ± 0.64; 95.00% CI: 0.18–0.49; and the PBI was 0.61 ± 0.94; ± 95.00% CI: 0.37–0.84.

For pregnant women in their third trimester of pregnancy, the OHI ranged from 1.65 ± 0.77; 95.00% CI: 1.46–1.84; the GI value was

0.64 ± 0.72; ± 95.00% CI: 0.46–0.81; and the PBI ranged from 1.18 ± 0.96; ± 95.00% CI: 0.42–1.94 (Table 1).

First trimester	Valid N	Mean	Confidence		Minimum	Maximum	Std.Dev.
			-95,00%	+95,00			
OHI	66	1,02	0,88	1,15	0	3	0,54
GI	66	0,33	0,18	0,49	0	3	0,64
PBI	66	0,61	0,37	0,84	0	3	0,94
Third trimester							
OHI	66	1,65	1,46	1,84	1	3	0,77
GI	66	0,64	0,46	0,81	0	2	0,72
PBI	66	1,18	0,95	1,42	0	2	0,96

Table 1. Values of OHI, GI and PBI at the first and third trimester.

First trimester	Valid N	Mean	Confidence		Minimum	Maximum	Std.Dev.
			-95,00%	+95,00			
OHI	66	1,02	0,88	1,15	0	3	0,54
GI	66	0,33	0,18	0,49	0	3	0,64
PBI	66	0,61	0,37	0,84	0	3	0,94
Third trimester							
OHI	66	1,65	1,46	1,84	1	3	0,77
GI	66	0,64	0,46	0,81	0	2	0,72
PBI	66	1,18	0,95	1,42	0	2	0,96

Table 2. Comparison of OHI, GI, PBI at the first trimester and third trimester.

The differences in the values of the OHI, GI, and PBI in pregnant women in their first trimester compared with the third trimester of pregnancy were as follows. The OHI value in the third trimester of pregnancy was significantly higher than in the first trimester [Z=4.46 and p<0.001 (p=0.000)]; GI in the third trimester of pregnancy was significantly higher than in the first trimester [Z=2.72 and p<0.01 (p=0.006)]; and PBI in the third trimester of pregnancy was significantly higher than in the first trimester (Z=3.46 and p<0.001) (Table 2).

The data relating to the detection of *S. mutans* in the saliva of pregnant women in their first trimester of pregnancy indicated that out of a total of 66 pregnant women, one (1.50%) woman

had negligible levels ($<10^3$ CFU / ml saliva), 14 (21.20%) had low levels (10^4 CFU / ml saliva), 39 (59.10%) had high levels (10^5 CFU / ml saliva), 10 (15.20%) had very high levels (10^6 CFU / ml saliva) of *S. mutans* in saliva, and in two (3.00%) women, 80.00% of the crown surface was colonized with bacteria (Table 3).

First trimester	Frequency	Percent	Valid Percent	Cumulative Percent
Negligible values	1	1,5	1,5	1,5
Low level of SM	14	21,2	21,2	22,7
Large presence of SM	39	59,1	59,1	81,8
Very large amounts of SM	10	15,2	15,2	97,0
High colonization with SM	2	3,0	3,0	100,0
Total	66	100,0	100,0	

Table 3. Streptococcus mutans values at the first trimester.

The data presented refer to the detection of *S. mutans* in saliva in pregnant women in the third trimester of pregnancy. Of the 66 pregnant women studied, 7 (10.60%) had a low level (10^4 CFU / ml saliva) of *S. mutans* in their saliva, 39 (59.10%) had a high level (10^5 CFU / ml saliva), and 20 (30.30%) had a very high level (10^6 CFU / ml saliva) of *S. mutans* in their saliva (Table 4).

Third trimester	Frequency	Percent	Valid Percent	Cumulative Percent
Low level of SM	7	10,6	10,6	10,6
Large presence of SM	39	59,1	59,1	69,7
Very large amounts of SM	20	30,3	30,3	100,0
Total	66	100,0	100,0	

Table 4. Streptococcus mutans values at the third trimester.

The cross-tabulation of *S. mutans* values obtained for the first and third trimesters of pregnancy revealed that one (100.00%) woman who showed negligible values of *S. mutans* in her saliva in the first trimester ($<10^3$ CFU / ml saliva), showed high levels in the third trimester (10^5 CFU / ml saliva). Of the 14 (100.00%) women who showed low levels of *S. mutans* in saliva in the first trimester (10^4 CFU / ml saliva), one (7.10%) woman showed low levels (10^4 CFU / ml saliva), 7 (50.00%) women showed high levels (10^5 CFU / ml saliva), and 6 (42.90%) women showed very large levels (10^6 CFU / ml saliva) of *S. mutans* in saliva in the third trimester. Of the

39 (100.00%) women who showed high levels of *S. mutans* in the first trimester (10^5 CFU / ml saliva), 5 (12.80%) women showed low levels (10^4 CFU / ml saliva), 23 (59.00%) women showed high levels (10^5 CFU / ml saliva), and 11 (28.20%) women showed very high levels (10^6 CFU / ml saliva) of *S. mutans* in their saliva in the third trimester.

Of the 10 (100.00%) pregnant women who showed very high levels (10^6 CFU / ml saliva) of *S. mutans* in their saliva in the first trimester, 7 (70.00%) women showed high levels (10^5 CFU / ml saliva) and 3 (30.00%) women showed very high levels (10^6 CFU / ml saliva) of *S. mutans* in their saliva in the third trimester.

Of the two (100.00%) pregnant women for whom 80.00% of the crown surface was colonized with bacteria in the first trimester, one (50.00%) showed low levels (10^4 CFU / ml saliva) and one (50.00%) showed high levels (10^5 CFU / ml saliva) of *S. mutans* in their saliva in the third trimester.

The cross-tabulation of *S. mutans* values for the first and third trimesters of pregnancy revealed a Fisher's exact test value of 6,409 and a p value >0.05 ($p=0.645$), and a Monte Carlo significance test (two-sided) value of 0.632–0.657, indicating no significant difference (Figure 1).

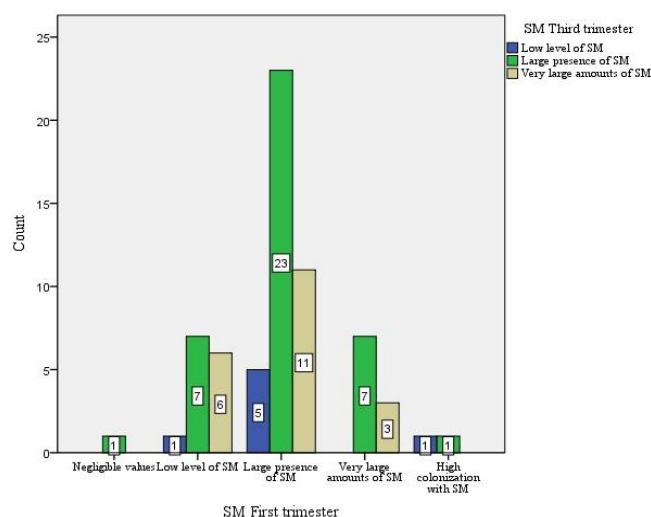


Figure 1. Comparison of Streptococcus mutans at the first trimester and the third trimester.

Correlations

OHI index * *S. mutans* / First trimester

The relationship between the OHI index and the prevalence of *S. mutans* in pregnant women in the first trimester showed a weakly

positive correlation that was not statistically significant (Spearman's rank order $R=0.05$ and $p>0.05$). With increased levels of *S. mutans* in saliva in the first trimester, the OHI index value increased but not statistically significantly.

OHI index * *S. mutans* / Third trimester

The relationship between the OHI index and the prevalence of *S. mutans* in pregnant women in the third trimester showed a weakly positive correlation that was not statistically significant (Spearman's rank order $R=0.05$ and $p>0.05$). With increased levels of *S. mutans* in saliva in the third trimester, the OHI index values increased but not significantly.

GI * *S. mutans* / First trimester

The relationship between GI and the prevalence of *S. mutans* in pregnant women in the first trimester showed a weakly positive correlation that was not statistically significant (Spearman's rank order $R=0.20$ and $p>0.05$). With increased levels of *S. mutans* in saliva in the first trimester, GI values increased but not significantly.

GI * *S. mutans* / Third trimester

The relationship between GI and the prevalence of *S. mutans* in pregnant women in the third trimester showed a very weak negative correlation that was not statistically significant (Spearman's rank order $R=-0.01$ and $p>0.05$). With increased levels of *S. mutans* in saliva in the third trimester, GI values decreased but not significantly.

PBI * *S. mutans* / First trimester

The relationship between PBI and the prevalence of *S. mutans* in pregnant women in the first trimester showed a moderately weak negative correlation that was not statistically significant (Spearman's rank order $R=-0.20$ and $p>0.05$). With increased levels of *S. mutans* in saliva in the first trimester, PBI values decreased but not significantly.

PBI * *S. mutans* / Third trimester

The relationship between PBI and the prevalence of *S. mutans* in pregnant women in the third trimester showed a weak negative correlation that was not statistically significant (Spearman's rank order $R=-0.06$ and $p>0.05$). With increased levels of *S. mutans* in saliva in the third trimester, PBI values decreased but not significantly.

Discussion

Our data indicated differences in the values of OHI, GI, and PBI in pregnant women in the first and third trimesters of pregnancy. This finding of significantly higher values in the third trimester of pregnancy correlated with the findings of previous studies by Vittek *et al.* and Lapp *et al.* which suggested that hormonal activity during pregnancy may predispose pregnant women to gingivitis and periodontitis^{15,16}. They pointed to progesterone as the possible cause of local inflammation. Loeet *et al.* and Miyazaki *et al.* reported that the frequency of periodontitis increased from the first to the third trimester, again suggesting that the periodontal tissues may be predisposed to periodontitis during pregnancy^{17,18}. Decreased oral hygiene in our subjects during the third trimester may be due to reduced self-care during the late stages of pregnancy and an unawareness of the particular importance of limiting the accumulation of dental plaque during pregnancy. This finding was in accordance with the findings of Agbelusi *et al.* and Pirie *et al.*, who associated gingival changes in early pregnancy with dietary changes, such as the increased consumption of sugary drinks and sweets, often to prevent nausea, that contribute to a lower pH of the saliva^{19,7}. In a study by Ho *et al.* looking at periodontal tissue changes in pregnant and non-pregnant women, gingival inflammation was found to be statistically significantly higher ($p<0.001$) in pregnant women in the third trimester²⁰. The authors connected gingival inflammation in pregnant women to the increased deposition of dental plaque biofilms. According to Pirie *et al.* the reduced maintenance of oral hygiene increases the abundance of anaerobic bacteria in saliva and in periodontal tissues and can cause worsening of the inflammatory reaction⁷. This is consistent with the results obtained in our study. They also pointed out that along with the increase in the OHI value, there was a progressive increase in the GI index value in all subjects⁷. Van der Reijden *et al.* showed that microorganisms associated with periodontitis can coexist with *S. mutans* and survive under acidic conditions, limited in their mutual interactions²¹. Therefore, individual variations of the host microflora may be responsible for the differences in *S. mutans* colonization between studies. Dental biofilm accumulates on all available solid tooth surfaces,

immediately after its removal by mechanical or chemical substances. If non-pathogenic bacterial flora is present and there is no invasion of potentially pathogenic microorganisms, the development of periodontal inflammation will not occur. However, if dental biofilm accumulates, the initial lesion indicating gingivitis develops after 24 hours¹¹.

Conclusions

The data from our study indicate poorer oral hygiene condition at the third trimesters of pregnancy. To avoid oral disease during the late stages of pregnancy, appropriate prevention measures are required in a timely manner, including maintaining high standards of oral hygiene and normal eating habits. One way to maintain effective oral and dental hygiene is by gargling using mouthwash. The use of mouthwash is one of the earliest prevention methods for caries due to reduced plaque attachment²².

Declaration of Interest

The authors report no conflict of interest.

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THE EFFECT OF DYNAMIC EXERCISES IN THE TREATMENT OF CERVICAL SPONDYLOSIS

Arjeta Azemi¹, Arbnore Ibrahimaj Gashi², Vujica Zivkovic³, Serjoza Gontarev⁴

¹ Center for physical therapy and rehabilitation in Klllokot, R. Kosovo

²University of Prishtina, Faculty of Medicine, Physiotherapy Department, R. Kosovo

³University "Ss. Cyril and Methodius" Faculty of Physical Education, Sport and Health Skopje, Macedonia

Corresponding Author

Arbnore Ibrahimaj Gashi

University of Prishtina, Faculty of Medicine, Physiotherapy Department, R. Kosovo

arbnore.gashi@uni-pr.edu

Abstract:

Introduction: Spondylosis presents cervical degenerative disorder that appears in cervical vertebrae. It is very common issue for the reasons that appear in people after age 30 which lead to higher numbers of problems connected with cervical region. **Purpose:** The main purpose of this paper is to determine the effectiveness of physical therapy in the treatment of cervical spondylosis.

Materials and methods: This research was prospective study which was conducted at the Center for physical therapy and rehabilitation, in Klllokot, Kosovo, during period 2013. To carry out the study according the ethical medical standards was received permission from rehabilitation center. The survey was short term lasting 10 days. The total number of patients included in the study was 60 of both sexes. Separation of patients in both groups (A and B) was done randomly. Group A of subjects was treated with: Thermotherapy, electrotherapy, dynamic exercises for the muscles of the cervical region against gravity, stretching and deep transverse massage, while the group B of subjects were treated with: Thermotherapy, electrotherapy, isometric exercises. Range of motion was measured with goniometer while the pain was estimated by Numerical rating scale of pain. Evaluation of patients was done before and after ten days of treatment.

Results: According our results, we found significant improvement in Group A regarding the mobility, pain and function. According our results dynamic exercises combined with deep transversal massage are more effective than isometric exercises.

Conclusion: This study confirms the hypothesis that short-term physiotherapy plays a significant role in the treatment of cervical spondylosis. Comparison between the two treatment techniques gives priority to dynamic exercises, contrary to isometric exercises. Our recommendation include: long-term study, the number of samples to be higher for assessing and comparing the effectiveness of the two treatment methods.

Keywords: Spondylosis, Pain, Exercises, Massage.

Introduction

Cervical spondylosis is a degenerative disorder present in the cartilage of the cervical joints, which may be asymptomatic or can be manifested with pain in the cervical region (Hirpara, Butler, Dolan, O'Byrne, & Poynton, 2012), (Egwu, Ajao, & Mbada, 2009).

The prevalence of pain in the cervical region in the general population ranges from 0.4% to 86.8% (Hoy, Protani, De, & Buchbinder, 2010). According to scientific data 15% -20% of patients report pain in the cervical region every year and 1.5% -1.8% seek medical help (Evans, 2014),(Lauche, et al., 2016). Pain in the cervical region is very problematic because it is presented to people after the age of 30, indicating a large number of disabilities linked to the cervical region such as lowering the quality of life and high cost in the health system (Takasaki & May, 2014).

For the treatment of the cervical region, many methods of physical therapy are applied, including manual therapy, physical agents, acupuncture etc. Regarding the short and long term effect of these methods there are many scientific contradictions (Wong, Shearer, Mior, Jacobs, & Cote, 2016).

The 14-day thermotherapy application has shown positive effects on pain reduction in the cervical region (Cramer, et al., 2012). After treating patients with different treatment methods including mobilization and manipulation, stretching, strength and flexibility, massage techniques and thermotherapy have shown functional improvement in patients with cervical disorders (Boissonnault & Badke, 2008).

The main purpose of this study was to evaluate the effectiveness of physical therapy in treatment of cervical spondylosis.

Material & methods

This research was prospective study which was conducted at the Center for physical therapy and rehabilitation “Banja e Klllokotit”, in Klllokot, Kosovo, during period 2013. To carry out the study according the ethical medical standards was received permission from rehabilitation center. The survey was short term lasting 10 days. The total number of patients included in the study was 60 of both sexes.

Separation of patients in both groups (A and B) was done randomly. Group A of subjects was treated with: Thermotherapy, electrotherapy, dynamic exercises for the muscles of the cervical region against gravity, stretching and deep transverse massage, while the group B of subjects were treated with: Thermotherapy, electrotherapy, isometric exercises. Range of motion was measured with goniometer while

the pain was estimated by Numerical rating scale of pain (NRS). Evaluation of patients was done before and after ten days of treatment. Inclusion criteria were patients with subacute and chronic phase with SC. While exclusion criteria were acute stage of disease, patients over the age of 60, patients with other cervical spine problems eg: intervertebral disk hernia on the cervical spine, spinal stenosis of the cervical region, etc.

Presentation of data is done through tables and graphs. Data processing is done with the InStat statistical package. From the statistical parameters are calculated: arithmetic average, standard deviation, as well as minimum and maximum value. For non-parametric data testing, the Mann-Whitney test was used, Verification of the tests for the degree of reliability was $P > 0.05$.

Results

Table 1. Right Rotation Parameters before Treatment by Groups

Right rotation before treatment	Group A	Group B	Total
N	30	30	60
Mean	55.9	61.0	58.4
DS	11.5	7.2	9.9
Min	40	40	40
Max	80	75	80
Mann-Whitney test	U'=501, P=0.303		

The mean rotation on the right side before the treatment of both groups was 58.40 (DS \pm 9.90), range between 40-80. Group A was 55.90 (DS \pm 11.50), range between 40-80. While group B showed higher mean levels for this movements which was 61.00 (DS \pm 7.20), range between 40-75. With the Mann-Whitney test we did not find any significant statistical difference between groups (U' = 501, P = 0.303 and $P > 0.05$), (Table 1).

Table 2. Left Rotation Parameters before treatment by groups

Left rotation before treatment	Group A	Group B	Total
N	30	30	60
Mean	66.2	68.2	67.2
DS	10.2	6.6	8.6

Min	45	50	45
Max	85	80	85
Mann-Whitney test	U'=488, P=0.578		

The mean rotation on the left side before the treatment of both groups was 67.2 (DS \pm 8.6), the range 45-85. Group A was 66.2 (DS \pm 10.2), range between 45-85. While group B showed higher mean levels for this movements which was 68.2 (DS \pm 6.6), range between 50-80. With the Mann-Whitney test we did not find any significant statistical difference between groups (U'=488, P=0.578 and P>0.05), (Table 2).

Table 3. Statistical parameters of pain before treatment by groups

NRS of pain before treatment	Group A	Group B	Total
N	30	30	60
Mean	6.8	6.9	6.8
DS	1.5	1.4	1.4
Min	4	4	4
Max	9	9	9
Mann-Whitney test	U'=462, P=0.864		

The mean level of pain according NRS before treatment in both groups was 6.8 (DS \pm 1.4), range between 4-9. Mean level of pain in group A was 6.8 (DS \pm 1.5), while group B was slightly higher 6.9 (DS \pm 1.4). With the Mann-Whitney test we did not find any significant statistical difference between groups (U'=462, P=0.864 and P>0.05), (Table 3).

Table 4. Right Rotation Parameters after treatment by groups

Right rotation after treatment	Group A	Group B	Total
N	30	30	60
Mean	63.8	63.3	63.6
DS	9.8	6.5	8.2
Min	50	43	43
Max	80	75	80
Mann-Whitney test	U'=465.5, P=0.824		

The mean rotation on the right side after treatment of both groups was 63.6 (DS ± 8.2), the range between 43-80. Group A was 63.8 (DS ± 9.8), range between 50-80. While group B showed higher mean levels for this movements after treatment which was 63.3 (DS ± 6.5). With the Mann-Whitney test we did not find any significant statistical difference between groups (U'=465.5, P=0.824 and P>0.05), (Table 4. and Graph. 1).

Graph. 1. Right Rotation Parameters after treatment by groups

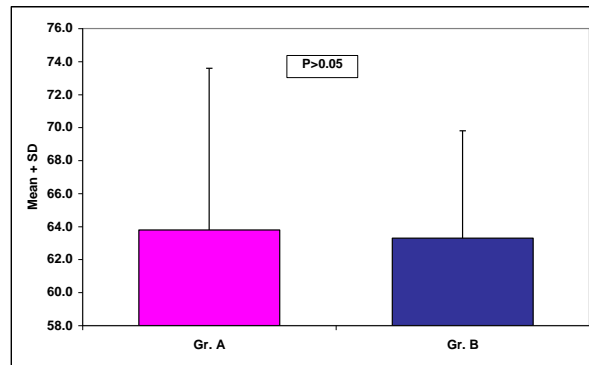


Table 5. Left Rotation Parameters after treatment by groups.

Left rotation after treatment	Group A	Group B	Total
N	30	30	60
Mean	72.4	70.0	71.2
DS	8.2	5.9	7.1
Min	56	54	54
Max	85	82	85
Mann-Whitney test	U'=583.0, P=0.048		

The mean rotation on the left side after treatment of both groups was 71.2 (DS ± 7.1), the range 54-85. Group A was 72.4 (DS ± 8.2), range between 56-85. While group B showed decreased mean levels for this movements which was 70 (DS ± 5.9). With the Mann-Whitney test we found significant statistical difference between groups (U'=583.0, P=0.048 and P<0.05), (Table 5 and Graph. 2).

Graph. 2. Left Rotation Parameters after treatment by groups.

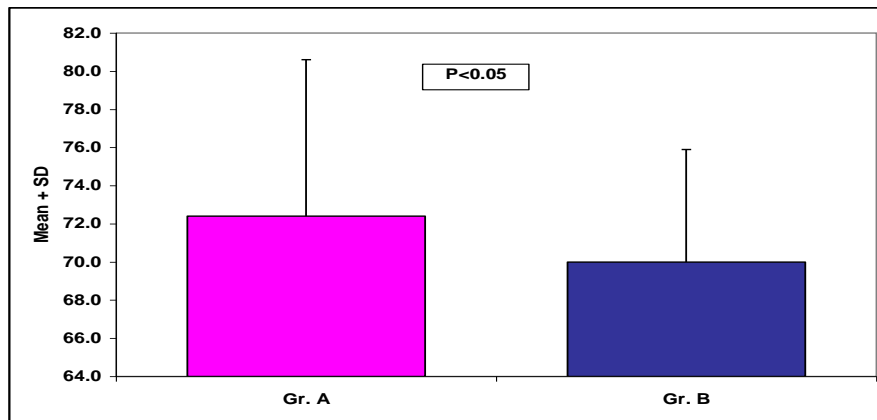


Table 6. Statistical parameters of pain after treatment by groups

NRS of pain after treatment	Group A	Group B	Total
N	30	30	60
Mean	2.0	3.7	2.9
DS	1.4	1.3	1.6
Min	0	1	0
Max	5	6	6
Mann-Whitney test	U'=730.5, P<0.0001		

The mean level of pain according NRS after treatment in both groups was 2.9 (DS \pm 1.6), range between 0-6. Mean level of pain in group A was 2 (DS \pm 1.4), while group B was slightly higher 3.7 (DS \pm 1.3). With the Mann-Whitney test we found significant statistical difference between groups (U'=730.5, P<0.01), (Table 6).

Dicussion

In this research were included 60 patients, of both genders, aged 40-60 years old, all participants were divided in randomized order into two groups and were treated for 10 days. The treatment protocol for the first group (A) has been the application of physical modalities, deep transverse massages and dynamic exercises while the other group (B) was treated with isometric exercises.

According to the overall research findings, physical therapy plays an important role in treating cervical spondylosis, reducing pain, increasing muscular strength and flexibility in the cervical region (Egwu, Ajao, & Mbada, 2009), (Boyles, Toy, Mellon, Hayes, & Hammer, 2011) (Hirpara, Butler, Dolan, O'Byrne, & Poynton, 2012) (Leaver, Refshauge, Maher, & McAuley, 2010) (Lauche, et al., 2016).

The two exercise protocols has shown to be very successful treatment strategies for people with cervical spondylosis. However, in group A, the exercises protocol showed to be more effective in increasing mobility and reducing pain than exercise that were applied in group B.

According our results, the level of pain was significantly decreased while cervical mobility was increased, in both groups but significantly in gr. A of exercise, these data can be compared with other research paper (Gemma V & Antonia, 2014), Boissonneult & Badke, 2008 (Boyles, Toy, Mellon , Hayes, & Hammer, 2011).

The role of dynamic exercises and deep transverse massages has been shown to be more effective in treatment of cervical spondylosis, the same results reported also other authors which concluded that dynamic exercise were more effective in cervical spondylosis treatment these data can be comparative to our results (Wong, Shearer, Mior, Jacobs, & Cote, 2016) (Forbush, Cox, & Wilson, 2011), (Cramer, et al., 2012), (Lauche, et al., 2016)

Based on the results of our research we can confirm that if we combine the exercises of both groups, the success of rehabilitation will be much more effective in cervical podylosis.

Conclusions

This study confirms the hypothesis that short-term physiotherapy treatment plays a significant role in treating cervical spondylosis. Comparison between two treatment techniques gives priority to dynamic exercises in spite of isometric exercises. Our recommendation consists of other long-term researches, with higher sample size for the evaluation and comparison of the efficacy of the two treatment methods.

No Conflicts of interest

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Corresponding Author

Arbnore Ibrahimaj Gashi

University of Prishtina, Faculty of Medicine, Physiotherapy Department, R. Kosovo

arbnore.gashi@uni-pr.edu

SHORT TERM PHYSICAL THERAPY TREATMENT FOR NECK PAIN

UDC: 615.82/84:617.53-085.8

(Original scientific paper)

Arbnore Ibrahimaj Gashi¹, Arjeta Azemi², Seryozha Gontarev³, Vujica Zivkovic³

¹University of Pristina, Medical Faculty, Physiotherapy Department, Pristina, Kosovo

²College of Medical Science and Diagnostic and Therapeutic Center "Rezonanca" Pristina, Kosovo

³University of "Ss. Cyril and Methodius" Faculty of Physical Education, Sport and Health" Skopje, Macedonia

Abstract

Neck pain among most patients is considered as simple neck pain without a specific cause, contributing factors in neck pain are considered poor posture, occupational activities, mechanical changes in spine, also anxiety and depression. The purpose of this research was to evaluate the effectiveness of short term physical therapy for treatment of neck pain. This research was carried out at the Diagnostic Therapeutic Centre "Rezonanca" in Pristina, this research was conducted during March-June in year 2017 at physiotherapy department, the study was prospective and short term. The total number of patients incorporated in the study was 25 out of 75, 30-80 years old of both sexes with pain in cervical region lasting more than three months. The survey was short term, lasting 21 days. Patients were treated with physical therapy protocol for 10 sessions. The evaluation of patients was done before and after treatment by Visual Analogue Scale of pain from 0-10. After statistical analysis with t-test we found important statistical significance assessing the degree of pain before and after treatment ($t = 7.201$, $P > 0.05$). Regarding the age group in relation with diagnoses we can notice that age group 47-52 years was the most influenced age and the frequency of patients was higher with diagnosed cervical pain radiated in the left arm. The combination of physical exercises and modalities of physical therapy plays a significant role in the treatment of cervical pain

Key words: neck pain, physical exercise, chronic stage

Introduction

Neck pain among most of the patients is considered as a simple neck pain without a specific cause, but the main cause of developing the symptoms is poorly understood, contributing factors in neck pain are considered poor posture, occupational activities, mechanical changes in spine, also anxiety and depression (Binder, 2007), while neck spondylosis is a degenerative disorder which may be asymptomatic or can be manifested with pain in the cervical region (Hirpara, Buttler, Dolan, O'Byrne, & Poynton, 2012), (Egwu, Ajao, & Mbada, 2009).

There are many definitions regarding the duration of the symptoms, of which the most accepted one is that neck pain may be acute, usually lasting less than 7 days, subacute, lasting more than 7 days but less than three months, and the third level is chronic pain, lasting more than three months (Misailidou, Malliou, Beneka, Karagianidis, & Godolias, 2010).

Neck pain is a common symptom in the population, the prevalence of pain in the general population ranges from 0.4% to 86.8% (Hoy, Protani, De, & Buchbinder, 2010). Pain in the cervical region may be present among people beyond the age of 30 indicating a large number of disabilities linked to the cervical region such as lowering the quality of life and high cost in the health system (Takasaki & May, 2014). The prevalence increases with longer prevalence periods and generally women have more neck pain compared to men. At least for 1-year prevalence, Scandinavian countries report higher mean estimates than in the rest of Europe and Asia (Fejer, Kyvik, & Hartvigsen, 2006). According to scientific data 15% -20% of patients report pain in the cervical region every year and 1.5% -1.8% seek medical help (Evans, 2014), (Lauche, et al., 2016).

The main challenge of physiotherapists is finding the best protocol to assess and to provide treatment for cervical pain, according to the evidence there are many studies that have evaluated the different forms of physical therapy, including manual therapy, physical modalities, exercise protocols, thermotherapy,

acupuncture etc. They all plays an important role in reducing pain, increasing muscular strength and flexibility in the cervical region (Egwu, Ajao, & Mbada, 2009), (Boyles, Toy, Mellon, Hayes, & Hammer, 2011) (Hirpara, Butler, Dolan, O'Byrne, & Poynton, 2012) (Leaver, Refshauge, Maher, & McAuley, 2010) (Lauche, et al., 2016) application of thermotherapy (Cramer, et al., 2012).

After treating patients with different treatment methods including mobilization and manipulation, stretching, strength and flexibility, massage techniques and thermotherapy, patients with cervical pain have shown functional improvements (Boissonnault & Badke, 2008). Regarding the short and long term effect of these methods there are many scientific contradictions that must be further studied in different stages of pain (Wong, Shearer, Mior, Jacobs, & Cote, 2016). The purpose of this research was to evaluate the effectiveness of short term physical therapy protocol for treatment of neck pain.

Material & methods

This research was conducted at the Diagnostic Therapeutic Centre "Rezonanca" in Pristina, during March-June year 2017 the study was prospective and short term, lasting 21 days. The total number of patients incorporated in the study was 25 out of 60, 30-80 years old of both sexes with pain in cervical region lasting more than three months, the pain was radiated on both shoulders, only one side.

Patients were referred for physiotherapy from doctors of general medicine, neurologists, orthopedist and neurosurgeons, patients received physiotherapeutic examination and rehabilitation plan for physical therapy for 10 sessions.

All patients were treated with physical therapy protocol by their therapist including: electrotherapy: application of transcutaneous electrical nerve stimulation (TENS) for 20 min in painful points of muscles in the cervical region, Ultrasound and application of thermotherapy for 15-20min. Then patients were treated with exercise program which included passive mobilization of cervical spine, stretching and strengthening with isometric contraction for main muscles on cervical region like deep flexors of neck, sternocleidomastoid muscles, sub-occipital muscles, trapezius and scalene muscles on both sides.

The evaluation of patients was done before and after treatment or after 10 sessions of physical therapy, pain was evaluated by Visual Analogue Scale of pain (VAS), this is subjective evaluation of pain by patients, they evaluated their pain from 0 minimal pain and 10 maximal pain that they felt.

The main criteria for inclusion in the research was chronic neck pain with a duration of more than 3 months with nonspecific cause. While exclusion criteria were acute stage of disease, pregnancy, patients with other cervical spine problems eg: intervertebral disk hernia on the cervical spine, spinal stenosis of the cervical region, hypertension, diabetes etc.

Presentation of data is done through tables. Data processing is done with the IBM SPSS Statistics. Statistical parameters that were calculated are: arithmetic average, standard deviation, as well as minimum and maximum value. Data testing was done for parametric with t-test. Verification of the tests for the degree of reliability was 95% and 99%, with $p < 0.05$ and $p < 0.01$.

Results

Table 1. Patients included in the study by age and sex.

Age	Sex				Total	
	F		M		N	%
	N	%	N	%	N	%
35-40	0	0	2	15.38	2	8
41-46	2	16.67	0	0	2	8
47-52	4	33.33	3	23.08	7	28
53-58	2	16.67	3	23.08	5	20
59-64	3	25	3	23.08	6	24
65+	1	8.33	2	15.38	3	12
Total	N	12	13	100	25	100
	%	48	-	52	-	100

The total number of patients included in this study was 25, higher frequency of age belonged to the age

group 47-52 years 28%. Regarding gender, the large number of patients were male of which most belonged to the age group of 47-52, 53-58 and 59-64 years and 23.08%, while female group were with higher frequency at age group 47-52 years old or 33.33% (Table 1).

Table 2. Statistical data about diagnosis related to age group and sex.

Age group			Sex				Total	
			F		M		N	%
			N	%	N	%	N	%
35-40	Diagnos	Cervicobrachialis dex	0	0	1	7.7	1	4
		Syndroma Cervicale	0	0	1	7.7	1	4
	Total		0	0	2	15.4	2	8
41-46	Diagnos	Cervicobrachialis sin	1	8.3	0	0	1	4
		Syndroma Cervicale	1	8.3	0	0	1	4
	Total		2	16.7	0	0	2	8
47-52	Diagnos	Cervicobrachialis bill	2	16.7	0	0	2	8
		Cervicobrachialis sin	1	8.3	3	23.1	4	16
		Syndroma Cervicale	1	8.3	0	0	1	4
Total		4	33.3	3	23.1	7	28	
53-58	Diagnos	Cervicobrachialis bill	0	0	3	23.1	3	12
		Cervicobrachialis dex	1	8.3	0	0	1	4
		Syndroma Cervicale	1	8.3	0	0	1	4
Total		2	16.7	3	23.1	5	20	
59-64	Diagnos	Cervicobrachialis bill	1	8.3	1	7.7	2	8
		Cervicobrachialis dex	0	0	1	7.7	1	4
		Syndroma Cervicale	2	16.7	1	7.7	3	12
Total		3	25	3	23.1	6	24	
65+	Diagnos	Cervicobrachialis dex	1	8.3	2	15.4	3	3
	Total		1	8.3	2	15.4	3	12
Total	Diagnos	Cervicobrachialis bill	3	25	4	30.8	7	28
		Cervicobrachialis dex	2	16.7	4	30.8	5	20
		Cervicobrachialis sin	2	16.7	3	23.1	6	24
		Syndroma Cervicale	5	41.6	2	15.4	7	28
	Total		12	100	13	100	25	100

From the results shown on Table 2. we can notice that from 25 patients included in the study, the higher number of patients were in the group of patients with diagnosed cervicobrachial syndrome bilaterally and cervical syndrome.

Regarding the age group in relation with diagnoses we can notice that age group 47-52 years was the most influenced age and the frequency of patients was higher with diagnosed syndrome cervicobrachial lateral sinister. While the lowest frequency of patients belonged to the age group 35-40 years old with only two diagnoses.

Table 3. Statistical parameters of pain before and after treatment.

	VAS before treatment	VAS after treatment
Average	6.08	3.36
SD	1.4	1.2
Min	4	1
Max	9	6
t-test, P-value	t=7.201, P>0.05	

The average pain measured with VAS before treatment was 6.08 with (SD \pm 1.4) in the interval of 4-9. After treatment, this value is reduced up to 3.36 (SD \pm 1.2), the interval of 1-6. After statistical evaluation, with the t-test we found important statistical significance assessing the degree of pain before and after treatment ($t = 7.201$, $P > 0.05$), (Table 3).

Discussion

The total number of patients included in this study was 25 out of 75, 30-80 years old of both sexes with pain in cervical region lasting more than three months, the higher frequency of age belonged to the age group 47-52. Regarding gender, the larger number of patients was male compared to female group shown in Table 1.

From the results shown on Table 2. we can notice that from 25 patients included in the study, the higher number of patients were in the group of patients with diagnosed sy. cervicobrachialis bilaterally and cervical syndrome.

Regarding the age group in relation with diagnoses we can notice that the age group 47-52 years was the most influenced age and the frequency of patients was higher with diagnosed sy. cervicobrachialis lat.sin. While the lowest frequency of patients belonged to the age group 35-40 years old with only two diagnoses.

The average pain measured with VAS before treatment was 6.08 with (SD \pm 1.4) in the interval of 4-9. After treatment, this value is reduced up to 3.36 (SD \pm 1.2) with statistical analysis with t-test we found important statistical significance assessing the degree of pain before and after treatment as shown on the table, also similar data is reported by other authors (Gemma V & Antonia, 2014), (Boissonnault & Badke, 2008), (Boyles, Toy, Mellon, Hayes, & Hammer, 2011), (Uthaikhup, Assapun, Wtcharasaksilp, & Jull, 2017).

Our study was short-term following up for 21 days and 10 sessions with physical therapy, application of exercise and manual mobilization (Vincent, Maigne, Fischhoff, Lanlo, & Dagenais, 2013) of the neck showed to be very effective in pain reduction, one study evaluated the combination of upper cervical and upper thoracic spine mobilization which indicated better results in pain management and overall short-term outcomes (Juchul, Eunsang, & Seungwon, 2018).

Another author (Ayub, Osama, Shakil-ur, & Ahmad, 2019) evaluated another method of mobilization called active and passive mobilization which showed to be very effective in treating cervical radiculopathy.

To reduce pain in muscles around the neck with latent trigger points according one study which reported that applying soft tissue release in sternocleidomastoid and suboccipital muscles had a positive impact in pressure pain threshold (Kim & Lee, 2018).

Applying strengthening exercise in cervical region has a positive impact in reducing pain, increasing muscular strength and flexibility, these data are consistent with other authors (Lauche, et al., 2016) (Boyles, Toy, Mellon, Hayes, & Hammer, 2011) (Hirpara, Butler, Dolan, O'Byrne, & Poynton, 2012) (Leaver, Refshauge, Maher, & McAuley, 2010). (Wong, Shearer, Mior, Jacobs, & Cote, 2016) (Forbush, Cox, & Wilson, 2011), (Cramer, et al., 2012),

Based on the results of our research we can confirm once again the significant role of physical therapy treatment in management of chronic neck pain which can be radiated or not in both shoulders.

Conclusion

The combination of physical exercises and modalities of physical therapy plays a significant role in the treatment of cervical pain, short-term physiotherapy for 21 days included 10 session plays a significant role in improvement of function and reducing pain for patients with chronic neck pain.

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THE PLACE OF TECHNETIUM 99M RADIOPHARMACEUTICALS FOR IMAGING OF NEUROENDOCRINE TUMORS

Armend Jashari

Clinical Service of Nuclear Medicine - Hospital and University Clinical Service of Kosovo
armendjashari@yahoo.com

Yll Kaçiu

Clinical Service of Nuclear Medicine - Hospital and University Clinical Service of Kosovo
yllifshmn@yahoo.com

Marija Anastasova

University Goce Delcev Stip, Faculty of Medical Sciences, Republic of North Macedonia
marija.15655@student.ugd.edu.mk

Emilija Janevik-Ivanovska

University Goce Delcev Stip, Faculty of Medical Sciences, Republic of North Macedonia
emilija.janevik@ugd.edu.mk

Abstract:

Neuroendocrine tumors, NETs are a heterogeneous group of neoplasms with different anatomic locations: in the gastrointestinal tract, bronchopulmonary in origin and in other sites, e.g. thyroid, breast, genitourinary system, head and neck. Usually, these tumors are locally advanced with distant metastases. They grow slowly and are therefore not easy to detect.

Beside Gallium-68 labelled somatostatin-receptor analogues, the most commonly used to locate NETs, their metastases and evaluate lesion nature and extension, some peptides have been used as molecules carrying Technetium-99m radioactive atoms. Radioactive peptides are the future of imaging and therapy for neuroendocrine tumors because they are specific and have a receptor-ligand interaction. They have greater stability because the changes are not natural/ changing the order of amino acids. Currently only few radiopharmaceuticals are capable to image cancer processes at the sub-cellular level. Diagnostics is possible because of gamma radiation emitted Technetium-99m radioactive atoms bound to molecules of the peptides. The radiation may be used to image the distribution of tumor cells in the patient body.

After evaluation of the published literature and our very limited experience, we can conclude that the clinical role of SPECT imaging using Technetium-99m radiopharmaceuticals in patients with NETs is very important, especially when PET is not available.

Keywords: Neuroendocrine tumors - NETs, technetium-99m, radiopharmaceuticals, somatostatin

The field of the paper: Medical Sciences and Health

Introduction

Neuroendocrine tumors (NETs)

Neuroendocrine tumors (NETs) are neoplasms characterized by the expression of general markers (neuron specific enolase, chromogranin, synaptophysin) and are hormone secretion products. They are very heterogeneous group of neoplasms that can occur at in part of the body despite having a shared origin from neuroendocrine cells. They arise from the endocrine cells within glands (adrenal medulla, pituitary, parathyroid) or from endocrine islets in the thyroid, the pancreas, or the respiratory and gastrointestinal tract.

Common types of NETs are located in the gastrointestinal tract or the pancreas and are collectively referred to as gastro-enteropancreatic neuroendocrine tumors (GEP-NETs). They constitute as well as heterogeneous group of tumors with their origin in neuroendocrine cells of the embryological gut (Öberg et al. 2012)

The NET classification has changed over the years as a result of scientific development and experiences obtained in clinical trials. The classification is mainly based on the nomenclature given by the WHO in 2010 and the classification of neuroendocrine neoplasms of the digestive system according to Rindi et al. since 2010:

1. well-differentiated neuroendocrine tumors (NETs) traditionally referred to as “carcinoid and pancreatic neuroendocrine (islet cell) tumors”, and
2. poorly-differentiated neuroendocrine carcinomas (NECs) traditionally referred to as “small cell NECs and large cell NECs”.

As result of that, GEP-NETs refers by WHO are well-differentiated NETs located in the esophagus, esophagogastric junction, abdomen, ampullary region, small intestine, appendix, colon and rectum and duct, liver and intrahepatic bile ducts, and extrahepatic bile ducts and pancreas). NETs with unknown primary tumor location (also known as CUP-NETs) are predominantly GEP-NETs.

A unique feature of well-differentiated GEP-NETs is their overexpression of somatostatin receptors (SSTR) on the tumor cells. Several types of tumors are known to significantly express SSTR. According to the EANM guideline, the following tumors have a high SSTRs expression (Virgolini 2010):

- Functioning and non-functioning gastroenteropancreatic neuroendocrine tumors (GEP-NET), gastrinoma, insulinoma, glucagonoma, VIPoma, etc.),
- Sympathoadrenal system tumors (phaeochromocytoma, paraganglioma, neuroblastoma, ganglioneuroma)
- Medullary thyroid carcinoma
- Pituitary adenoma
- Medulloblastoma
- Merkel cell carcinoma
- Meningioma

Out of the five subtypes of human somatostatin receptors (sstrs) which have been identified, most abundant in GEP-NETs is sstr2, followed by equal amounts of sstr1 and sstr5, lower amounts of sstr3 and hardly any sstr4 (Johnbeck et al. 2014, Maxwell et al. 2015). Poorly-differentiated NECs hardly express somatostatin receptors.

Imaging of Neuroendocrine tumors

Many different imaging techniques are used to localize and differentiate GEP-NET. Nuclear medical techniques try to contribute to the diagnosis and early detection of NET by using highly specific radiopharmaceuticals, as well as to monitoring the efficacy of therapy.

Radiolabeled peptides are very promising vectors for imaging and therapy for neuroendocrine tumors. As specific for binding to somatostatin receptors, they represent the right choice, but also the challenge of which radionuclide will be used and which ligand to obtain the most appropriate response.

These peptides are synthetic analogues of somatostatin and have great stability because the changes are not natural and there is a change in the order of the amino acids.

Imaging procedures for the diagnosis of neuroendocrine tumors (NETs) in nuclear medicine use pure gamma emitter radionuclides such as technetium-99m or PET radionuclides (positron emission tomography).

NET imaging radiopharmaceuticals used to visualize neuroendocrine tumors are similar in molecular structure to synthesized hormones or are involved in various metabolic and cellular processes of tumor cells. They may be somatostatin analogues (111-In DTPA / 99mTc-Octreotide, 68Ga-DOTA), catecholamine analogues and metabolites (131I / 231I-MIBG, 18F / 11C-DOPA, 11C-HTP, 11C-HP analog 18F-FDG).

One of the most promising NET radiopharmaceuticals for diagnostic purposes that allows widespread use is Technetium 99mTc-HYNIC-Tyr3-Tektrotyd. It is a radiopharmaceutical indicated for the diagnosis of pathological lesions in which somatostatin receptors are overexpressed (especially subtypes 2 and, to a lesser extent, subtypes 3 and 5) and which can be visualized with it. Another advantage is that technetium-99m is used as the radionuclide as the most commonly used and available radionuclide.

Technetium-99m - radionuclide for SPECT imaging of NETs

The physical characteristics of technetium-99m are suitable for imaging procedure using gamma cameras (SPECT, SPECT / CT), because they deliver a low radiation to the patient and provide better image quality with lower doses injected in a form of radiopharmaceutical. The radiation dose that the patient receives after the applied activity is very small and does not cause harmful effects.

Generally, a radiopharmaceuticals are pharmaceutical preparation that is usually administered intravenously to a patient containing radioactive isotope such as Technium-99m and specific molecule for target organ in the body. Technetium-99m (^{99m}Tc) has a low-energy gamma rays of 140-keV that are emitted and easily pass through the human body. It has a half-life of six hours, which is long enough to examine metabolic and functional processes, but still short enough to minimize the radiation dose to the patient.

Even if the use of radiopharmaceuticals labelled with positron emitters radionuclides challenged the field of SPECT tracers, over 70% of diagnostic investigations are still performed with this single isotope for imaging of bone, renal, hepatic, hepatobiliary, cardiac, and oncological diseases or other pathologies.

Because imaging of neuroendocrine tumors has become one of the most actual areas in nuclear oncology, Technetium-99m radiopharmaceutical have been proposed in an attempt to provide high-quality imaging and possible sensitivity at a reduced cost, time, and radiation doses.

Tc-99m HYNIC-TOC provides a high imaging quality for the staging of carcinoids, and it seems to be the method of choice for detection of the primary focus in patients with metastases from the unknown primary tumor. The

sensitivity in comparison with CT is higher for primary lesions and liver and abdominal lymph node metastases. The usage of this radiopharmaceutical has substantially changed the management of GEP-NET patients.

The aim of this publication is to stress the importance and the place of Technetium 99m radiopharmaceuticals for imaging of neuroendocrine tumors and show that is still the most used radionuclide in the clinical nuclear medicine practice. Through the preparation of this study we want to demonstrate the quality of diagnosis and follow-up therapy of neuroendocrine tumors. The introduction of new radiopharmaceuticals for imaging with radioactive isotope Technetium-99m, available in all nuclear medical institutions, allows timely diagnosis even where positron emission tomography is unavailable. In order to achieve high specific binding and safety after application of this relatively new radiopharmaceutical, it is necessary to ensure proper quality control, which will guarantee the same.

Material and methods

Material for this paper is the most relevant literature from the main databases of scientific and literary data related to the Technetium-99m radiopharmaceutical introduced and used for imaging of neuroendocrine tumors. This was the starting point for our research work and it will be used for comparison of the obtained results.

The compilation and comparative method allowed us to create and draw our own conclusions using an individual and specific approach.

Results from the research material and discussion

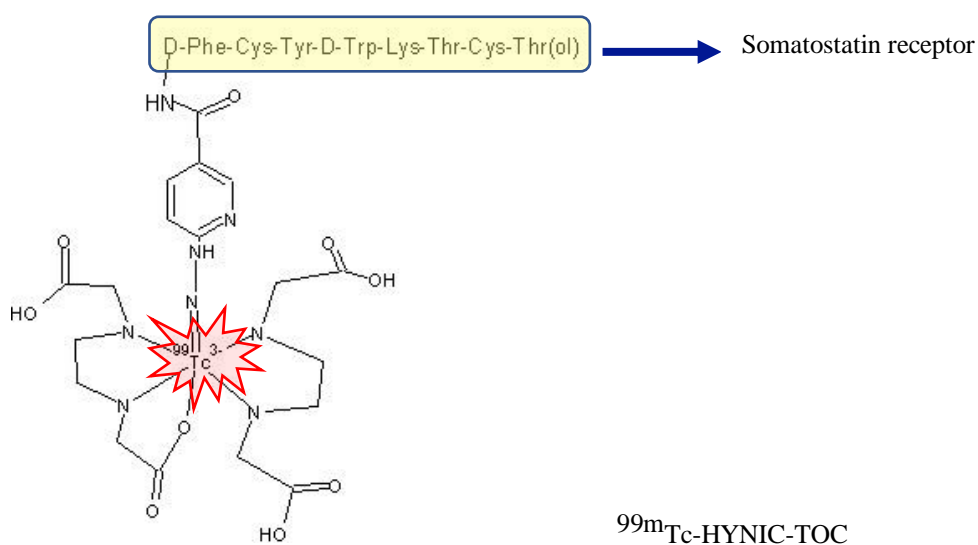
Visualization of tumors expressing somatostatin receptors (sst) using radioactively labeled peptides, somatostatin analogues, has become an integral part of nuclear oncology.

Using Technetium-99m for this purpose resulted in a readily available technique with very good image quality, high sensitivity detection of cancer and lower average effective dose for the patient tested.

Technetium-99m is considered to be a suitable radionuclide for somatostatin receptor scintigraphy (SRS). It is the workhorse of the nuclear medicine physician because of its short half-life (6 hrs.) and emission of gamma radiation with the energy of 141 keV. The wide availability and cost-effectiveness of ^{99m}Tc are of major importance for routine clinical applications. Several chelators were investigated to provide efficient and stable ^{99m}Tc labeled somatostatin analogs with high affinity to somatostatin receptors, among them conjugates of 6-hydrazinonicotinamide (HYNIC) found their way to the clinics.

The HYNIC core with N-hydroxysuccinimidyl hydrazinonicotinamide (NHS-HYNIC, HYNIC) has become one of the most popular and effective BFCs used for ^{99m}Tc labeling of somatostatin analogs. It has initially been developed for radiolabeling of polyclonal immunoglobulin and was then recommended for preparation of hydrazino-modified proteins and synthesis of ^{99m}Tc -protein conjugates and chemotactic peptides and HYNIC as bifunctional chelator (BFC) was introduced for ^{99m}Tc labeling of octreotide and TOC (Tyr3-octreotide) with high efficiency.

Figure 1. Structure of ^{99m}Tc -HYNIC-TOC, Tektrotyd



HYNIC ligands are particularly convenient for radiolabeling biomolecules, with ^{99m}Tc being the active ester most readily combined with small molecules, proteins, or different targeting vectors.

However, even if the metal-organohydrazino unit can be easily prepared from the metal-oxo core by a simple condensation reaction, the intimate details of the chemistry are more complex and dependent upon reaction conditions and the presence of co-ligands, which can influence the stability, hydrophilicity, and pharmacokinetics of the resulting ^{99m}Tc -compounds.

Nevertheless, a wide range of bioactive molecules have been labelled with the HYNIC strategy, among which the most investigated are undoubtedly the somatostatin derivatives for neuroendocrine tumor (NETs) imaging (Mikołajczak.2016). Most published data concern the commercially available ^{99m}Tc -[HYNIC, Tyr(3)]octreotide (^{99m}Tc -HYNIC-TOC, Tektrotyd) prepared through a two-vial kit formulation containing HYNIC- [D-Phe1, Tyr3-Octreotide] and EDDA as a co-ligand. The complex is formed by a technetium atom bound to a ligand composed of two functional parts, and a HYNIC group linked to an octapeptide.

The EDDA ancillary ligand is required to stabilize the coordination geometry. ^{99m}Tc -HYNIC-TOC represents a selective receptor imaging agent for neuroendocrine tumors and, even if the true molecular structure of ^{99m}Tc -HYNIC-TOC is not exactly determined, its biological properties can be understood by considering the bioactive group octreotide and selectively targeting the somatostatin receptors (SSR) overexpressed on the membranes of neuroendocrine tumors.

^{99m}Tc -HYNIC-TOC binds plasma proteins 33-51%, at a later time, 20 hours after injection, whereas it was substantially lower directly after injection (Decristoforo, 2000). Activity is accumulated mainly in the liver, spleen, kidneys and, in lesser quantity, in the thyroid.

^{99m}Tc -HYNIC-TOC is rapidly cleared from the circulation, with a short effective half-life. It appears as the radioactive radiopharmaceuticals is eliminated mainly by renal excretion. Cumulative urine excretion of radioactivity over 24h has been reported to be 24-64% (Decristoforo, 2000). Some gastrointestinal excretion was also observed in images, but the image quality was impaired due to low count rate at later time points.

As ^{99m}Tc -HYNIC-TOC is given only as a single injection, characterization of its elimination is however considered sufficient.

The risk for pharmacokinetic drug-drug interactions has not been addressed in the application of ^{99m}Tc -HYNIC-TOC and any interaction studies have been performed.

All published data that we evaluated confirm that ^{99m}Tc -HYNIC-TOC can be implemented in routine use in smaller nuclear medicine departments where there is regular use of technetium-99m. Experience using this gamma radionuclide as well as established quality control techniques provide reliability in its application.

According to the protocol given by the manufacturer in our radiopharmaceutical laboratory, we made a standard operational procedure for quality control of ^{99m}Tc -HYNIC-TOC which should be realized before the application to the patient.

This procedure determines the percentage of ^{99m}Tc -HYNIC-TOC, as well as the percentage of possible unwanted chemical forms in the form of radiochemical impurities that reflect the quality of the imaging.

Conclusions

Even today, Technetium-99m is the radionuclide of choice for diagnostic applications in nuclear medicine. Its ideal physical and chemical properties make it particularly suitable for use everywhere, especially in countries and institutions where PET technology is unavailable, especially when it comes to diagnosing and monitoring the treatment of neuroendocrine tumors.

From a practical point of view, the introduction and use of new technetium-99m-labeled radiopharmaceuticals, such as ^{99m}Tc -Tektrotyd, will indicate its importance in diagnosing and monitoring patients with neuroendocrine tumors, especially for the diagnosis of pathological lesions. over-expressed (especially subtype 2 and, to a lesser extent, subtype 3 and 5) and which can be visualized with it.

In order to achieve high specific binding and safety after application of this relatively new radiopharmaceutical, it is necessary to ensure proper quality control, which will guarantee the same.

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IMPROVING OF RADIOPHARMACEUTICAL STANDARDS AT NUCLEAR MEDICINE CLINICAL SERVICE IN KOSOVO

Armend Jashari^{1,2}, Emilija Janevik Ivanovska

¹ Hospital and University Clinical Service of Kosovo - HUCSK

² Nuclear Medicine Department in Hospital and University Service of Kosovo

Abstract

In conventional Nuclear Medicine it is very important that all materials and products administered into the human body, including ^{99m}Tc radiopharmaceutical kits are safe and constant high quality in producing the required effects. Manufacturers have an obligation to make certain that all released products are suitable for their intended use. All radiopharmaceuticals must conform to specifications that assure products are of suitable quality and are adequate and safe for patient administration.

Quality Assurance (QA) ensures consistent good quality service and involves several steps and adherence to pre-planned procedures, contributing to achieving the highest quality for the final products intended use.

Quality control (QC) is an essential part of QA and is necessary to ensure an adequate QA programme to protect patients from unnecessary ionizing radiation.

QC is the part of QA programme that assesses whether the performance level required of the service has been reached by documenting measurements obtained from tests and checks performed in various areas in nuclear medicine, such as all equipment's and radiopharmaceuticals. These results may then be compared to preset standards to make certain that the results are within the established limits.

According to the European Association of Nuclear Medicine (EANM), the quality of all radiopharmaceutical products should be determined prior to release for human administration and a QC program should be in place at any hospital department that deals with radiopharmaceutical kit preparation. Although most of the responsibility of radiopharmaceutical quality belongs to the manufacturer of the pharmaceutical, the nuclear medicine department

producing the final product for human administration (radiopharmaceutical) also has the responsibility of ensuring that the radiopharmaceutical produced is up to standard QC of the Radionuclides can be done according the Callahan guidelines for the elution of generators and the preparation of kits on-site and also the parameters that should be tested in QC program.

According to Early & Sodee (1995), the radionuclide purity and the radiochemical purity are two areas that are of utmost importance when determining the quality of the radiopharmaceutical. Therefore, these two parameters should always be included in a basic QC programme if it is not possible to set up a comprehensive QC program.

The main parameters that are necessary to be tested about radiopharmaceuticals are Radionuclide purity, Chemical purity, and pH of the eluate.

The goal of this paper is to a review the quality standards of radionuclides and radiopharmaceuticals in a nuclear medicine service in University and Hospital Service in Kosovo.

Keywords: Quality Control (QC), Quality Assurance (QA), radiopharmaceuticals

Introduction

The handling of radiopharmaceuticals is potentially hazardous. The level of risk depends in particular upon the types of radiation emitted and the half-lives of the radioactive isotopes. A continuous assessment of the effectiveness of the Quality Assurance system is essential to prove that the procedures applied in the Radiopharmacy Department lead to the expected quality.

Radioactive products should be stored, processed, packaged and controlled in dedicated and self-contained facilities. The equipment used should be reserved exclusively for radiopharmaceuticals. The radiopharmacy has to be organized in such a way as to minimize the risk of cross contamination and mix-up. Only the necessary equipment should be located there. Access to the controlled areas should be via a gowning area and should be restricted to authorized personnel. Guests and technical staff should follow appropriate rules for access, which should be described in a special instruction. After repairs the premises have to be cleaned

and decontaminated appropriately.

The technetium-99m generator and the working area for the preparation of technetium-99m labelled radiopharmaceuticals should be located in a controlled area. The room must be approved for work with open radioactive sources. The solutions of ^{99m}Tc eluate and the ready preparations have to be stored in well-shielded conditions. Manufacturers have an obligation to make certain that all released products are suitable for their intended use. All radiopharmaceuticals must conform to specifications that assure products are of suitable quality and are adequate and safe for patient administration.

Quality Assurance ensures consistent good quality service and involves several steps and adherence to pre-planned procedures, contributing to achieving the highest quality for the final products intended use.

Radiopharmacy in the Nuclear Department is covering all requirements related to the Hospital Radio/Pharmacy. New equipment for the radiolabeling was installed and complete Standard Operating Procedures for the labelling with Technetium-99m were written and introduced. Quality control (QC) is an essential part of QA and routine work that was established in our nuclear department.

Radiochemical purity of all radiolabeled radiopharmaceuticals started to be evaluated daily and quality control of the generator (Radionuclide purity, Chemical purity, and pH of the eluate) regularly every week following the recommendation of the European Association of Nuclear Medicine (EANM), that the quality of all radiopharmaceutical products should be determined prior to release for human administration and a QC program should be in place at any hospital department that deals with radiopharmaceutical kit preparation.

Devices used for radiochemical purity determination such as a dose calibrator, gamma counter, gamma camera, thin layer chromatography scanner, high performance liquid chromatography radioactivity detector and autoradiography apparatus, require determination of background activity each time when used for measurements and regularly verification of detection linearity and accuracy of measurement.

Other equipment used for preparation of radiopharmaceuticals such as water baths, thermometers, heating plates etc. have to be checked for correctness of the settings.

A system of planned preventive maintenance and calibration should be operated to ensure that all facilities and equipment used in the preparation of radiopharmaceuticals are regularly maintained and calibrated where appropriate. Records and logs should be kept for all equipment irrespective of whether maintenance and calibration is performed in house or by external contractors.

Although most of the responsibility of radiopharmaceutical quality belongs to the manufacturer of the pharmaceutical, the nuclear medicine department producing the final product for human administration (radiopharmaceutical) also has the responsibility of ensuring that the radiopharmaceutical produced is up to standard.

The aim of this publication is to introduce quality standards of preparation and quality control of radiopharmaceuticals before clinical application in a Nuclear Medicine service in University and Hospital service in Kosovo.

Materials and methods

Material for this paper is the most relevant literature from the main guides and databases for good radiopharmacy practice introduced and used for improving of radiopharmaceutical standards in daily bases in a unit of Nuclear Medicine.

Results

Discussion

Conclusion:

Radiopharmacy is an integrated part of the Nuclear Medicine Department and according to the high European standards and requirements. Nuclear Medicine service in University and Hospital service in Kosovo is established in 1973 as first radio-pharmacy in the country and now by meeting the necessary standards is implementing quality daily work. Having new equipment,

regularly employed radiopharmacist and physicist and standardized protocols, our department starts to follow all criteria for recognized work.

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Ronald J. Callahan¹, Henry M. Chilton², James A. Ponto³, Dennis P. Swanson⁴, Henry D. Royal⁵, and Allegra D. Bruce¹

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COST-EFFECTIVE QUALITY CONTROL METHOD FOR RADIOCHEMICAL PURITY OF ^{99m}Tc-TECTROTYD USED IN A HOSPITAL RADIOPHARMACY UNIT

Armend Jashari

Clinical Service of Nuclear Medicine - Hospital and University Clinical Service of Kosovo
armendjashari@yahoo.com

Jasna Grozdanovska

University Goce Delcev Stip, Faculty of Medical Sciences, Republic of North Macedonia
jasna.15752@student.ugd.edu.mk

Yll Kaçiu

Clinical Service of Nuclear Medicine - Hospital and University Clinical Service of Kosovo
yllifshmn@yahoo.com

Liljana Makraduli

Replek farm – Skopje
liljana.makraduli@gmail.com

Ljubica Adzi-Andov

Amedela Laboratory, Skopje
bube.petrovska@yahoo.com

Emilija Janevik-Ivanovska

University Goce Delcev Stip, Faculty of Medical Sciences, Republic of North Macedonia
emilija.janevik@ugd.edu.mk

Abstract

The new Radiopharmacy in Nuclear Medicine department in the Hospital and University Service of Kosovo apply the policy that all products administered into the human body, especially the new one are safe and show a constant high quality in producing the required effects. To ensure the efficacy of radiopharmaceuticals prepared at department in the Hospital and University Service of Kosovo, we introduced a cost-effective routine chromatographic method.

The radiochemical purity (RCP) of ^{99m}Tc-Technetium labelled radiopharmaceuticals (RP) is important to ensure optimal scintigraphic image quality. In a new hospital radiopharmacy unit it may not be possible to use compendial analytical methods or expensive equipment for radiochemical purity analysis, but all radiochemical analysis methods should however be validated against compendial or otherwise proven methods.

Our goal was to optimize the radiolabeling protocol for the regular use of ^{99m}Tc-Tektrotyd and to establish chromatographic method for quality control after labelling as a part of our daily diagnostic procedures for assessment of NET patients labelled with ^{99m}Tc-Technetium was for the first time used to identify medical problems related to overexpression of somatostatin receptors, particularly subtype 2 and, to a lesser extent subtype 3 and 5.

Methods:

Tektrotyd or HYNIC – (D-Phe¹, Thy³-Octreotide) trifluoroacetate (Polatom) radiopharmaceutical were reconstituted with about 2 000 MBq of freshly eluted sodium pertechnetate as described by the manufacturer and spiked with eluate of the same generator to obtain a range of impurity concentrations. Samples of technetium-^{99m} Tektrotyd were spotted on 1x15 cm ITLC-SG strips and developed in appropriate mobile phases described by the manufacturer. Each strip was immediately cut into 30 pieces of 0.5cm and the radioactivity of each piece was measured in a dose calibrator (Capintec, Inc).

The percentage of RCP for each ITLC strip was calculated using the total the radioactivity and the radioactivity from each segment as the total present radioactivity from which the basic radioactivity was subtracted. The present plotted radioactivity in the obtained peaks corresponded to the distribution of radioactivity, i.e. to the present complex of ^{99m}Tc Tektrotyd.

Results and Discussion: The proposed method proved to be accurate and precise within the RCP range of approximately 90% to 100% in comparison of the producer requirements.

Conclusion: The proposed method is suitable as a reliable low-cost method for limited resource settings and small hospital radiopharmacy unit.

Keywords: Quality control, Radiochemical purity, Instant Thin Layer Chromatography Medium (ITLC), ^{99m}Tc -Tektrotyd,

The field of the paper: Medical Sciences and Health

Introduction

Nuclear Medicine needs optimal quality radiopharmaceuticals for accurate diagnosis and therapy of diseases (IAEA 2008; Dondi 2011). As the use of Nuclear Medicine increases in developing countries, so does the need for radiopharmaceuticals.

The increased need for visualization of neuroendocrine tumors and the lack of PET did oblige us to introduce the ^{99m}Tc -Tektrotyd. ^{99m}Tc -Tektrotyd is a radiopharmaceutical for diagnostic use only. It is a radiopharmaceutical indicated for the diagnosis of pathological lesions in which somatostatin receptors are overexpressed (especially subtypes 2 and, to a lesser extent, subtypes 3 and 5) and which can be visualized with it. The physical characteristics of ^{99m}Tc are more suitable for gamma camera imaging, with lower patient radiation and better image quality with lower radiation doses.

With the increasing demand for radiopharmaceuticals (Faria 2015), it is of paramount importance to ensure that only safe and effective products are administered to patients (IAEA 2016). Even though most radiopharmaceuticals are used to be prepared in small scale units, quality assurance programs should be implemented (Elsinga et al. 2010). Internationally, guidelines for Good Radiopharmacy Practice recommend that only validated analytical methods should be used to evaluate product quality before patient use. Analytical Quality Control (QC) methods form part of quality assurance, helping to assess the quality of the product. According to Ajay et al., validation of analytical methods provides evidence that the method is suitable for its intended use, and thus helps to ensure provision of safe and effective final products (Ajay et al. 2012). The consequence of using unvalidated non-compendial methods could result in the administration of unknown substances, poor quality images and/or an unnecessary radiation exposure of patients (Amin et al. 2011; Vincenti et al. 2016).

In radiopharmacy, one of the most frequently used analytical criteria is radiochemical purity (RCP) of the product. The radiochemical purity of a preparation is the fraction of the total radioactivity in the desired chemical form of the radiopharmaceutical. During the labelling process, radiochemical impurities may be present as a result of incomplete radiolabelling or decomposition products due to the presence of oxidizing or reducing agents, radiolysis or change of temperature and pH (Saha 2010; Millar et al. 2009; Loveless 2009; Mambilima 2016).

RCP can be assessed by various analytical techniques, for example paper, thin layer, or liquid chromatography and electrophoresis. Radiochromatography involves the separation of components in solution (depending on their affinity with the chromatography materials), and the measurement of the distribution of radioactivity on the chromatogram (Saha 2010).

A variety of methods for quantification of distribution of radioactivity on radio-TLC plates are described in literature. Decristoforo and Zolle provide an overview of seven methods used for ^{99m}Tc radiopharmaceuticals, including cutting and counting in a scintillation counter, chromatogram scanning, analysis by linear analyzer and phosphor-imager autoradiography (Decristoforo et al. 2007). The methods differ regarding sensitivity, resolution and linearity, but differences in time required per analysis and the cost of equipment of each of these methods are considerable, making their adoption in resource developing countries challenging (Loveless 2009).

At Radiopharmacy in Nuclear Medicine department in the Hospital and University Service of Kosovo in Prishtina there is no chromatogram scanner or more advanced equipment with which the distribution of radioactivity or chromatograms of radiopharmaceuticals can be analyzed. Although the technical and analytical possibilities are limited, new methods and new radiopharmaceuticals are being introduced in our institution.

An alternative method was therefore sought, namely counting sections of the chromatography strips corresponding to the distribution of the different radiochemical species with a contamination monitor available in the Nuclear Medicine department in Prishtina.

Prior to employing it in daily practice, this proposed method had to be validated. The work described here was performed to validate a cost-effective method for quantifying the distribution of radioactivity on ITLC chromatography strips (Millar 2009).

Material:

The study was conducted in the Department of Nuclear Medicine, in a laboratory for radiopharmacy using appropriate equipment that provides protection from ionizing radiation, suitable for the use of technetium- ^{99m}Tc . The realization of this study is based on the idea of how to contribute to having a good safe new radiopharmaceutical product, using a radiopharmaceutical kit, ^{99m}Tc -Tektrotyd.

In this study we used:

- Tektrotyd (POLATOM), kit for preparation of radiopharmaceuticals (TEKTROTYD 2015),
 - ^{99m}Tc –pertechnetate obtained off ^{99}Mo / ^{99m}Tc generator - POLATOM with power of 16 GBq
- And material for quality control of the final labelled radiopharmaceutical:
- ITLC SG pad - Merck - Silica gel impregnated pad,
 - Methyl ethyl ketone (MEK),
 - A mixture of acetonitrile and water in a volume ratio of 1: 1, 1 mL
 - syringe with needle for subcutaneous injection,
 - Appropriate equipment for measuring radioactivity (eg dose calibrator)

Methods:

For the preparation of ^{99m}Tc -Tektrotyd we used fresh sterile sodium pertechnetate (^{99m}Tc s) solution for injection without oxidant (eluate of $^{99}\text{Mo}/^{99m}\text{Tc}$ radionuclide generator) (up to 2000 MBq) in accordance with radiopharmaceutical preparation instructions (Figure 1).

Analysis and quality control of a labeled radiopharmaceutical ^{99m}Tc -Tektrotyd was performed by introducing validated and efficient methods based on detection and identification of ^{99m}Tc -Tektrotyd and determination of radiochemical purity (Parisella 2012).

Determination of radiochemical purity (RCP)

Radiochemical purity using Instant thin layer chromatography (ITLC) was performed on Silica gel strips with different mobile phases:

- Acetone (Ac) to determine the percentage of the free $^{99m}\text{TcO}_4^-$ fraction ($R_f=0.9-1.0$)
- Acetonitrile (ACN) 50% for the ^{99m}Tc -colloid fraction ($R_f=0.0 - 0.3$)

Five μl of ^{99m}Tc -Tektrotyd samples was spotted on ITLC-SG strips. Within 5 minutes the strip was cutted in 30 pieces at 0.5 cm from the origin. The radioactivity from each portion of the strip was measured in NaI (TI) crystal counter / dose calibrator. Each strip was placed flat in the bottom of a 10 cm deep container and the counter placed at the dose calibrator to ensure that the counting geometry was the same for all samples. Each count rate reading was recorded with background count rate subtracted. From these values, the percentage of radiochemical purity of each strip was calculated.

A drop of the same Tc- 99m eluate used for kit reconstitution (and to spike the radiopharmaceuticals) was used to run a control analysis under the same conditions as those of the radiopharmaceutical samples.

The calculation the percentage of radioactivity of ^{99m}Tc -Tektrotyd using the following formula:

$$100\% - (A + B).$$

Limit: minimum 90 per cent of the total activity.



Figure 1. Fume Hood - Safeflow- Class II Biohazard Safety Cabinet in which the procedure of labelling was performed

Results

Preparation of technetium ^{99m}Tc - Tektrotyd kit was realized according to the aseptic procedure provided by the manufacturer Polatom, showed in the Figure 1 and Figure 2:

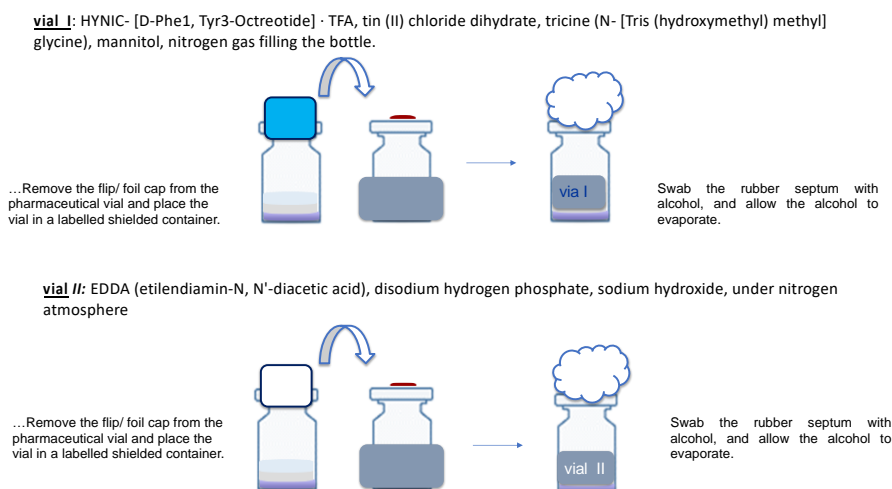


Figure 1. Method of preparation – the constitution of two vials

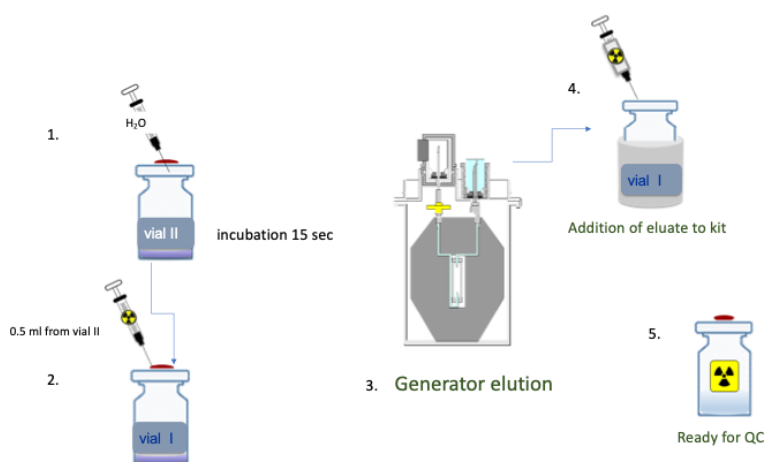


Figure 2. Procedure of labelling with ^{99m}Tc -pertechnetate

Quality control

Determination of radiochemical purity was performed using one of the alternative chromatographic procedures, A or B, as described.

For four ^{99m}Tc -Tektrotyd samples RCP was $93 \pm 0,43$, under the limit of 90% to 100% as required, with activities between 2 and 3 MBq. The mean RCP of triplicate analyses at each sample RCP level for this radiopharmaceutical is shown in Table 1.

Table 1 Radiochemical purity of ^{99m}Tc -Tektrotyd

Series	^{99m}Tc -Tektrotyd (complex)	$^{99m}\text{Tc O}_4^-$ (free unbound radionuclide)	^{99m}Tc colloid species
1	$94,44 \pm 0,39$	$0,67 \pm 0.38$	$4,89 \pm 0.32$
2	$93,44 \pm 0,41$	$1,45 \pm 0.43$	$5,11 \pm 0.35$
3	$93,74 \pm 0,44$	$1,29 \pm 0.30$	$4,97 \pm 0.29$
4	$93,66 \pm 0,46$	$0,69 \pm 0.41$	$5,65 \pm 0.31$
Mean \pm CV	$93 \pm 0,43$	$1,03 \pm 0.40$	$5,16 \pm 0.34$

Mean \pm CV; N=3; CV - coefficient of variation

Obtained results for the percentage of the radioactive complex of ^{99m}Tc -Tektrotyd and percent of free unbound radionuclide and ^{99m}Tc colloid species are showed on and Figure 3 and Figure 4.

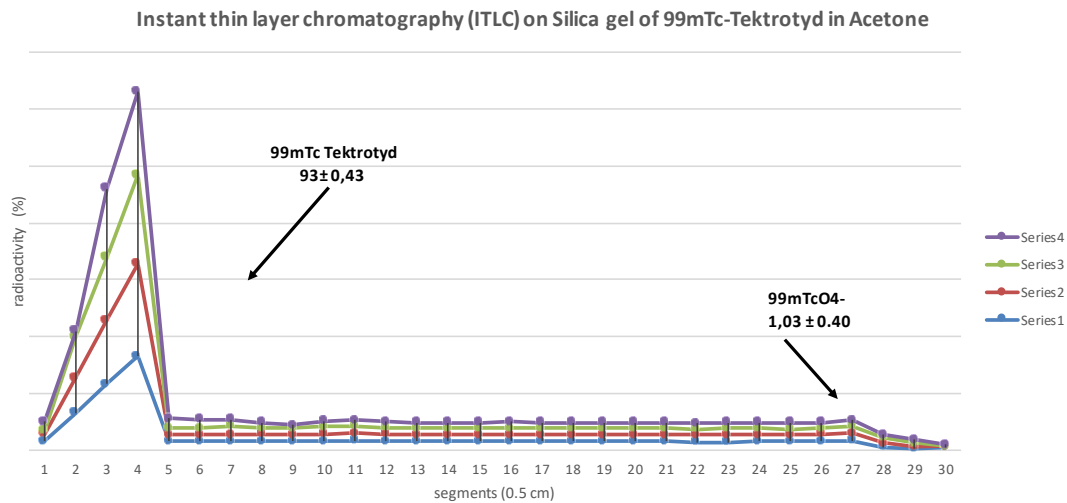


Figure 3. Instant ITLC showing ^{99m}Tc -Tektrotyd in Aceton as a developing medium

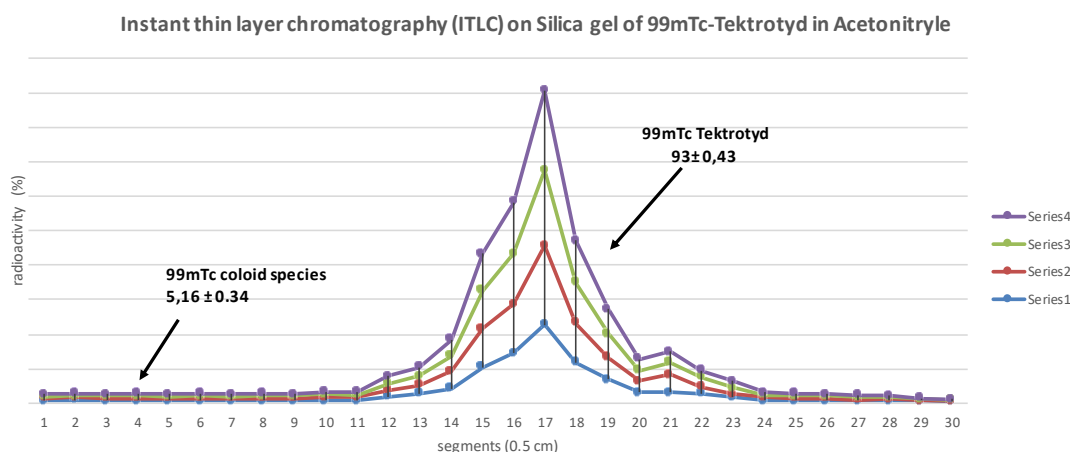


Figure 4. Instant ITLC showing ^{99m}Tc -Tektrotyd in Acetonitrile as a developing medium

Discussion

Many Nuclear Medicine units and radiopharmacies strive to upgrade their quality management systems, aiming at harmonization and standardization to meet internationally recommended Good Practice guidelines as far as possible. Guidelines for radiopharmacy practice have been published, amongst others by the IAEA to assist with standardization of radiopharmacies (IAEA 2008a, IAEA 2008b). In developing countries, it is not always possible to meet all good radiopharmacy practice requirements. There is therefore a risk that radiopharmaceuticals prepared or compounded in such sub-optimal facilities may not meet the required safety and efficacy standards.

Recommended analytical methods are supplied with package inserts from radiopharmaceutical kits and by pharmacopoeia monographs. In radiopharmacies with limited funding, it may be difficult to follow the recommended analytical methods due to lack of adequate equipment and limited availability of consumables. Other disadvantages of many of the recommended methods include the time required to complete the tests, which delays administration of the short-lived products to patients, and use of relatively large volumes of solvents when solid phase extraction (SPE) or HPLC are used (Seetharaman et al 2004). Thus, there is a clear need for practical, simple, faster (Hammes et al. 2004; Mihon et al. 2016) and low-cost methods. Any alternative methods should be validated prior to use (Leonardi et al. 2012).

The current study describes the validation of a cost-effective quantification method for instant thin layer radiochromatography strips available at Nuclear Medicine Department, Hospital and University Clinical Service of Kosovo in Prishtina.

In validation of analytical methods, reference standards are generally used for comparison purpose. Certified reference standards for ^{99m}Tc impurities do not exist in the radiopharmaceutical industry due to the short half-life and short shelf life of the radiolabeled complexes as well as absence of a stable isotope of ^{99m}Tc . In our study, samples containing varying concentrations of ^{99m}Tc -Tektrotyd were obtained by spiking with adequate quantities of sodium pertechnetate and measured with a validated method for comparison with the proposed alternative counting method. Seetharaman et al. used a similar approach to determine linearity of their method (Seetharaman et al. 2006).

Limitations of this study include the non-availability of reference standards for Tc-99m labelled compounds since there are no stable isotopes of technetium (Todde et al. 2014).

In validation studies, sample preparation should be done very carefully, and effects like possible adhesion of impurities to containers used in preparing samples should be considered in the design of the tests. Furthermore, spiking samples with pertechnetate may result in additional labeling of the Tc-99m-radiopharmaceutical, which may cause an additional uncertainty in the outcome of the experiments.

Conclusion

Through the preparation of this study it is expected to obtain results that will aim to increase the quality of diagnosis and follow-up therapy of neuroendocrine tumors.

The introduction of a new radiopharmaceutical ^{99m}Tc -Tektrotyd containing the radioactive isotope ^{99m}Tc Technetium, available in all nuclear medical institutions, enables timely diagnosis even where positron emission tomography is unavailable. In order to achieve high specific binding and safety after application of this relatively new radiopharmaceutical, it is necessary to ensure proper quality control, which will guarantee the same. The value of the successful validation of the proposed method is twofold: Firstly, it has increased the awareness of the importance of validation among staff in our unit, and secondly, a cost-effective method is now available and can be used in any other low-income Nuclear Medicine units.

From a practical point of view, we hope that this research will indicate the importance of using new radiopharmaceuticals labeled with technetium-99m, as in this case ^{99m}Tc -Tektrotyd, in diagnosing and monitoring patients with neuroendocrine tumors, especially for the diagnosis of which somatostatin receptors are overexpressed (especially subtype 2 and, to a lesser extent, subtype 3 and 5) and which can be visualized with it.

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Implementation and monitoring of oral rabies vaccination of foxes in Kosovo between 2010 and 2013—An international and intersectorial effort

Boris Jakobson^a, Izedin Goga^b, Conrad M. Freuling^c, Anthony R. Fooks^{d,j},
Valdet Gjinojci^e, Beqe Hulaj^b, Daniel Horton^{d,i}, Nicholas Johnson^d,
Jeton Muhaxhiri^f, Ilir Recica^f, Dan David^a, Richard O'Flaherty^{f,g},
Nick Taylor^h, Tony Wilsmore^h, Thomas Müller^{c,*}

^a Rabies Department, Kimron Veterinary Institute, 20250 Bet Dagan, Israel

^b Kosovo Veterinary Laboratory, Industrial Zone, 10 000 Pristina, Kosovo

^c Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, 17943 Greifswald-Isle of Riems, Germany

^d Wildlife Zoonoses and Vector-borne Diseases Research Group, Animal Health and Veterinary Laboratories Agency, Weybridge, UK

^e Food and Veterinary Agency, Industrial Zone, 10 000 Pristina, Kosovo

^f Control and/or Eradication of Animal Diseases, EuropeAid/132620/C/SER/XK, Pan Livestock Services Ltd, Industrial Zone, 10 000 Pristina, Kosovo

^g Support for the control and eradication of animal diseases, IPA Multibeneficiary Project Western Balkans, EuropeAid/129988/C/SER/MULTI, Opera S.c.a.r.l., 00197 Rome, Italy

^h Veterinary Epidemiology and Economics Research Unit (VEERU) & PAN Livestock Services Ltd., University of Reading, School of Agriculture, Policy and Development, Reading RG6 6AR, UK

ⁱ School of Veterinary Medicine, Faculty of Health & Medical Sciences, University of Surrey Guildford, Surrey, GU2 7TE, UK

^j Department of Clinical Infection, Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, Liverpool, Merseyside, L69 7BE, UK

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ABSTRACT

The European Union has used instrument for pre-accession (IPA) funds to provide technical assistance and supplies for the eradication, monitoring and control of rabies in several pre-accession countries. As a result, since 2010, multi-annual oral rabies vaccination (ORV) programmes for eliminating fox rabies have been launched in six Western Balkan countries.

Here the implementation of the ORV programme in Kosovo, the smallest of the West Balkan countries, is described. Associated challenges under difficult political conditions, potential biases, and the results of rabies surveillance and monitoring of ORV campaigns (bait uptake and immunisation rates) since 2010 are reported.

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Introduction

In a geopolitically important position representing the South-eastern Gateway to Europe, the Balkans, and in particular the Western Balkans, has been a cultural and politically unstable region for centuries. The term Western Balkans is more a political than a geographical designation for a region of Southeast Europe that is not yet part of the European Union (EU). However, with Croatia being a member state (MS) since July 2013 and Serbia, Bosnia and

Herzegovina, Montenegro, Kosovo, Macedonia and Albania aiming at joining the EU in the future this term may no longer be applicable.

Rabies, a zoonotic disease with a uniquely high fatality rate (Jackson, 2013; Fooks et al., 2014), has been endemic in the Western Balkans for centuries (Mutinelli et al., 2004). About the same time as strict sanitary measures and mass parenteral vaccination resulted in elimination of the age-long plague of dog-mediated rabies in the early 1990s (Mutinelli et al., 2004), fox-mediated rabies became a new challenge (Müller et al., 2012a). Following the emergence of fox rabies, assumed to originate in Kaliningrad at the beginning of the 1940s, the disease spread rapidly over the continent. As a result large parts of Central and Western Europe were already affected by the mid-1970s. Surveillance data suggest that

* Corresponding author. Tel.: +49 38351 71659; fax: +49 38351 71151.
E-mail address: thomas.mueller@fli.bund.de (T. Müller).

fox rabies invaded the Western Balkans from neighbouring regions in Hungary, Romania and Slovenia (www.who-rabies-bulletin.org; (Mutinelli et al., 2004). For reasons that are not fully understood, the sylvatic rabies epidemic in the Western Balkans only advanced southwards slowly. Although certainly confounded by a lack of adequate surveillance and laboratory diagnosis at the time, fox rabies did not emerge in Serbia, Kosovo and Macedonia until 1986, 1998 and 2011, respectively (Kirandjiski et al., 2012; Mutinelli et al., 2004). With the emergence of the disease in Northern Greece in 2012 the fox rabies epidemic reached its southeastern-most extension (Tsiodras et al., 2013).

Due to the strict implementation of oral fox rabies vaccination (ORV) programmes, large parts of Western and Central Europe have been officially declared free from fox-mediated rabies. Consequently the boundaries of the disease-free area have been pushed towards the eastern and southern borders of the EU (Cliquet and Aubert, 2004; Freuling et al., 2013; Müller et al., 2012a). As complete elimination of terrestrial rabies from the EU appears to be feasible in the short or medium term, the EU is giving top priority to rabies control (Freuling et al., 2013). Attention has now shifted towards the elimination of the disease in countries neighbouring the EU (Demetriou and Moynagh, 2011; Müller et al., 2012a). The EU animal disease eradication fund is being used to support ORV programmes in Kaliningrad and parts of Belarus and Ukraine. Similarly, funds dedicated to support the accession process are being deployed to support ORV in the Western Balkans (Müller et al., 2012a). These funds are channelled through the instrument for pre-accession (IPA), which is being used to procure supplies of rabies vaccine and to provide relevant technical assistance to support eradication programme activities (Demetriou and Moynagh, 2011). Since 2010, multi-annual ORV programmes have been launched in six Western Balkan countries, whilst plans are in place for a nationwide ORV programme in Albania commencing in 2014 (Demetriou and Moynagh, 2011; Müller et al., 2012a). Kosovo is the smallest of the West Balkan countries. The last confirmed case of autochthonous human rabies on the territory of Kosovo occurred in 1954. In October 2007, two cases of rabies in foxes were reported in the region near the border with the Former Yugoslav Republic of Macedonia (FYROM). Although no further rabies cases have been reported since, being sure of absence of disease in Kosovo is difficult because (i) rabies is endemic in neighbouring countries of FYROM, Serbia, Montenegro and Northern Albania ([who-rabies-bulletin.org](http://www.who-rabies-bulletin.org)), (ii) borders are porous to wildlife, and (iii) of insufficient surveillance to rule out endemic infection in Kosovo.

The IPA programme supported control programmes for rabies and classical swine fever (CSF) through projects managed by the European Union Office in Kosovo. This report covers activities undertaken within the framework of one such project (EuropeAid/127852/D/SER/KOS), which provided technical assistance to the Food and Veterinary Agency of Kosovo (FVA) and Kosovo Food and Veterinary Laboratory (KVL). Here we describe the implementation of the ORV programme and associated challenges, discuss potential biases, and report the results of rabies surveillance and monitoring of ORV campaigns (bait uptake and immunisation rates) in the Republic of Kosovo since 2010.

Materials and methods

Study area

Kosovo has an area of 10,908 km². It lies between latitudes 41° and 44° N, and longitudes 20° and 22° E and is bordering Serbia to the North and East, Montenegro to the Northwest, Albania to the Southwest and FYROM to the South. Kosovo is surrounded by

several mountain ranges with peaks as high as 2656 m. Within the territory, Kosovo is subdivided into two large plain areas, one to the north-east and one to the south-west, covering approximately 36% of the territory and lying at elevations between 400 and 700 m above sea level. These plain areas are divided by central highland ranges rising to elevations of about 1000 m. The population density in the country was last reported at 1.8 million in 2011 (Anon., 2011).

Implementation of rabies surveillance and oral rabies vaccination

In Kosovo, the FVA of the Ministry of Agriculture, Forestry and Rural Development of Kosovo is in charge of rabies control and the implementation of the rabies eradication programme using ORV. Initially, diagnostic capacities at the KVL in Pristina had to be established. Prior to testing field samples KVL staff received intensive in-service training by the Kimron Veterinary Institute, Israel (KVI-IL), on performing rabies diagnostic tests using reference material provided and the drafting and implementation of standard operating procedures (SOPs) for handling samples. Technical assistance and support concerning implementation of rabies surveillance and operational activities for ORV campaigns was further provided by the Animal Health and Veterinary Laboratory Agency, UK, the Friedrich-Loeffler-Institute, Germany, EuropeAid, and the Veterinary Epidemiology and Economics Research Unit (VEERU) & PAN Livestock Services Ltd. of the University of Reading, UK.

Oral rabies vaccine

A SAD-derived oral vaccine (SAD Berne strain) with a national registration in another EU Member State was used in all ORV campaigns conducted in Kosovo since 2010. The bait casing contained 150 mg tetracycline (TC) to assess bait uptake in the target species. Before each ORV campaign, vaccine baits were shipped from the manufacturer to Kosovo in refrigerated vehicles and stored at –20 °C by a local contractor until distribution in the field. The maintenance of the cold chain was confirmed using temperature data loggers that were shipped and stored together with the baits. The titre of each batch of vaccine virus was checked on arrival to ensure it was above the minimum recommended effective dose (Anon., 2005). Vaccine titres were independently determined at the WHO Collaborating Centre for Rabies Surveillance and Research at the Friedrich-Loeffler-Institute (FLI), Germany, in three independent runs using 5 out of 25 baits. The mean vaccine titres of the different batches used as determined both by the manufacturer and FLI are shown in Table 1.

Oral vaccination campaigns

Between 2010 and 2013, with the exception of 2012, ORV campaigns were conducted twice a year, in spring and autumn when environmental conditions were optimal. With an area of 9200 km² almost the entire territory of Kosovo was covered. In 2012, due to a delay in budgetary approval the spring ORV campaign had to be abandoned, hence vaccine baits were distributed only in an autumn campaign.

Vaccine baits were mainly distributed using fixed-wing aircrafts (Cessna 172) equipped with the SURVIS-system for automatic dropping and registration of individual bait positions (for details see Müller et al., 2012c). In general, an average flight altitude of 300–500 m above ground and an average speed of 170–180 km/h were applied. Initially, during the first four ORV campaigns a flight-line distance of 1000 m and a bait density of 23 baits/km² were used. A modification of the dispersal scheme was applied during the campaigns as follows: in a 5 km wide vaccination belt bordering neighbouring countries (2000 km²) the flight-line distance was reduced to 500 m. The flight-line distance in the other parts

Table 1

Time point of vaccination, titre of vaccine batches used, number of baits purchased, distributed and bait density applied in oral rabies vaccination (ORV) campaigns in Kosovo during 2010 and 2013.

ORV campaign	Time period	Vaccine batch No	Titre (manufacturer)	Titre (FLI)	Flight-line distance in meters	Number of baits purchased	Baits distributed	Average bait density/km ²
1	27–29 May 2010	1017	7.8		1000	459,200	234,600	25.5
		1117	7.6			140,800		
2	26 October–04 November 2010	1017	7.8	5.76	1000		362,000	39.3
		1117	7.6	5.4				
3	27 April–02 May 2011	4218	7.8	7.18	1000	300,000	300,000	32.6
4	17–20 October 2011	5618	7.8	6.77	1000	121,500	300,000	32.6
		5717	7.8			178,500		
5	13–19 November 2012	2219	7.6		500	250,000	250,000	27.2
6	06–10 May 2013	3220	7.8	6.92	500	250,000	250,000	27.2
7	18–21 October 2013	8120	7.9	8.02	500	250,000	250,000	27.2

of Kosovo (7200 km²) remained unchanged using a bait density of 42 and 30 baits/km², respectively. During the seven ORV campaigns conducted between 2010 and 2013 a total of 1,946,000 vaccine baits were dispersed (Fig. 1; Table 1). For all campaigns, the flight sectors were adapted in advance according to natural or man-made landscape features. In the mountain areas in the south and west of the country the flight lines had to be adjusted according to the topography. Hand distribution of baits was an essential complementary measure, especially in areas with a high density of settlements and non-flying zones. Baits were also provided to NATO's Kosovo Force (KFOR) to be hand dispersed in and around KFOR military bases.

Communication, public awareness and training

As the competent authority for human health and communicable diseases, the Public Health Institute (PHI) in the Ministry of Health was involved in public information campaigns. These campaigns informed and educated the general public on the threat of rabies and risk mitigating measures. The latter comprised information on wildlife ORV campaigns and on how to deal with inadvertent contact with vaccine baits distributed by aerial dispersal. Prior to vaccine distribution in the field, public awareness campaigns were organised including notices in newspapers, TV advertisement and billboard posters. Written materials were published in both Serbian and Albanian languages. Frontline public health workers were informed in advance of vaccine dispersal. Private veterinarians, local authorities and the general public were encouraged to report any suspicion of rabies disease to the Food and Veterinary Agency. With support from beneficiary authorities and the European Commission Liaison Office, a documentary on rabies was broadcast on local television to launch World Rabies Day in Kosovo.

The PHI and seven regional health institutes were responsible for administration of post-exposure prophylaxis in humans that came in contact with rabies, suspect animals or vaccine baits.

Rabies surveillance and monitoring of ORV campaigns

Sampling in the frame of rabies surveillance (examination of all animals suspected of having rabies, those found dead as well as road kills) and monitoring of bait uptake and seroconversion (8 foxes from hunting bag per 100 km² of vaccinated area and year) followed recent EFSA recommendations (Cliquet et al., 2010). To evaluate the efficacy of ORV campaigns and to implement adequate rabies surveillance and monitoring, a technical assistance project team was deployed which operated countrywide. As part of the terms of reference, 20 one-day training sessions for hunters and veterinarians were given at Regional Hunting Associations' offices. During those training courses field kits (card boxes containing labelled plastic bags, tubes, submission forms) for sample

collection were distributed to the trainees. Furthermore, collection of samples, e.g. head, blood and bone tissue (femur) to be submitted for testing for rabies diagnosis, serology and bait-uptake, respectively, was demonstrated. For collection of samples, hunters and private veterinarians were offered an incentive of €25 per sample regardless of originating from a hunted or a fox found dead. Veterinarians who checked, packed and dispatched samples received a €1.50 incentive payment. The laboratory examination of samples was performed at KVL.

Bait disappearance rate

Hand placement of baits, by gamekeepers and members of the technical assistant project team, was used to monitor bait disappearance. Baits were hand placed in the field at two and five specific locations during the first, second ORV campaigns, respectively, and seven locations in each case during the third and fourth ORV campaigns. Around 40–50 baits were placed at each location 50 m apart at marked positions with daily follow-up to count baits taken. The disappearance rate was finally calculated 72 h after distribution in the field.

Rabies surveillance

For rabies diagnosis, brain tissue (cerebellum, medulla oblongata, Ammon's horn) of animals submitted for testing was examined for viral antigen using the standard fluorescent antibody test (FAT) (Dean et al., 1996). FAT-negative results of animals involved in human exposure and FAT-inconclusive results were confirmed using the rabies tissue culture infection test (RTCIT; Webster and Casey, 1996), mouse inoculation test (MIT; Koprowski, 1996) or real-time polymerase chain reaction (RT-qPCR) as described (Hoffmann et al., 2010). Partial sequencing of rabies viruses (RABV) was conducted as described (David et al., 2007).

Bait uptake and seroconversion

The presence of the biomarker (bait-uptake) in foxes collected post vaccination was detected by demonstration of tetracyclin (TC)-induced fluorescence in bones (mandible, femur) or dentine of the canine tooth of animals using a method described elsewhere (Johnston et al., 1999; Linhart and Kenelly, 1967). ORV-induced rabies virus specific antibodies (Ab) were determined using a commercial blocking enzyme-linked immunosorbent assay (ELISA, Biopro, Czech Republic) as described (Wasniewski et al., 2013). For verification and comparison, 10% of serum samples of the first four ORV campaigns were tested at the Rabies Laboratory at KVI-IL (accredited and EU-approved laboratory for the pet travel scheme) on the presence of virus neutralising antibodies (VNA) using the

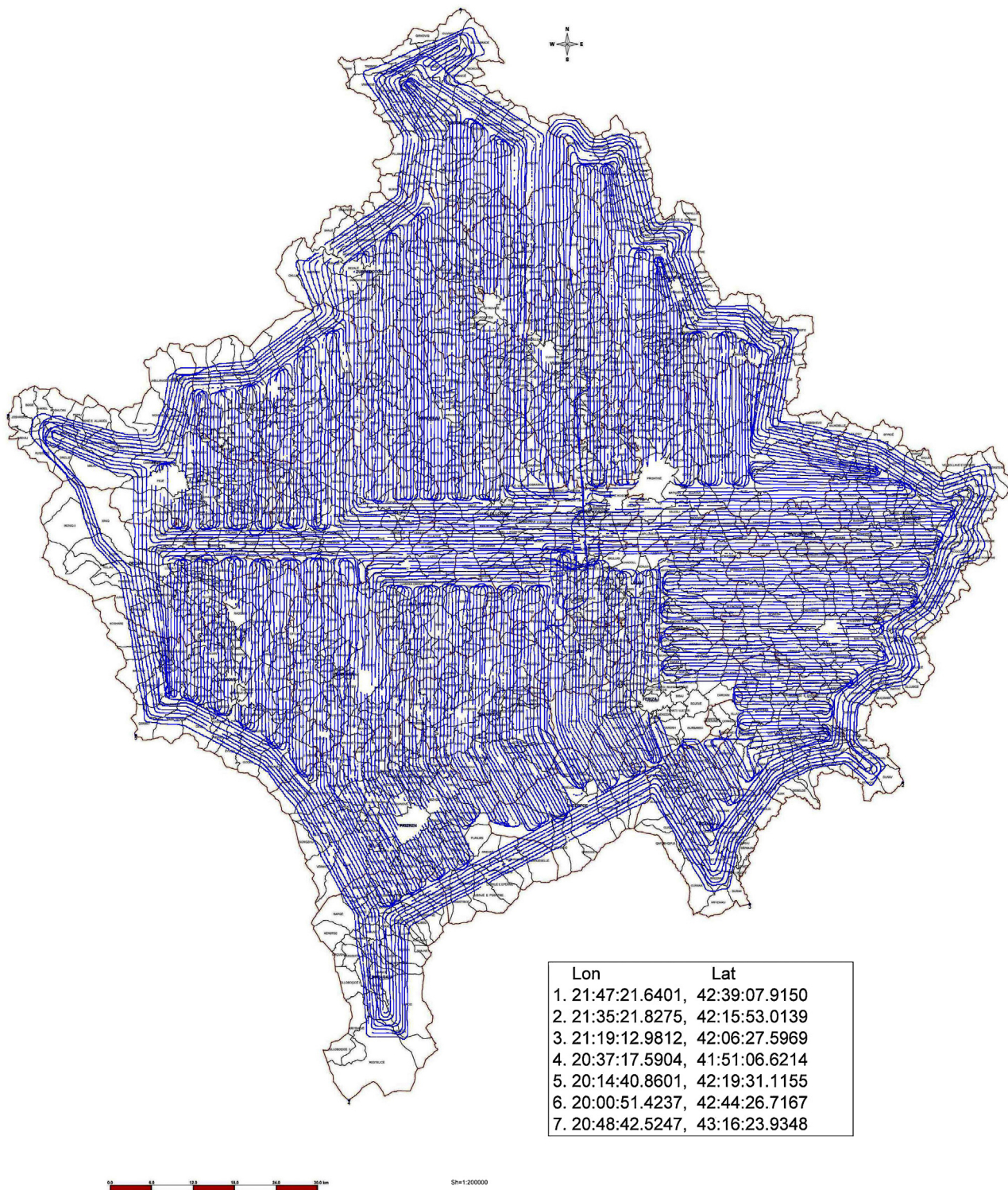


Fig. 1. Individual flight routes and computer-supported automatic recording of exact bait drop positions (Lon = longitude, Lat = latitude) for the autumn oral rabies vaccination (ORV) campaign 2013. Few examples (1–7) of Gauss–Krueger coordinates of bait drop positions are shown. Areas not covered represent high mountain ranges in the border area between Serbia, FYROM and Montenegro where difficult terrain prevents low operating altitudes.

rapid fluorescent focus inhibition test (RFFIT) (Smith et al., 1973), with modifications as described (Cox and Schneider, 1976).

Data and statistical analysis

For each ORV campaign vaccination areas and the location of animals submitted for testing were digitized and converted into

a GIS database (ArcGIS©, Esri Inc., Redlands, California, USA), as previously described (Staubach et al., 2001). The 95%-confidence intervals (CI) for bait uptake and seroconversion rates were calculated and differences between two consecutive ORV campaigns tested for significance using the chi-square test (χ^2 test or the Fisher exact test (*F*-test) alternative when necessary) as described (Sokal and Rohlf, 1995). Kappa statistics on concordance of TC and

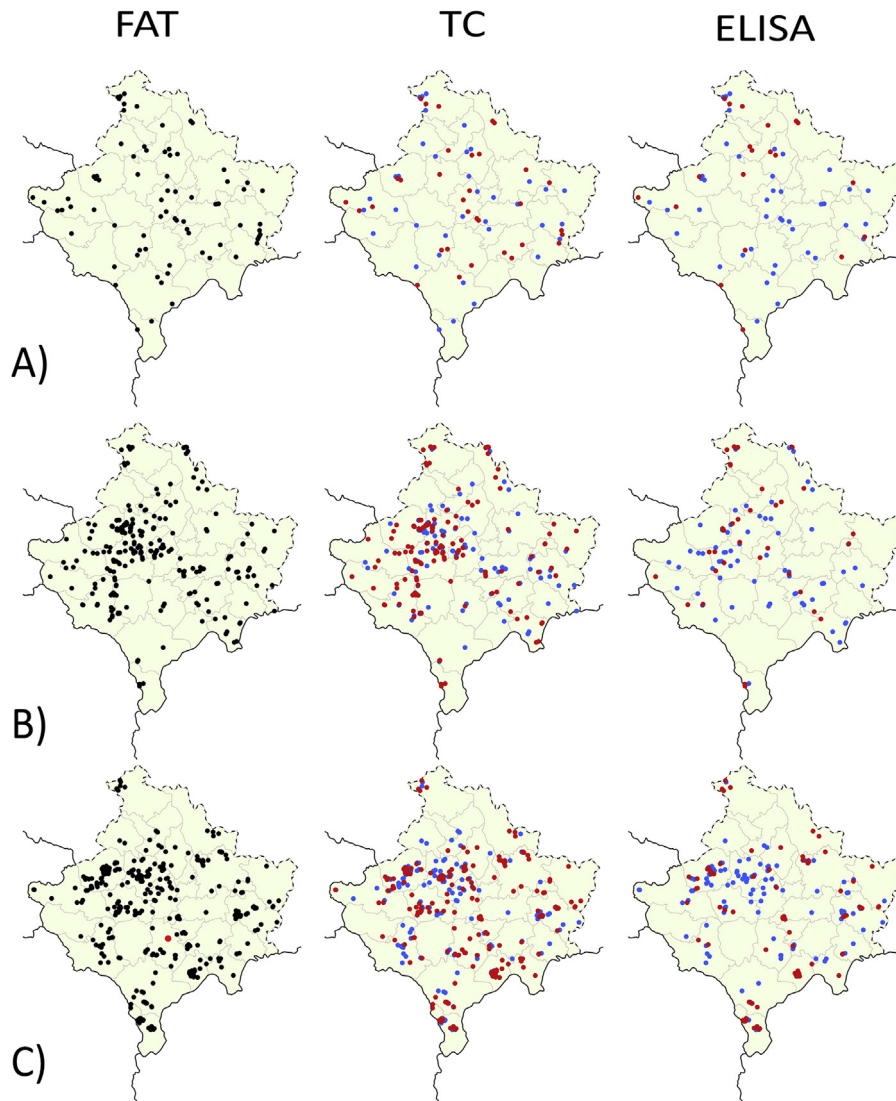


Fig. 2. Map showing districts of Kosovo and the geographical origin of animal specimens collected in the frame of rabies surveillance and monitoring of ORV campaigns and tested for the presence of rabies virus antigen (FAT), tetracycline specific fluorescence (TC) and rabies-specific Ab (ELISA) between 2010 and 2013. In each case, data for two consecutive campaigns are shown: (A) 1st and 2nd campaign; (B) 3rd and 4th campaign; (C) 6th and 7th campaign (for details see Table 2). Blue/black and red dots represent negative and positive/suspect (FAT) animals, respectively.

Ab results were conducted using free ©2014 GraphPad Software (<http://graphpad.com/quickcalcs/kappa1/>).

Results

Rabies surveillance

Since re-implementation of rabies surveillance in June 2010, the number of suspect animals submitted for rabies diagnosis continuously increased. By February 2014 a total of 728 animals (86.3% foxes, 13.7% non-target species) had been tested for rabies at FVL. Table 2 only refers to the foxes tested. During the first four ORV campaigns the number of animals tested exclusively comprised foxes, except for one submission (dog). Since the autumn campaign 2012, an increasing number of non-target species were submitted for rabies diagnosis including wolves (*Canis lupus*; N=23), marten (*Martes martes*; N=80), badgers (*Meles meles*; N=8), wildcats (*Felis silvestris*; N=5) and roe deer (*Capreolus capreolus*; N=1). During the entire observation period only 3 suspect dogs, one in 2010 and two in 2013, were submitted for testing following the involvement in

human biting incidents. There were no submissions of farm animals during the reporting period. GIS analysis revealed that animals had been submitted from almost all districts; the location of sample submissions for two consecutive ORV campaigns in each case is shown in Fig. 2. Rabies could not be detected in any of the animals tested by FAT at KVL (Table 2). However, one fox from the village of Suhareke (Fig. 2C) obtained in September 2012 with non-specific fluorescence was re-tested during a training course at KVI-IL and at FLI in 2014. Whilst RT-qPCR was positive, RTCIT and MIT were negative. Subsequent partial sequencing of a 606 bp region of the amino terminus of the nucleoprotein (N) gene sequence of the Kosovo rabies virus showed a 99% sequence identity with rabies virus isolated from Greece (KC771887; KC836502), FYROM (JQ973886; JQ973885) and Serbia (JQ973879; JQ973877).

Monitoring of ORV campaigns

The bait disappearance rate after 72 h in the field for the first four ORV campaigns ranged between 75.5% (first campaign) and 94.8% (fourth campaign). During June 2010 and February 2014, a

Table 2
Estimated bait uptake (tetracycline) and seroconversion rates (ELISA) in foxes obtained during the seven oral rabies vaccination (ORV) campaigns conducted in Kosovo between 2010 and 2014. Lower (TTU) and upper (TTO) 95% confidence intervals (CI) are indicated.

ORV campaign	Sampling period	IFA			Tetracycline			ELISA						
		Tested	Positive	Positive in %	Tested	Positive	Positive in %	Tested	Positive	Positive in %				
1	June 2010–October 2010	38	–	30.56	36	11	30.56	15.51	45.60	32	7	21.88	7.55	36.20
2	November 2010–April 2011	41	–	60.53	38	23	60.53	44.99	76.07	33	14	42.42	25.56	59.29
3	May 2011–October 2011	126	–	45.00	120	54	45.00	36.10	53.90	62	12	19.35	9.52	29.19
4	November 2011–April 2012	125	–	67.74	124	84	67.74	59.51	75.97	38	22	57.89	42.20	73.59
5	December 2012–April 2013	2	–	100.00	2	2	100.00	100.00	100.00	1	1	100.00	100.00	100.00
6	May 2013–October 2013	170	–	35.09	171	60	35.09	27.93	42.24	93	11	12.90	5.26	18.39
7	November 2013–data	166	–	79.52	166	132	79.52	73.38	85.66	98	69	70.41	61.37	79.45
	Total	668	–	55.71	657	366	55.71	51.91	59.51	357	136	38.10	33.06	43.13

total of 657 and 356 foxes from the entire vaccination area were tested for TC-specific fluorescence and rabies-specific Ab, respectively (Fig. 2). Bait uptake in foxes ranged between 30.6 and 79.6% in the spring campaign 2010 and autumn campaign 2013, respectively, with an average bait uptake of 55.7%. Of 356 foxes tested for rabies specific Ab during the observation period 136 (38.1%) were positive by ELISA with seroconversion rates in the target population ranging between 19.3 and 70.4% (Table 2). Concordance between ELISA and RFFIT was checked using 17 serum samples (8 negative and 9 positive in ELISA). Whilst all ELISA-negative samples had VNA titres <0035 IU/mL, VNA titres of ELISA-positive varied from 1.73–4.73 IU/mL. Two ELISA-positive samples had VNA titres <0.2 IU/mL. Concordance between TC and Ab results of individual ORV campaigns ranged between 60 and 86.7% with the lowest concordance observed after the second ORV campaign. The strength of agreement was highest after the seventh ORV campaign (Table 3). The bait uptake and seroconversion rates steadily increased over time ($P < 0.01$). This trend was only interrupted after the sixth campaign when both bait uptake and seroconversion rates were lowest ($P < 0.001$) compared to the preceding and following campaigns (Table 2).

Of 23 wolves, 80 martens and 2 badgers tested in frame of the ORV monitoring, 8 (34.7%), 45 (56.2%) and 2 (25%) were positive for TC with 5 (21.7%), 7 (8.6%) and 3 (37.5%) having rabies-specific Ab by ELISA, respectively. All remaining animals (wild cats, Roe deer) were negative in both tests.

Post-exposure prophylaxis in humans

No human cases of rabies had been reported in Kosovo for at least 30 years. During the observation period a total of 29 post-exposure prophylaxis treatments (PEP) were administered to humans that came in contact with rabies suspect dogs, wildlife and other animals. This equals a PEP rate between 0.5 and 1.6 per 100,000 inhabitants. No inadvertent contact of humans with vaccine baits had been reported during distribution to the PHI or any of the seven regional health institutes.

Discussion

The implementation of rabies control programmes using ORV has been a challenge for veterinary authorities (Stöhr and Meslin, 1996), particularly in densely populated areas in Central Europe (Selhorst et al., 2006). Therefore, in retrospect it is a remarkable achievement that the responsible authorities in Kosovo were able to implement the EU-funded ORV programme in a relatively short period of time given that the prevailing preconditions were challenging. In fact, the country had only gained independence from Serbia 2 years before implementation of the programme. The newly developed political, administrative, socio-economical as well as socio-cultural structures were only just in place, whilst the country further struggled with both outer and inner political tensions. For instance, the entire administration and regulations had to be adopted in congruence with the EU standards. Another challenge was and still is the ethnic separation. Although inhabited mostly by Albanians, Kosovo has a large Serb minority. This posed a problem, especially on the northern border of Kosovo with Serbia, where the Serbian minority population is concentrated in the four most northern municipalities. Here, the ethnic minority still look to Serbia for administration and governance and seek to avoid participation in Kosovo Government programmes. Therefore, the responsible authorities and supporting projects put special emphasis on encouraging the Serbian minority veterinarians and hunters to participate in ORV activities (a Serbian veterinarian is a member of the Project Steering Committee) and involved

Table 3
Concordance between the presence of tetracycline (TC, biomarker) and rabies specific Ab (Ab) as determined in ELISA for individual ORV campaigns conducted in Kosovo between 2010 and 2014.

ORV campaign	Sampling period	Foxes tested both for TC and Ab	TC pos Ab pos	TC pos Ab neg	TC neg Ab pos	TC neg Ab neg	Agreement in %	Kappa	95% CI	Strengths
1	June 2010–October 2010	31	5	5	2	19	77.42	0.439	0.097–0.782	Moderate
2	November 2010–April 2011	30	8	9	3	10	60	0.227	0.09–0.545	Fair
3	May 2011–October 2011	60	11	18	0	31	70	0.387	0.2–0.774	Fair
4	November 2011–April 2012	38	22	10	0	6	73.68	0.41	0.156–0.663	Moderate
5	December 2012–April 2013	-	-	-	-	-	-	-	-	-
6	May 2013–October 2013	94	11	27	0	56	71.28	0.327	0.168–0.485	Fair
7	November 2013–April 2014	98	67	11	2	18	86.73	0.65	0.48–0.82	Good

international KFOR forces. Furthermore, diagnostic capacities and chains of command had to be initiated from the beginning as there was no well-functioning veterinary infrastructure in place. However, the results of this study show that the concerted actions of veterinary and public authority and private project management were able to fulfil the task. Hence, the rapid implementation of ORV in Kosovo in 2010 is an example for an efficient intersectorial collaboration under the ‘One Health Concept’ (Zinsstag et al., 2011).

In addition to these political challenges, the epidemiology of rabies in the region prior to the first vaccination campaign in spring 2010 was unclear. The last confirmed case of autochthonous human rabies on the territory of Kosovo occurred in 1954 presumably as a result of a spillover from dog-mediated rabies. Previous to this project, rabies surveillance in Kosovo was inadequate and it was not until two cases of fox rabies were reported in the region near the Macedonian border in 2007, that it was apparent that the disease front had reached Kosovo. Occasional detection of the disease in neighbouring Northern Albania (Korro et al., 2009), Serbia, Montenegro and FYROM, the latter after strengthened rabies surveillance programmes (Kirandjiski et al., 2012), supported the assumption that fox rabies was more widespread in the region and could have been endemic but undetected in Kosovo. The results of our surveillance activities in the target population with one PCR-positive result in a fox sample from Suhareke in 2013 (Fig. 2C) corroborate this hypothesis. Because rabies had not been reported for some time veterinary and public health authorities were informed that there had been a suspect rabies case in a fox to raise awareness and enhance surveillance.

Although not confirmed by RTCIT, which prevents declaration of a rabies case based on case definition, 99% sequence identity to rabies viruses isolated from neighbouring regions (Picard-Meyer et al., 2013; Tasioudi et al., 2014) suggests a low-level persistence of rabies in the form of spatio-temporal moving infection clusters within a highly immunized fox population (Tischendorf et al., 1998). In this respect, it could also be evidence of transboundary migration of rabid foxes from other endemic areas, as demonstrated for other Balkan regions by phylogenetic analysis (Johnson et al., 2006; Turcitu et al., 2010; Kirandjiski et al., 2012; Picard-Meyer et al., 2013).

Despite huge efforts to strengthen rabies surveillance in Kosovo during the past 4 years it was still not optimal as only few suspect dogs and other domestic animals were submitted for rabies diagnosis. In Kosovo, there is a large population of “street” dogs (reputedly unowned but live in and around cities and villages) whereas “feral” dogs are rare or do not exist. Vaccination of dogs and cats older than 3 months is obligatory and free of charge in Kosovo (paid from the Kosovo Consolidated Budget), with media promotions and approximately 50,000 doses of inactivated rabies vaccines provided annually to the private veterinary sector. Despite this availability, there is unfortunately no data on the vaccination coverage in the dog population available. Obligatory dog registration is being implemented in Kosovo but an unknown percentage of dogs are still unprotected against rabies, leading to potential contacts between unprotected owned dogs, street dogs and potentially rabid wildlife. Thus, those animals should be included in future rabies surveillance as recommended to avoid the risk of rabies becoming established in the dog population (Cliquet et al., 2010). Nevertheless, the risk of rabies in Kosovo can be considered very low. This is also supported by the relatively low number of PEP administered during the observation period.

As rabies had been unreported prior to the implementation of ORV in Kosovo this programme cannot be regarded as a measure to control and eliminate the disease, but instead to prevent further spread and/or re-introduction. Therefore, in our case the rabies incidence cannot be used as a parameter to monitor the effectiveness of the ORV campaigns, but other indirect parameters

need to be used. Vaccination coverage (seroconversion) and bait uptake as biological parameters clearly showed an effect of the ORV campaigns on the target population, but with an overall average of 55.7% (CI 95%, 51.9–59.5%) the bait uptake only reached sufficient levels during some years (Table 2). The use of seroconversion levels as an indicator of ORV success is controversial (Jakobson et al., 2006). Experimental and field data showed the validity of this method to assess vaccination coverage in red foxes (Cliquet et al., 2012; Müller et al., 2001). However, the presence of VNAs may not always be a reliable parameter as an indicator of a successful oral rabies vaccination (Hanlon et al., 2002). Also, the vaccination coverage threshold of 70% may not be necessary to control the disease (Thulke and Eisinger, 2008), as demonstrated in Germany (Müller et al., 2012b). In general, bait uptake and seroconversion rates of spring campaigns were lower compared to autumn campaigns. Nevertheless, speculations on the reasons for the low seroprevalence include unfavourable climatic conditions at the time of bait distribution, particularly for the first round of ORV when the average daily temperature in Kosovo was 24 °C. Furthermore, two vaccine batches tested at the FLI had a titre lower than the minimum effective titre needed (Tables 1 and 2). Whether those low vaccine titres were the reason for the low concordance between TC and seroconversion after the second ORV campaign is difficult to prove because of the low samples size (Tables 2 and 3). The veterinary authorities formally complained to the vaccine producer but it was decided to carry out vaccination with those batches in order to avoid a complete loss of herd immunity. In the following campaigns stronger specifications as regards the vaccine titre were defined. With the exception of these two vaccine batch failures, all other technical monitoring parameters, e.g. temperature logging, bait placement, etc., showed no deviations from the expected values and the vaccination strategy chosen generally followed those recommendations of the EU (Commission, 2002) with bi-annual campaigns during spring and autumn. The only slight modifications were a relatively high bait density and a flight-line distance of 1 km due to topographical features (Table 1, Fig. 1).

A more likely explanation for the observed lower levels of seroconversion after spring campaigns is that they may reflect the seasonal turnover of the fox population, in which there are additions of young naive foxes to the population during the summer (and therefore to the hunted sample taken at this time) (Table 2). Unfortunately, the age of the animals was not determined or recorded for a large proportion of submitted foxes thus precluding a detailed analysis. However, it is very likely that the sample size of those campaigns was biased towards juvenile foxes which, depending on the birth period and the time point of vaccine bait distribution, are either immune incompetent at the time of vaccination or develop an impaired immune response due to maternal antibodies (Bernardi and Ito, 2000; Müller et al., 2001, 2002).

The overall objective of this IPA project was to improve the animal health situation in Kosovo, particularly regarding rabies and through the elimination of the disease from animal populations in Kosovo contribute to an improved human and animal health status as well as an improved trading status for Kosovo. Given that all countries surrounding Kosovo have implemented ORV, it is likely that rabies in the entire Balkan region will be eliminated in a few years' time. Our analysis shows that ORV in Kosovo contributed to this larger control effort.

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REVIEW ARTICLE

Passive surveillance of American foulbrood in the Republic of Kosovo: geographic distribution and genotype characterization

Beqë Hula^a, Izedin Goga^{b*} , Armend Cana^a , Xhavit Merovci^a, Franca Rossi^c , Simone Crudele^c, Luciano Ricchiuti^c  and Franco Mutinelli^d 

^aVeterinary Laboratory, Food and Veterinary Agency, Pristina, Kosovo; ^bAgricultural and Veterinary Faculty, University of Pristina, Pristina, Kosovo; ^cIstituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy; ^dIstituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

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American foulbrood (AFB) is one of the most devastating infectious diseases of honey bees with a worldwide distribution. Based on clinical symptoms of AFB, the disease has been thought to have long existed in the Republic of Kosovo honey bee colonies. This passive surveillance study was intended to confirm the presence and determine the distribution of AFB in the Republic of Kosovo, based on the examination of clinical symptoms, rapid immunochromatographic tests, and microbiological and molecular identification of the etiological agent *Paenibacillus larvae*. From 2007 until 2019, official veterinary inspectors collected 2027 brood combs from a total of 373 apiaries clinically suspected as an AFB outbreak. Samples were collected in 26 out of 38 municipalities of the country predominantly during spring and autumn. *P. larvae* were isolated from 219 out of 373 apiaries, i.e., 58.71%. All samples with AFB clinical symptoms of the disease were positive in the immunochromatographic test. The presence of the AFB was demonstrated in 24 municipalities. Seventeen positive apiaries from different regions were randomly selected in the years 2016 to 2018 in order to characterize the isolates circulating most recently by ERIC genotyping. The presence of both ERIC I and II genotypes, as well as of a biotype with a profile slightly different from ERIC I and II, was observed. The results indicate a need for a change in approach in future control strategies and training programs for early diagnosis and improved management of apicultural practices.

Keywords: American foulbrood; Republic of Kosovo; *Paenibacillus larvae*; ERIC typing; passive surveillance

Introduction

American foulbrood (AFB), with a worldwide distribution, is one of the most severe and highly contagious honey bee diseases affecting bee brood with the potential to destroy an entire bee community (Alippi et al., 2004). AFB is caused by the Gram-positive, spore-forming bacterial species *Paenibacillus larvae* that comprises different genotypic groups distinguished by PCR targeted to the Enterobacterial Repetitive Intergenic Consensus (ERIC) sequences (Versalovic et al., 1991).

The strains that were previously known as *P. larvae* subsp. *larvae* (Heyndrickx et al., 1996) were grouped within ERIC types I and II, and the strains known as *P. larvae* subsp. *pulvifaciens* were grouped within ERIC types III and IV (Genersch et al., 2006). The genotypes ERIC I and II are regularly isolated from infected honey bee colonies worldwide, while ERIC III and IV are represented by few historical isolates in culture collections (Genersch, 2010).

The clinical symptoms of AFB are darkened, sunken and perforated cell caps, characteristic unpleasant odor and sticky larval remains when drawn out with a matchstick (OIE, 2019).

The disease can be spread by spores when transporting honey bees, queen bees and beehives, as well as theft, or when using tools and inventory, beekeeper clothes and feeding contaminated pollen or honey (Gochnauer & Corner, 1987).

With a total of 135,750 honey bee colonies and 6,453 beekeepers in the Republic of Kosovo, the beekeeping industry belongs to the second most important livestock sector after the cattle sector with 70.44% of professional, 23.32% amateur and 7.23% part-time beekeepers according to the Kosovo beekeeper's association.

In the Republic of Kosovo, AFB is a notifiable disease, and beekeepers receive compensation for each affected honey bee colony that is officially notified and destroyed. This strategy is intended to stimulate beekeepers to promptly report the disease to the competent authority and to minimize the damage to apiaries.

Nevertheless, beekeepers have reported increased mortality of honey bee colonies in recent years so that this study was designed to confirm the presence, distribution and genotype characterization of AFB in bee colony in the Republic of Kosovo through passive surveillance. Here we present data showing the presence of AFB in honey bee colonies with suspected clinical symptoms.

*Corresponding author. Email: izeding@yahoo.com

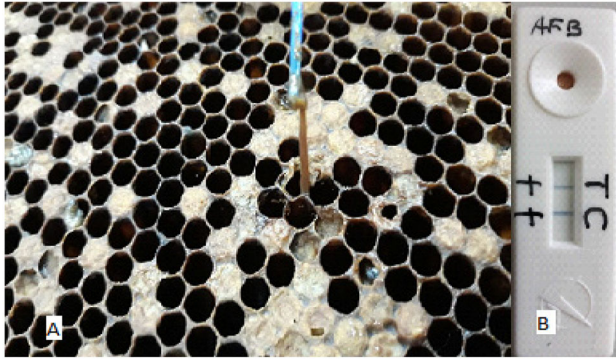


Figure 1. AFB diagnosis based on (A) clinical signs and (B) analysis with the AFB Diagnostic Test Kit. In the latter, both control (C) and test (T) lines are stained indicating a positive result.

Materials and methods

Visual inspection for AFB

A total of 2027 brood combs were examined for symptoms of AFB during a passive surveillance program conducted from 2007 until 2019. Signs such as irregular and patchy brood pattern, sunken and perforated cell caps, dark-colored or greasy looking larvae as well as stickiness in the matchstick test on larval remains were evaluated in 373 clinically suspected outbreaks in 26 out of 38 municipalities of the whole territory of Republic of Kosovo, most of them in summer and autumn.

Suspected samples of brood combs were collected by local veterinary services and transported at 4-8 °C to the laboratory to be examined as described below.

Rapid immunochromatography

Larvae with AFB symptoms were tested with the AFB lateral flow test - Diagnostic Test Kit (Vita Europe, London, UK) according to manufacturer instructions (<https://www.vita-europe.com/beehealth/products/afb-diagnostic-test-kit/>).

Isolation of the AFB causative agent

To isolate *P. larvae*, bee larvae showing typical symptoms of AFB were suspended in 1 mL of sterile distilled water (SDW) and heated at 80 °C for 15 minutes in a water bath to kill *P. larvae* vegetative forms, the non-spore forming bacteria and fungi (Ritter, 2003). Heat-treated larval suspensions were transferred onto Columbia agar supplemented with 5% sheep blood (Hornitzky & Karlovskis, 1989), and onto MYPGP-agar plates. MYPGP-agar contained 10 g Mueller-Hinton broth, 15 g yeast extract, 3 g K₂HPO₄, 1 g Na-pyruvate, 20 g agar and 20 ml 10% glucose (Dingman & Stahly, 1983). In order to inhibit contaminants of the genera *Bacillus* and *Brevibacillus*, as other *Paenibacillus* species, nalidixic acid (Hornitzky & Clark, 1991) and pipemidic acid (Alippi, 1995) were added to culture media. Plates were incubated for 3 days at 37 °C under

microaerophilic conditions in jars containing CampyGen™ (Thermo scientific, Basingstoke UK). Colonies were transferred on a glass slide and tested with a drop of 3% (v/v) H₂O₂ for catalase activity. Catalase-negative isolates were retained for further characterization. Presumptive *P. larvae* isolates were grown in tryptic soy broth (TSB, Biolife Italiana Srl., Milan, Italy) and incubated as stated above before DNA extraction for genotypic assays.

DNA extraction

Crude DNA extracts were prepared from a single bacterial colony per sample according to Rossi et al. (2018) using the Nucleospin® Tissue kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). DNA was quantified by using the Qubit 3.0 fluorometer (Thermo Fisher Scientific, Milan, Italy) according to the instructions and using the provided standards and staining solutions.

PCR tests

Presumptive *P. larvae* isolates were identified at the species level by the species-specific qPCR test described by Rossi et al. (2018) using the qPCR BIO SyGreen Mix Separate-ROX (PCR Biosystems, Resnova S.r.l., Rome, Italy).

Rep-PCR for ERIC genotype determination was carried out with the 2X Emerald Amp GT PCR Master Mix (Takara, Diatech Labline, Jesi, AN, Italy) adjusting dNTPs and MgCl₂ concentrations to those described by Genersch et al. (2006). Fifty ng of DNA were added to 20 µL PCR reactions. Ten µL of PCR reaction were electrophoresed on 1.5% (w/v) agarose gels in IX Tris-acetate buffer (TAE), prepared from TAE 50X (Thermo Fisher Scientific, Milan, Italy), stained with Sybr Safe DNA Gel Stain (Thermo Fisher Scientific) and run at 100V for 1.30 h. Gels were photographed with the UVITEC Essential V6© Gel Documentation System (Eppendorf, Milan, Italy).

Results

Out of 373 brood comb samples from apiaries tested visually and with the immunochromatographic diagnostic tool, 219 from 24 municipalities were positive results with 100% concordance between the two tests (Figure 1).

Results were confirmed by bacteriological examination on Columbia blood agar and selective MPYGP medium with growth of presumptive *P. larvae* colonies in all cases.

Based on these results, the AFB distribution in the municipalities taken into account in this investigation is shown in Table 1.

In order to verify *P. larvae* genotypes and the possible geographical distribution, from a total of 219 isolates, only 17 *P. larvae* isolates were chosen from the

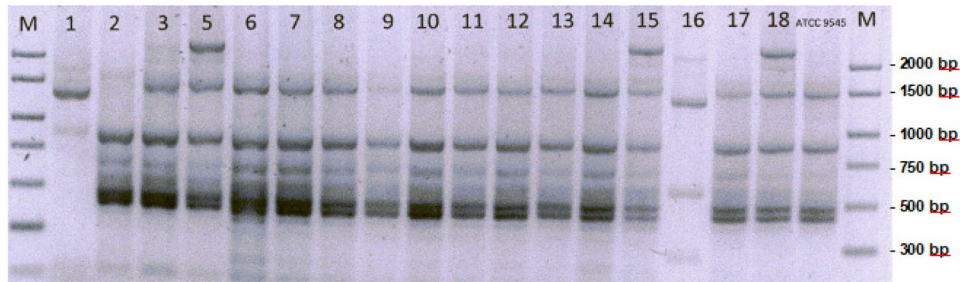


Figure 2. ERIC profiles of *P. larvae* strains circulating in the Republic of Kosovo in the years 2016–2018. Numbers correspond to isolate labels, whose origin is as follows: 1, Deqan; 2, Malisheve; 3, Junik; 5, Lipjan; 6 Prishtinë; 7, Prizren; 8, Gjilan; 9, Viti; 10, Deqan; 11, Dragash; 12, Skenderaj; 13, Suhareke; 14, Vushtrri; 15, Peje; 16, Kamenice; 17, Ferizaj; 18, Prishtinë. M, PCR 50 - 2,000 bp Marker (Sigma Aldrich, Milan, Italy), type strain ATCC 9545, positive control was used.

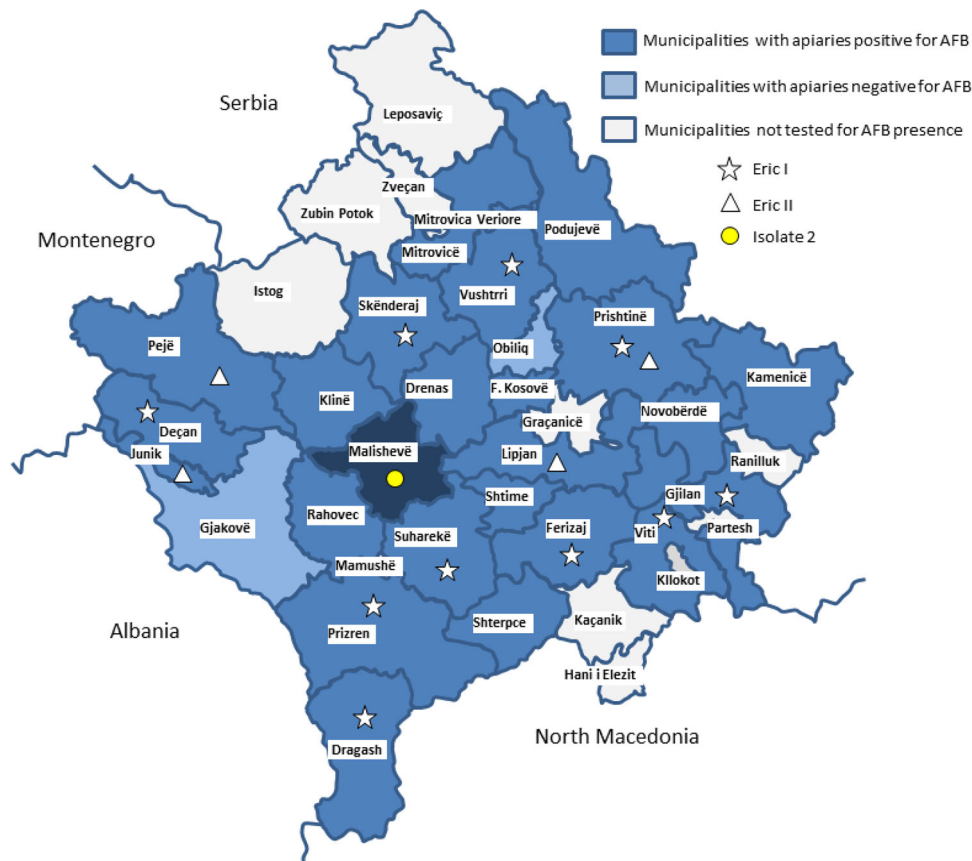


Figure 3. Occurrence of AFB in the municipalities of the Republic of Kosovo and distribution of ERIC I and ERIC II genotypes as well as isolate 2.

period 2016–2018 for further investigation. These 17 isolates that belonged to 15 municipalities of different geographical areas (two isolates from Deqan and Prishtinë and one isolate respectively from Malisheve, Junik, Skenderaj, Lipjan, Prizren, Gjilan, Viti, Dragash, Suhareke, Vushtrri, Peje, Kamenice and Ferizaj) were identified by a species-specific Real Time PCR test and characterized by ERIC typing. Results confirmed the affiliation to the species *P. larvae* for 15 isolates.

The majority of samples showed a profile that resembled either ERIC I or ERIC II genotype, based on the presence of bands indicative for these genotypes, one close to 1500 (present in all isolates except isolate

2) and the other close to 1000 bp (Genersch et al., 2006), one band close to 700 bp, and a double band below 500 bp that in the images presented in other studies appears as a single strong band different electrophoresis conditions (Bassi et al., 2015). Based on species-specific qPCR results and ERIC typing, isolates 1 and 16 did not belong to the species *P. larvae*, though they exhibited similar colony morphologies. The band longer than 2000 bp, distinctive of the ERIC II genotype, was not always strongly evident, as, for example, for isolate 3, which in the particular analysis shown in Figure 2 does not almost show that band, while in other experiments it was more evident. Therefore, 10 isolates

were attributed to the ERIC I genotype and 4 to the ERIC II genotype, while isolate 2, showed a profile not attributable to any of the two for the absence of the band of size close to 1500 bp (Figure 2).

Discussion

This is the first report on AFB distribution in the apiaries of the Republic of Kosovo. The passive surveillance program revealed a wide diffusion of AFB caused by strains with genetic profiles of the most common ERIC genotypes, ERIC I and II. However, also strains with slightly different profiles that should be better characterized genetically and with respect to the pathogenic potential can be present, as indicated by the ERIC genotype of isolate 2. This finding is in line with a recent paper describing a new ERIC genotype circulating in Spain (Beims et al., 2020).

PCR analysis confirmed the diagnosis carried out by immunochromatographic and bacteriological tests for all isolates tested except two. This is indicative that isolation of the AFB etiological agent in some cases can fail due to the lack of specificity of the culture media (Crudele et al., 2020).

The total of 2027 brood combs tested from 373 apiaries during passive surveillance from 2007 to 2019 showed a high prevalence (58.71%) of AFB in apiaries which is considered is an indication that the disease spread is out of control in the Republic of Kosovo. The reason can be that Kosovar beekeepers in general, and amateur and part-time beekeepers in particular, have not enough knowledge about AFB and honey bee disease control strategies, so that a high risk of disease spread among them exists. Another issue is that existing apiaries are spatially too close to each other, thus favoring the spread of diseases through bee contacts during robbing, as well as robbing from unmanaged AFB dead hives. This severe condition suggests the necessity of applying active disease surveillance strategies at the clinical and preclinical level. In particular, the number of *P. larvae* spores in honey, which positively correlates with the prevalence of AFB outbreaks in the territory (Von der Ohe & Dustmann, 1997), could provide an indication of AFB risk at hive and apiary level. This information could be useful to raise the attention of beekeepers and to allow an early diagnosis, which is crucial to the control of AFB spread (Figure 3).

Conclusions

This is the first report on the presence, distribution and genotype characterization of AFB in the Republic of Kosovo. Both ERIC I and ERIC II genotypes were detected. Despite constant efforts to control AFB in Kosovo it does not appear to be under control. This highlights the need for a change in the approach of future control strategies. Early detection of the spore load of infectious agent in honey, as well as training

programs for best management apiculture practices, are necessary to reduce AFB prevalence and prevent the further spread of the disease.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Izedin Goga  <http://orcid.org/0000-0003-2421-0259>
 Armend Cana  <http://orcid.org/0000-0001-6034-4903>
 Franca Rossi  <http://orcid.org/0000-0002-7443-1538>
 Luciano Ricchiuti  <http://orcid.org/0000-0002-9946-0554>
 Franco Mutinelli  <http://orcid.org/0000-0003-2903-9390>

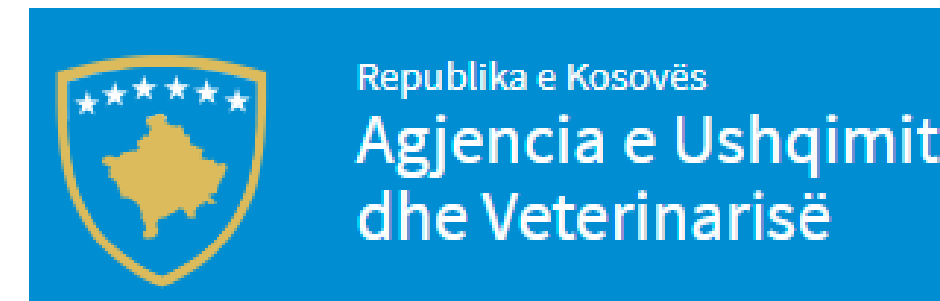
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Pesticide Residues in Honey in Kosovo a Case Study

B. Hulaj¹, I. Goga¹, K. Wallner², B. Fritz², A. Rama^{*3}
Food and Veterinary Agency, Prishtina, Kosovo¹,
University of Hohenheim, Stuttgart, Germany,²
Higher Colleges of Technology, Sharjah, U.A.E.³



ABSTRACT

The objective of this study was to assess the presence of some organochlorine and organophosphorus compounds pesticide residues (34) of locally produced honey collected from individual apiaries during August, 2017 from Peja region in Kosovo. In the present study, a total of 17 honey samples collected have been quantitatively screened by Gas Chromatograph-Electron Capture Detector (GC-ECD). The recovery rate ranged from 78.2 to 98.0%. The method provided limits of detection (LOD) in the range of 0.001-0.168 µg/kg. The limit of quantification (LOQ) was 0,003 µg/kg only for flumethrin 0,005 µg/kg). Residues of pesticides were found in a considerable number of honey samples 8 out of 17 or 47.1%. GC-ECD analysis demonstrated that the minimum and maximum level of coumaphos residue, as only present pesticide in samples was 3.4–39.1 µg/kg. Six samples (35.3%) exceeded the European Union maximum residue levels (EU MRLs). Positive samples with exceeded MRL Residue of Coumaphos 6/17 owing to its applications to control Varroa destructor, indicating that the chemicals used by apiculturists inside the hives in order to control disease are the main pollutants of the produced honey. The obtained results of this study imply that more emphasis should be given to a continuous monitoring programme for pesticide residues in honey, accompanied by an education programme to beekeepers on proper hive management, at the national level to protect consumer health.

INTRODUCTION

Currently, Kosovo has 6,453 beekeepers with 70,664 bee-hives distributed in the territory of Kosovo with an average production for bee society 9.55 kg honey or 674 T / year whereas the import from 2014 is around 140 t / year. (MAFRD, 2015). Honey is a natural product of bees and is recognized as a food with nutritional properties and is known like a food with valuable therapeutic applications. However, bee products can also be a source of toxic substances, such as antibiotics, pesticides and heavy metals due to environmental pollution and misuse of beekeeping practices. Honey bees collect pollen and nectar from the surrounding flowers and then they may return to hives collecting significant amounts of toxic contaminants, in addition the honeybees are also exposed to pesticides and antibiotics administered by beekeepers as part of the hive to control some infestations such as Varroa destructor, Acarapis woodi and Paenibacillus larvae (Fell & Cobb, 2009; Genersch, Evans, & Fries, 2010); Residues of organochlorine insecticides in honey can possibly be dangerous for people, because of the insecticides mutagenic, teratogenic and embriotoxic activities. Coumaphos, is an organophosphate insecticide widely used to control varroosis in bee colonies and for the treatment of ectoparasitoses in livestock. Coumaphos targets cholinergic signaling through acetylcholine esterase inhibition and comprises the majority of excitatory neurotransmission in the Varroa's nervous system (Millar and Denholm 2007). In Kosovo honey has never been included in regular pesticide residues controls. The aim of our study was to estimate the amounts of residues from organochlorine insecticides in Kosovar honey.



MATERIALS AND METHODS

The objective of this study was to assess the presence of some organochlorine and organophosphorus compounds pesticide residues (34) of locally produced honey collected from individual apiaries during August, 2017 from Peja region in Kosovo. In the present study, a total of 17 honey samples collected have been quantitatively screened by Gas Chromatograph-Electron Capture Detector (GC-ECD) at Apicultural State Institute, University of Hohenheim, Germany. All honey samples were labeled either according to their botanical and geographical origin as suggested by the beekeepers. The samples were stored at room temperature in the dark until analysis. One honey sample was checked to be free of any of the targeted pesticides and it was used as blank honey for calibration curve.

RESULTS

Residues of pesticides especially coumaphos was found in a considerable number of honey samples 8 out of 17 or 47.1%. GC-ECD analysis demonstrated that the minimum and maximum level of coumaphos residue, as only present pesticide in samples was 3.4–39.1 µg/kg. Six samples or 35.3% of the detected pesticide exceeded the European Union maximum residue levels (EU MRLs). Positive samples with exceeded MRL Residue of Coumaphos 6/17 owing to its applications to control Varroa destructor, indicating that the chemicals used by apiculturists inside the hives in order to control disease are the main pollutants of the produced honey.

CONCLUSIONS

This research studied the presence of pesticides in honey for consumption in Kosovo. Overall, it was confirmed, the primary determined hypothesis, that there is presence of pesticides in honey. It is noteworthy that in these study the pesticide detected/quantified can present a risk to human health but also they pose a risk to bees as increased mortality rate in developing larvae, reduced longevity in adult bees which were exposed to coumaphos as immatures, and higher number of queen cells under construction in treated hives. Inevitably, the utilization of acaricides in apiculture has resulted in residues being frequently found in bee products. Finally in Kosovo there is no published study about the presence of pesticides in honey. The lack of information about pesticide residues in honey in Kosovo implies the necessity to determinate the pollution of those bees' products in the country. In that way, the aims of this study were to identify currently used pesticides and common pesticide management practices and to validate a multiresidue analysis methods. As this is the first ever study on possible presence of pesticides in honey in Kosovo, frequent analytical surveillance by food control agencies is highly recommended to control the incidence of pesticide contamination in Kosovo especially in bee products.

Table 1. Honey samples analyzed to detect possible presence of pesticides in 2017

Honey	No. of samples	No. of positive samples	No. of negative samples	Positive samples Mean±SD
Local	17	8 (47.1%)	9 (52.9%)	11.81(±11.53)

Table 2. Coumaphos levels (µg/kg) in positive samples

Sample No.	Sample Code	Level (µg/kg)
1	518/17	6.8
2	520/17	9.9
3	522/17	7.4
4	523/17	39.1
5	524/17	12
6	527/17	3.4
7	528/17	3.6
8	530/17	12.3

Prüfauftrag: Rückstandsanalyse

Analytlen SOP P-1-001 (Z)	Ergebnis (µg/kg)	Analytlen SOP P-1-008	Ergebnis (µg/kg)
Brompropylat	n.d.	Thymol	n.d.
Coumaphos	39,1	Paradichlorbenzol	n.d.
Fluvalinat	n.d.		
Tetradifon	n.d.	Analytlen SOP P-1-010	Ergebnis (µg/kg)
Acrinathrin	n.d.	Acetamidiprid	n.d.
Chlorfenvinphos	n.d.	Cyprodinil	n.d.
Dimoxystrobin	n.d.	Flonicamid	n.d.
Alpha-Cypermethrin	n.d.	Thiacloprid	n.d.
Lambda-Cyhalothrin	n.d.	Tebuconazol	n.d.
Iprodion	n.d.	Prothioconazol	n.d.
Tolyfluanid	n.d.	Metconazol	n.d.
Beta-Cyfluthrin	n.d.	Thiophanat-methyl	n.d.
Myclobutanil	n.d.	Prochloraz	n.d.
Deltamethrin	n.d.	Pyraclostrobin	n.d.
Boscalid	n.d.	Fluopyram	n.d.
Kresoxim-Methyl	n.d.		
Esfenvalerat	n.d.	Analytlen SOP P-1-005	Ergebnis (µg/kg)
Chlorpyrifos-methyl	n.d.	N,N-Diethyl-m-toluamid (DEET)	keine Analyse
Azoxystrobin	n.d.	Dimethylphenylformamid (DMF)	n.d.
		Dimethylanilin (DMA) / Amitraz	n.d.

(Z) akkreditiert nach DIN EN ISO/IEC 17025

Bestimmungsgrenze

n.d. = keine Rückstände nachweisbar (nicht detektierbar) <

Table 3. The list of pesticides examined

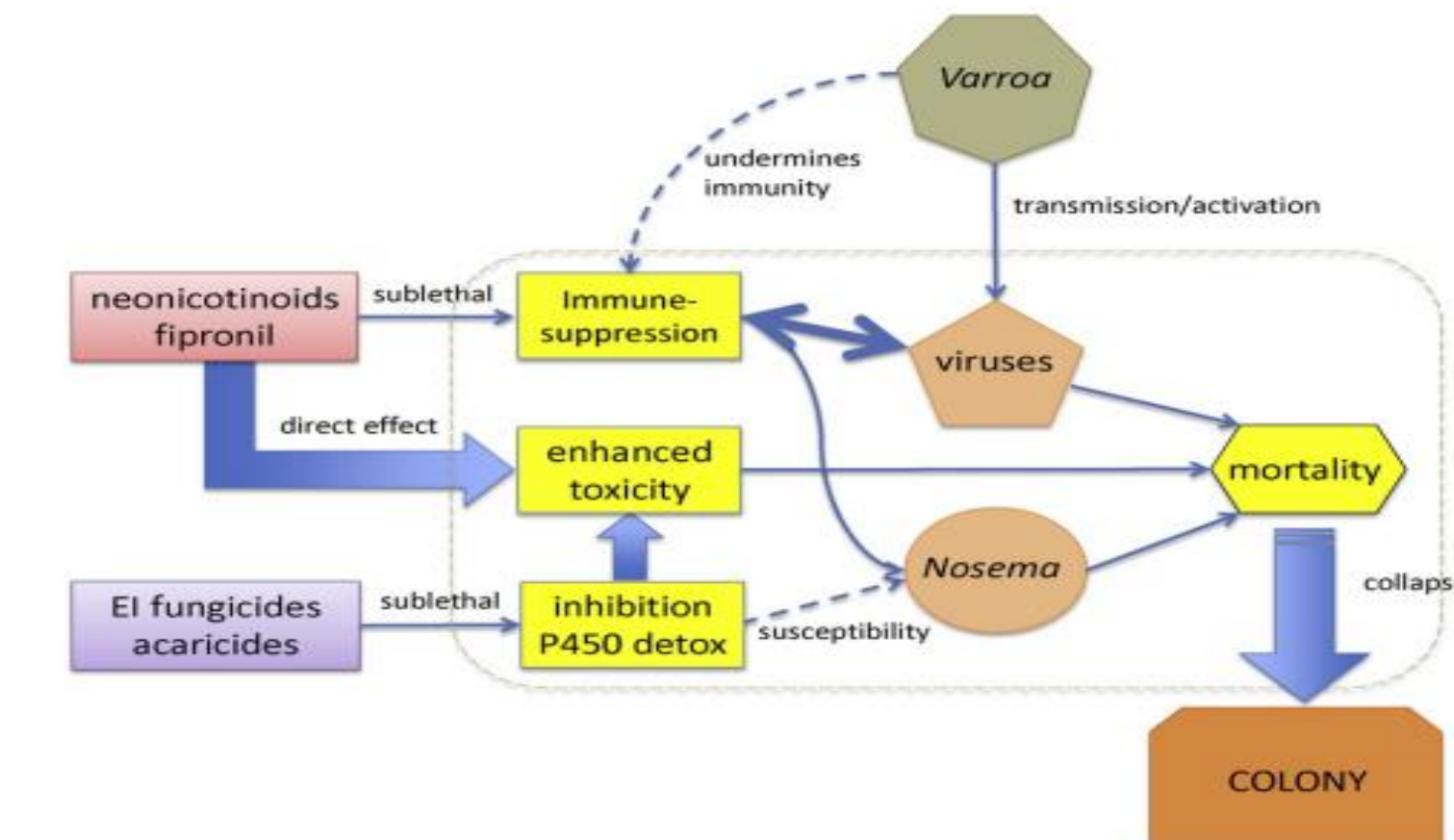


Fig. 1 Interactions between pesticides, parasites and pathogen stressors in relation to honey bee colony collapses

Survey of the prevalence of *Salmonella* species on laying hen farms in Kosovo

B. Hulaj,^{*,1} P. Çabeli,[†] I. Goga,^{*} N. Taylor,[‡] C. Hess,[§] and M. I. Hess[§]

^{*}Kosovo Food and Veterinary Agency, Zona Industriale Pn 10000 Prishtine, Kosovo; [†]Faculty of Veterinary Medicine, University of Agriculture Tirana, Albania; [‡]Veterinary Epidemiology and Economics Research Unit (VEERU), School of Agriculture, Policy, and Development, University of Reading, Reading, RG6 6AR, UK; and [§]Clinic for Poultry and Fish Medicine, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria

ABSTRACT A survey on the prevalence of *Salmonella* (*S.*) species was carried out on 39 layer farms in Kosovo between April and September 2012. In total 367 samples, comprising feces, dust, eggs, and internal organs from dead birds, were investigated using bacteriological culture methods. Additionally, data on the location of the farm, the total number of birds on the farm, age of birds, and laying performance were collected. *Salmonella* were isolated from 38 samples obtained from 19 (49%) farms. The most common serovar identified was *Salmonella* Enteritidis, found on 18 farms. The most common *S.* Enteritidis phage type was PT29 followed by PT6, PT7, PT21, PT13a, PT8, PT14b, and PT4. One *S.* Enteritidis isolate was not typable. Six farms had more than one phage type. Furthermore, serovar *S.* Bovismorbificans also was found in samples from 3 farms. Flock size or production stage was not associated with the probability of iso-

lating *Salmonella*. The only flock factor found to be significantly associated was percent hen/day production: It was 2.8 times more likely to isolate *Salmonella* from flocks with production above 80% hen/day production compared to flocks producing at a lower level. Analysis of antimicrobial resistance patterns of 30 isolates revealed that all isolates were sensitive to gentamicin, ampicillin, sulphamethoxazole trimethoprim, and oxytetracycline, and 29 (97%) were sensitive to ciprofloxacin. All isolates showed intermediate resistance or were resistant to minocycline and cloxacillin. Twenty-six isolates (86%) had intermediate resistance to amoxicillin and 27 isolates (90%) were fully resistant to streptomycin. The present survey revealed a high prevalence of *Salmonella* Enteritidis in layer flocks in Kosovo, indicating that table eggs have to be suspected as an important source of human salmonellosis.

Key words: *Salmonella*, Kosovo, prevalence, survey, layers

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INTRODUCTION

In the 1980s, intensive poultry production based on what is now Kosovo territory ran to about 10 million broilers per year plus a standing flock of about one million laying hens. Afterwards, political turbulences led to a decline of the poultry sector but since 2000 the poultry industry has recovered, with currently more than half a million laying hens in about 80 flocks supplying 80% of table eggs consumed in Kosovo (the rest being imported). Layer flock sizes range from 2,000 to 80,000, and most layer farms have only one house, although a few larger farms have up to four.

Human salmonellosis is a major public health concern in Europe, mainly caused by the serovar Enteritidis (EFSA, 2006; EFSA and ECDC, 2012). In Kosovo *Salmonella* (*S.*) Enteritidis was isolated from 45% of

247 cases of human gastro-enteritis reported to the Institute of Public Health in Pristina in 2014 (Institute of Public Health, Pristina, 2014). Outbreaks in humans often are related to contaminated poultry meat and eggs (Patrick et al., 2004; Jackson et al., 2013; Middleton et al., 2014). The link between *S.* Enteritidis in humans and the consumption of contaminated poultry products, especially undercooked and raw eggs, has been well documented (Coyle et al., 1988; Hogue et al., 1997; Palmer et al., 2000; De Buck et al., 2004). Commercial layer farms can be a significant reservoir of *Salmonella* infection and pose a threat to humans (Garber et al., 2003; EFSA, 2005; Dewaele et al., 2012). However, a *Salmonella* infection is usually not associated with clinical signs in chickens, arguing for specific strategies by the government or industry to protect public health.

Antimicrobial resistance (AMR) is of growing public health concern, especially with the appearance of multi-drug resistant microorganisms. Zoonotic bacteria that are resistant to antimicrobials are of special concern since they might compromise effective treatment

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¹Corresponding author: bhulaj@yahoo.com

regimes in humans. It is therefore relevant to assess the nature and extent of AMR in *Salmonella* found in poultry. In 2009, in the European Union, the occurrence of resistance in *Salmonella* isolates from salmonellosis cases in humans was high for ampicillin and tetracyclines, and moderate for sulphonamides, whereas resistance to the critically important antimicrobials for human medicine, cefotaxime (a third-generation cephalosporin) and ciprofloxacin (a fluoroquinolone), was relatively low (EFSA and ECDC, 2011). In the United States, Han et al. (2013) found 30 out of 54 (56%) *Salmonella* isolates from a variety of human, chicken meat, and egg-associated sources were resistant to at least one antimicrobial agent tested.

The survey reported in this paper was carried out to estimate the prevalence of *Salmonella* in egg-laying farms in Kosovo along with the identification of serotypes, phage types, and antimicrobial resistance patterns.

MATERIALS AND METHODS

Sampling Plan

The survey was carried out between April and September 2012. The method used was based on the technical specifications document (SANCO/34/2004 Rev3) annexed to Decision 2004/665/EC published by the European Commission concerning the baseline study to estimate the prevalence of *Salmonella* species in flocks of laying hens across the European Union (EC, 2004). On the basis of an expected 50% farm prevalence, to give 95% confidence interval with a precision of $\pm 10\%$, a sample size of 44 farms out of the total 80 farms in Kosovo would be needed. Due to some practical limitations it was possible to sample 39 farms, selected randomly across 13 municipalities of Kosovo. This resulted in a 95% confidence interval for the prevalence estimate with a precision of $\pm 15\%$.

Sample Collection

All layer farms in Kosovo at the time of the survey operated caged systems. All except one of the sampled farms had only one house. Therefore only one house was sampled on all farms except the largest farm that had 80,000 hens in 4 houses, where 2 houses were sampled. As required by the technical specification for caged systems, 5 samples (each about 60 g) of naturally mixed feces representative of the whole house were taken from droppings belts, scrapers, or deep pits. Two dust samples (each about 25 g) were taken, one from the floor and one from the fan housing. All feces and dust samples were collected into separate sterile containers. Thirty eggs were collected from different places around the house. These numbers and types of samples were taken from each of the 2 sampled houses on the large

farm. The intention was also to collect 2 fresh carcasses from each farm, but in practice only 11 carcasses (up to 24 h old) of dead chickens were collected, one from each of the 11 farms.

Salmonella Culture and Typing Method

Salmonella culture and typing was carried out in the Food and Veterinary Laboratory of the Kosovo Food and Veterinary Agency. The method used for the culture of *Salmonella* was according to ISO 6579:2002 (ISO, 2002). From each feces and dust sample, 25 g of feces or dust material was mixed in 225 mL of buffered peptone water (BPW, CM 059, Oxoid Limited, Basingstoke, UK). For the egg samples, pools were created using one mL of yolk from each of 15 eggs to make 2 15 mL pools per farm. Each 15 mL pool of mixed egg yolk was mixed into 135 mL of BPW. From carcasses, the liver, spleen, and intestines were harvested and 25 g of the pooled and macerated material was mixed into 225 mL of BPW. Each of these inoculated BPW mixtures was then incubated initially at 37 °C for 18 to 24 h.

Three separate and equally spaced drops of the inoculated broth (0.1 mL total) were placed on the surface of a modified semi-solid Rapaport Vassiliadis (MSRV) medium with novobiocin (1868-17 Difco) plate. The plates were examined after 24 and 48 h incubation at 41.5°C for suspect *Salmonella* growth. Suspected colonies were streaked onto Brilliant Green agar (CM 0263, Oxoid Limited, Basingstoke, UK), Xylose-Lysine-Desoxycholate Agar (XLD CM 0469, Oxoid Limited, Basingstoke, UK), Xylose-Lysine-Tergitol 4 (113919 Merck KGaA, Darmstadt, Germany), and Brilliance™ *Salmonella* agar (CM 1092, Oxoid Limited, Basingstoke, UK) and incubated at 37 °C for a further 24 hours.

Suspect *Salmonella* colonies were confirmed by serotyping according to the Kauffman-White scheme (Popoff, 2001). Phage typing of *Salmonella* is a useful typing tool for subcategorizing the more common *Salmonella enterica* serovars, i.e., *S. Enteritidis* and *S. Typhimurium*. Isolates of *S. Enteritidis* were phage-typed according to the World Health Organization collaboration center Colindale schemes (Ward et al., 1987).

Thirty *Salmonella* isolates were tested by disc diffusion for their in vitro sensitivity to 8 antimicrobials. The test was performed using the protocol from Bauer et al. (1966). Antimicrobial discs (Oxoid Limited, Basingstoke, UK) were placed on inoculated Mueller Hinton Agar plates using a disc dispenser. The discs used contained the following antibiotics: streptomycin (S 10mcg); gentamicin (Cn 10mcg); ampicillin (AMP 10mcg); amoxicillin (AML 2mcg); cloxacillin (OB 5mcg); ciprofloxacin (CIP 1mcg); sulphamethoxazole + trimethoprim (SXT 25mcg); oxytetracycline (OT 30mcg); minocycline (MH 30mcg).

Table 1. Total samples taken and the numbers of positive samples (isolates), by sample type.

Type of sample	Total samples from all farms	Number of samples from positive farms	Number of positive samples	% positive (of all samples)	% positive (of samples taken on positive farms only)
Feces (5 × 60 g pools per farm)	200	95	22	11.0%	23.2%
Dust swabs (2 × 25 g pools per farm)	80	38	13	16.3%	34.2%
Eggs (2 × 15 eggs pooled per farm)	76	38	2	2.6%	5.3%
Internal organs (up to one carcass per farm)	11	7	1	9.1%	14.3%
Total samples – all types (tested pools)	367	178	38	10.4%	21.3%

Data Collection and Analysis

For the purposes of estimating the population prevalence, the primary sampling unit was the farm. Farms were subsequently designated as positive or negative according to the presence or absence of *Salmonella* in one or more of the samples. At the time of sample collection, a brief information sheet also was filled in. This covered the location, total number of birds on the farm, production stage of flock in months (time since start of lay), the percent hen/day egg production, appearance of any clinical disease, and the number of carcasses found on the day of sampling.

Ninety-five percent confidence intervals for percentage estimates were calculated using the Wilson score intervals method, with correction for population size, (Wilson, 1927; Wallis, 2013) as provided in the statistical toolbox at *OpenEpi.com*. This method provides exact, non-symmetrical confidence intervals that are robust even when sample size is small or the percentages are close to zero or 100%. To test for differences in percentages between groups the Chi squared test was used as a test for homogeneity among multiple groups. A Fisher or mid-*P* exact test was used as a test for difference between 2 groups, which is also summarized using relative risk (RR) with confidence intervals calculated using the Taylor series method (O'Brien et al., 1994) as provided in the statistical toolbox at *OpenEpi.com*. Statements about statistical significance of differences are based on the probability (*P*) value for the test statistic being less than or equal to 0.05 as the arbitrary criterion for significance.

RESULTS

Salmonella Prevalence

From 367 samples tested, *Salmonella* was isolated from 38 samples: 22 isolates from feces, 13 from samples of dust, 2 from eggs, and one isolate from poultry internal organs (Table 1). With respect to sample type, the highest prevalence of positive samples was for the pooled dust samples. If samples from positive farms are

Table 2. Types of samples positive for *Salmonella* on the survey farms.

Types of samples positive for <i>Salmonella</i>	Number of farms
All samples negative	20
Positive samples	19
<i>Egg only</i>	1
<i>Dust swab only</i>	3
<i>Feces only</i>	10
<i>Feces and dust swab</i>	4
<i>Feces, dust swab, and internal organs</i>	1
Total	39

considered only, 34% of the dust pools tested yielded *Salmonella* isolates, compared with 23% of the pooled feces samples, a relative risk of 1.48 (although this tendency was not statistically significant with a mid-*P* exact *P*-value of 0.2038). Pooled egg samples had the lowest prevalence of positive samples, with only 5.3% of the pooled samples from positive farms yielding *Salmonella* isolates, a relative risk compared to feces pools of 0.23 (statistically significant, with a mid-*P* exact *P*-value: 0.0119).

Of the 39 farms sampled in the survey, 19 tested positive for *Salmonella* in one or more samples (Table 2), giving an estimated farm level prevalence of *Salmonella* in Kosovo layer farms of 48.7% (95% confidence interval: 33.9 to 63.8%) (Table 3). Only 2 different serovars were identified: *S. Enteritidis* and *S. Bovismorbificans*. *S. Enteritidis* was found on 18 of the 19 positive farms, giving an estimated farm level prevalence of *S. Enteritidis* in Kosovo layer farms of 46.2% (95% confidence interval: 31.6 to 61.4%). *S. Bovismorbificans* was found on 3 of the farms, giving an estimated farm level prevalence of *S. Bovismorbificans* on Kosovo layer farms of 7.7% (95% confidence interval: 2.7 to 20.3%). *S. Bovismorbificans* was found on 2 farms along with *S. Enteritidis* and on one farm as the only serovar.

Table 2 provides details of the types of samples from which *Salmonella* was isolated on the survey farms. On 15 of the 19 positive farms, *Salmonella* was isolated from one or more of the feces samples. On 10 of these farms, feces samples were the only samples to be positive. *Salmonella* was isolated from dust samples on

Table 3. Farm level prevalence of *Salmonella* among layer farms in the survey.

	Number of farms sampled	Positive farms: number (%)	(95% c.i.) ¹
Overall	39	19 (48.7%)	(33.9 to 63.8%)
By region			
Ferizaj (south/east)	4	1 (25.0%)	(4.6 to 70.0%)
Gjilan (east)	6	4 (66.7%)	(30.0 to 90.3%)
Peje (west)	13	9 (69.2%)	(42.4 to 87.3%)
Pristina (centre/east)	4	0 (0.0%)	(0.0 to 49.0%)
Prizren (south)	12	5 (41.7%)	(19.3 to 68.1%)
<i>Overall Chi-Square: 7.903</i>	<i>p-value: 0.995</i>		
By two groups of regions			
Gjilan + Peje	19	13 (68.4%)	(46.0 to 84.6%)
The rest	20	6 (30.0%)	(14.6 to 51.9%)
		<i>Relative risk: 2.28</i>	<i>(1.09 to 4.76)</i>
<i>Fisher exact (2-tail) P-value: 0.03633</i>	<i>Mid-P exact (2-tail) P-value: 0.02107</i>		
By flock size category			
<5,000	18	9 (50.0%)	(29.0 to 71.0%)
5,000 < 10,000	10	5 (50.0%)	(23.7 to 76.3%)
10,000 < 20,000	7	3 (42.9%)	(15.8 to 75.0%)
> = 20,000	4	2 (50.0%)	(15.0 to 85.0%)
<i>Overall chi-square: 0.1173</i>	<i>P-value: 0.990</i>		
By two flock size groups			
<5,000	18	9 (50.0%)	(29.0 to 71.0%)
> = 5,000	21	10 (48.0%)	(28.3 to 67.6%)
<i>Relative risk: 1.05</i>	<i>(0.55 to 2.00)</i>		
<i>Fisher exact (2-tail) P-value: > 0.9999</i>	<i>Mid-P exact (2-tail) P-value: 0.888</i>		
By production stage			
< = 9m	18	10 (56%)	(33.7 to 75.4%)
> 9m	21	9 (43%)	(24.5 to 63.5%)
<i>Relative risk: 1.30</i>	<i>(0.68 to 2.47)</i>		
<i>Fisher exact (2-tail) p-value: > 0.6392</i>	<i>Mid-P exact (2-tail) p-value: 0.4526</i>		
By hen/day production			
< = 80%	22	6 (27%)	(13.2 to 48.2%)
> 80%	17	13 (76%)	(52.7 to 90.4%)
		<i>Relative risk: 2.80</i>	<i>(1.35 to 5.83)</i>
<i>Fisher exact (2-tail) P-value: > 0.005702</i>	<i>Mid-P exact (2-tail) P-value: 0.003126</i>		

¹c.i.: Confidence interval. For proportion/percentage these are Wilson score intervals; for relative risk these are Taylor series.

8 farms, on 5 of which feces samples were also positive. *Salmonella* was isolated from eggs on only one farm (where all other samples were negative) and from dead bird organs on only one farm (of 11 farms where carcasses were collected) where feces and dust samples were also positive.

The farm level prevalence of *Salmonella* was calculated for farms grouped according to different categories among the variables captured on the questionnaire: location (grouped into five administrative regions), flock size, the production stage, and production level (Table 3). The prevalence was calculated regardless of serovar, although *S. Enteritidis* was found on all but one of the positive farms. Layer farms are unevenly geographically distributed, with concentrations of poultry farms in the regions of Prizren, in the south, and Peje, in the west. The distribution of number of birds per farm was highly skewed, with most flocks being less than 6,000 birds (minimum 2,400; median 5,200; maximum 80,000 and interquartile range 3,600 to 10,000). There was just one farm with 80,000 birds kept as 4 flocks in 4 houses. This was the only farm with more than one house. The flocks sampled were between 4 and 18 mo into production (median 10; interquartile range 8 to 12). Percent hen/day production at the time of sampling varied between 60 and 95% (median 80%; interquartile range 75 to 85%). There was a trend for

production to decrease with increasing time into production: 67% of flocks 9 mo or less into production had over 80% hen/day production, compared with only 24% of those over 9 mo (mid-*P* exact *P*-value: 0.00958).

Table 3 shows that *Salmonella* prevalence was significantly higher among farms in 2 regions, Gjilan and Peje, compared with the rest (these 2 regions are geographically at opposite sides of the country, east and west). Flock size or production stage was not associated with different prevalences. The only flock factor found to be significantly associated with different prevalences was percent hen/day production: It was 2.8 times more likely to isolate *Salmonella* from flocks with production above 80% hen/day production compared to flocks producing at a lower level.

Phage Types

All the isolates of *S. Enteritidis* were phage typed. Table 4 shows the phage types of *S. Enteritidis* identified and the proportion of positive farms from which each phage type was isolated. The most common *S. Enteritidis* phage type was PT29, which was isolated from 5 (28%) of the positive farms. However, PT6, PT7, and PT21 also were found frequently, each being present on 4 (22%) of the positive farms (Table 4). The other phage types isolated were PT13a (3 farms, 17%), PT8,

Table 4. Phage types of *S. Enteritidis* identified on 18 *Salmonella* positive farms.

Phage type	Number of farms ¹	Percentage of the 18 positive farms
nPT29	5	27.8%
nPT6	4	22.2%
nPT7	4	22.2%
nPT21	4	22.2%
nPT13a	3	16.7%
nPT8	2	11.1%
nPT14b	2	11.1%
nPT4	1	5.6%
Untypeable	1	5.6%

¹Six farms had more than one phage type (details in text).

PT14b (each found on 2 farms, 11%), and PT4, the least common *S. Enteritidis* phage type, found on only one farm. Six farms had combined infections with more than one phage type: types 7 and 21; types 8 and 21; types 7 and 29; types 6 and 13a; types 4 and 6; types 7, 8, and 13a.

Antimicrobial Resistance Patterns

The results of the antimicrobial sensitivity testing of 30 of the *S. Enteritidis* and *S. Bovimorficans* isolates are shown in Table 5. All isolates were sensitive to gentamicin, ampicillin, sulphamethoxazole trimethoprim, and oxytetracycline, and 29 (97%) were sensitive to ciprofloxacin. All isolates showed intermediate resistance or were resistant to minocycline and cloxacillin. Twenty-six isolates (86%) had intermediate resistance to amoxicillin, and 27 isolates (90%) were fully resistant to streptomycin.

DISCUSSION

This survey found *Salmonella* on almost half of the poultry layer farms sampled in Kosovo. *S. Enteritidis*, the serovar most frequently associated with human illness in relation to eggs (EFSA, 2006; EFSA, 2010), was found on 18 of the 19 positive farms. *S. Bovismorbificans* was the only other serovar isolated. Therefore, of the 5 serovars given top priority by the EU because of their public health significance, *S. Enteritidis*, *S.*

Typhimurium, *S. Virchow*, *S. Infantis*, and *S. Hadar*, only one was isolated from the farms.

The high flock prevalence of *S. Enteritidis* is similar to that found in some EU countries by baseline surveys carried out between October 2004 and September 2005 (EFSA, 2007). In those surveys the flock prevalence of *S. Enteritidis* was similarly high or higher in Czech Republic (59.4%), Poland (54.6%), Spain (48.2%), Portugal (47.7%), and Lithuania (44.4%). High flock prevalence of *S. Enteritidis* infection in layer flocks also has been found outside Europe, e.g., Min Chin Im et al. (2015) found 34 infected out of 67 flocks (51%) tested in a survey in Korea. This demonstrates that Kosovo is not unusual in facing a high flock prevalence of *S. Enteritidis* in its newly developing poultry sector. Nevertheless, across the EU as a whole the baseline surveys found a range of flock prevalence of *S. Enteritidis*, from quite low (e.g., Austria, 9.5%, UK, 6.2%, and the Netherlands, 6.1%), through intermediate levels (e.g., Germany, 22.8% and Hungary, 32.2%) to the high prevalences mentioned above.

In the baseline surveys carried out in the EU, dust samples had a higher likelihood of being positive compared to feces samples (EFSA, 2007). A similar tendency was found in this survey, although, because more feces samples were taken and tested on each farm, more positive feces samples were found overall, and it was more common to find a farm positive on the basis of a positive feces sample than a positive dust result. This result suggests that dust sampling could be a more sensitive method of surveillance for *Salmonella* than feces sampling. Isolation of *Salmonella* from dust may be easier than from fresh feces because *Salmonella* is relatively more resistant to desiccation than many competitor organisms (Miura et al., 1964; Davies and Wray, 1996; Davies and Breslin, 2003a). Dust sampling might pick up the presence of infection over a longer retrospective period and also infection in the environment (from contaminated feed and from wild birds), while feces samples reflect more closely the current infection status of the birds present at the time of sampling.

Only 5.3% of the pooled egg samples tested from the positive layer flocks in the survey yielded *Salmonella*.

Table 5. Antimicrobials included in AMR testing of the *Salmonella* isolates, and the resulting sensitivity.

Antimicrobial class and sub-classes	Active ingredient in the disc	Sensitivity/resistance
Aminoglycoside	streptomycin (S 10mcg)	3/30 sensitive 27/30 resistant
Aminoglycoside – 2 deoxystreptamine	gentamicin (Cn 10mcg)	30/30 sensitive
Penicillin – aminopenicillin	ampicillin (AMP 10mcg)	30/30 sensitive
	amoxicillin (AML 2mcg)	4/30 sensitive
Penicillin – penicillinase-resistant	cloxacillin (OB 5mcg)	26/30 intermediate 0/30 sensitive 30/30 resistant
2 nd generation quinolone (fluoroquinolone)	ciprofloxacin (CIP 1mcg)	29/30 sensitive 1/30 intermediate
Sulphonamide + diaminopyrimidine	Sulphamethoxazole + trimethoprim (SXT 25mcg)	30/30 sensitive
Tetracyclines	oxytetracycline (OT 30mcg)	30/30 sensitive
	minocycline (MH 30mcg)	11/30 intermediate 19/30 resistant

The EU member state baseline surveys did not routinely include eggs in the survey sample, but in several other studies of naturally *Salmonella* infected laying flocks the proportion of infected eggs also was found to be low (often below 3%) (Humphrey et al., 1991; de Louvois, 1993; Henzler et al., 1994; Kinde et al., 1996; Advisory Committee on the Microbiological Safety of Food, 2001). Arnold et al. (2012) found similarly low percentages of contaminated eggs from infected layer flocks and the rate of contamination was much higher for shells than for contents. Gole et al. (2014) demonstrated an association between indoor environmental contamination by *S. enterica* and contamination of eggs on layer farms in Australia. Arnold et al. (2012) also found the rate of eggshell contamination was higher per infected bird in flocks with high within-flock prevalence of *Salmonella* infection, possibly due to a correlation between high *Salmonella* prevalence and poor hygiene standards. This means that high prevalence flocks could contribute disproportionately to eggs with contaminated shells. In a survey in Korea, Min Chin Im et al. (2015) found lower rates of *Salmonella* detection inside eggs (5%) and eggshells (17%) relative to detection from environmental dust samples (40%) on layer farms. Sampling on a *Salmonella* infected layer farm in Spain (Garcia et al., 2011) detected *Salmonella* in 92% of feces samples and 34% of samples from eggshells, but no *Salmonella* spp. were detected in the egg contents. Even what may be perceived as a low proportion of egg production contaminated with *Salmonella* may pose a significant risk for human health considering the large number of eggs consumed. It is therefore important to reduce the risk of egg *Salmonella* contamination and the numbers of *Salmonella* bacteria present.

In this survey, flock size was not associated with the risk of *Salmonella*. This differs from the findings of other surveys. For example, in a survey by Snow et al. (2007), the highest prevalence of *Salmonella* occurred in the largest farm size category (30,000 birds or more). In the current survey, most flocks contained less than 6,000 birds. Only 2 farms had 30,000 birds or more, and of these 2, the largest was negative for *Salmonella*. Hence, increased risk was not associated with increasing flock size in this survey. This is possibly related to the fact that in Kosovo the larger flocks tend to be managed by owners who have a higher level of training and knowledge. In comparison, the relatively small-scale flocks of up to 6,000 birds often are managed by non-specialized managers with little training. In particular, understanding and application of biosecurity and hygiene measures are poor. In contrast, a survey in Barbados found that the odds of testing positive for *Salmonella* were 10 times higher on large farms, compared to small farms, and the authors related this to the finding that more small farms cleaned and disinfected poultry facilities quarterly or more often than large farms did (Aimey et al., 2013). All the flocks in Kosovo used caged (battery) systems, which also were found to have higher risk for *Salmonella* in other sur-

veys (Snow et al., 2007). This survey showed a significantly higher probability of isolating *Salmonella* from flocks with higher production levels (greater than 80% hen/day production). This might be explained by increased physiological stress on the birds leading to increased likelihood of shedding *Salmonella*.

Phage typing of *S. Enteritidis* was performed for the first time in Kosovo during this survey. Nine phage types of *S. Enteritidis* were detected. The most common *S. Enteritidis* phage type was PT29. Phage types PT6, PT7, and PT21 also were frequently found in more than 20% of the positive farms. The least common *S. Enteritidis* phage type was PT4 in contrast to other EU countries where PT4 is the most or more common phage type (EFSA, 2007). Improvement of the regular sampling of flocks would be useful in monitoring infection levels. Phage typing of any *Salmonella* isolates could show possible linkages between seemingly sporadic cases, which could help in recognizing the spread of infection among flocks.

The antimicrobial sensitivity testing revealed a mixture of sensitivity and resistance of the isolates to different classes of antimicrobial. Most isolates were resistant to the aminoglycoside, streptomycin, but 100% were sensitive to gentamicin. All were resistant to the penicillinase-resistant penicillin, cloxacillin, and most had intermediate resistance to the aminopenicillin, amoxicillin, but 100% were sensitive to ampicillin. Almost two-thirds of the isolates were resistant to the tetracycline, minocycline, but 100% were sensitive to oxytetracycline. 100% were also sensitive to sulphamethoxazole and trimethoprim and all but one were sensitive to ciprofloxacin. In contrast to the findings here, a survey of layer flocks in UK, in which 177 *Salmonella* isolates were tested against 16 antimicrobials, 77% were sensitive to all 16, and no more than 15% of isolates were resistant to any single antimicrobial (Snow et al., 2007). In a survey of layer farms in Korea, 93 out of 101 isolates were fully susceptible to a range of antimicrobials (Min Chin Im et al., 2015). Although based on only a small number of tested isolates, the high level of resistance observed in this survey is cause for concern.

Because *Salmonella* is an important cause of food-borne disease in humans, the EU agreed to a program for the reduction of *Salmonella* of public health significance in farm animals under Regulation EC No 2160/2003. In view of the findings of this survey, Kosovo might consider following a similar program, at least with respect to the commercial poultry sector. Good cleaning and disinfection practice has previously been shown to be effective in reducing *Salmonella* overall (Garber et al., 2003, Davies and Breslin 2003b). Inactivated *Salmonella* Enteritidis vaccines, when used in conjunction with good hygiene and disinfection practices, also have been shown to decrease the presence of *Salmonella* Enteritidis in layer flocks (de Freitas Neto et al., 2008). In conclusion, the results of this survey show that *Salmonella* enterica, particularly

S. Enteritidis, occurs in the commercial large-scale laying hen production in Kosovo, indicating that table eggs could be an important source of human salmonellosis in Kosovo. Kosovo should consider taking steps to address this threat to human health.

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Seasonal variation in bait uptake and seropositivity during a multi-year biannual oral rabies fox vaccination programme in Kosovo (2010–2015)

Nick Taylor^{a,d}, Izedin Goga^{b,e,*}, Valdet Gjinovci^c, Jeton Muhaxhiri^d, Ilir Recica^d, Beqe Hulaj^e, Boris Yakobson^{f,d}, Tony Wilmshire^{a,d}

^a Veterinary Epidemiology and Economics Research Unit (VEERU) & PAN Livestock Services Ltd., University of Reading, School of Agriculture, Policy and Development, Reading RG6 6AR, UK

^b Agricultural and Veterinary Faculty, University of Pristina, Bulevardi Bill Clinton, 10000 Pristina, Kosovo

^c Food and Veterinary Agency, Industrial Zone, 10 000 Pristina, Kosovo

^d Control and/or Eradication of Animal Diseases, EuropeAid/132620/C/SER/XK, Pan Livestock Services Ltd, Industrial Zone, 10 000 Pristina, Kosovo

^e Kosovo Veterinary Laboratory, Industrial Zone, 10 000 Pristina, Kosovo

^f Rabies Department, Kimron Veterinary Institute, 20250 Bet Dagan, Israel



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ABSTRACT

The European Union supported programmes for rabies control in Kosovo between 2010 and 2015, including spring and autumn biannual oral vaccination campaigns targeting foxes. Throughout the programmes foxes were obtained to provide samples for monitoring the campaigns. This paper explores the seasonal pattern of bait uptake and seropositivity in the fox population. Bait uptake varied by season and by the phase of the project supporting the programme (the main differences between phases being the number of baits distributed and flight line separation). Seropositivity varied by season and by titre of the vaccine used in the preceding campaign. The analyses also suggested a negative effect of higher daytime temperature on bait uptake, and possible association between geographic location of sampling and concordance between bait uptake and seropositivity, but the dataset was too unbalanced to support robust conclusions on these detailed aspects. Descriptive summaries of the data and the multilevel analyses showed that the proportion of sampled foxes that were positive for bait uptake and the proportion seropositive were both high through winter, following the autumn campaigns, and declined through spring and summer, with a low point in samples collected during the time when juvenile foxes are typically dispersing from their birth dens. The percentage of foxes positive for bait uptake was below 30 % (first project phase) and 40 % (second project phase) from mid-July to mid-October following a spring campaign, compared with around 70 % (first project phase) and 80 % (second project phase) in the periods between autumn and the following spring campaigns. As could be expected, the percent of samples that were seropositive followed a similar seasonal pattern, with some additional variation associated with the titre of vaccine used. This seasonal pattern is likely because the population sampled in the late summer months includes increasing numbers of young foxes that could not have been effectively exposed to the spring vaccination campaign, and would have lost any possible maternal immunity by late summer.

The main finding of high levels of bait uptake and seroprevalence through winter, following the autumn campaigns, declining through summer despite the implementation of spring campaigns, supports advice that countries lacking financial resources to support biannual campaigns should focus resources on once per year vaccination in late autumn or early winter. This pattern also indicates that a fox population may rapidly become naïve after cessation of vaccination programmes, therefore strongly coordinated regional programmes and good surveillance will be needed.

Abbreviations: ORV, oral rabies vaccination; IPA, Instrument for Pre-Accession; FLI, Friedrich Loeffler Institute; TET, tetracycline biomarker; ELISA, enzyme-linked immunosorbent assay

* Corresponding author at: Agricultural and Veterinary Faculty, University of Pristina, Bulevardi Bill Clinton, 10000 Pristina, Kosovo.

E-mail addresses: nick.taylor@panveeru.net (N. Taylor), izeding@yahoo.com (I. Goga), valdet.gjinovci@rks-gov.net (V. Gjinovci), jetonm@gmail.com (J. Muhaxhiri), rilir@hotmail.com (I. Recica), bhulaj@yahoo.com (B. Hulaj), boris.yakobson@gmail.com (B. Yakobson), tony.wilmshire@panveeru.net (T. Wilmshire).

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1. Introduction

Rabies is a zoonotic viral disease, which causes an acute encephalitis with a high fatality rate in domestic and wild mammals and humans (Jackson, 2013).

The Western Balkans has been suffering from rabies for centuries (Mutinelli et al., 2004). While dog mediated rabies was eliminated in the early 1990s, fox mediated rabies became the new challenge (Müller et al., 2012a). Fox mediated rabies invaded the Western Balkans from neighbouring regions in Hungary, Romania and Slovenia (Mutinelli et al., 2004; McElhinney et al., 2011) and slowly advanced southwards. Fox rabies was reported in central Serbia, Kosovo and North Macedonia in 1986, 1998 and 2011 respectively (Kirandjiski et al., 2012; Mutinelli et al., 2004; McElhinney et al., 2011). The fox rabies epidemic in the region reached its south-eastern most extension with the emergence of the disease in the north of Greece in 2012 and 2013 (Tsioupras et al., 2013; Tasioudi et al., 2014)

In October 2007, two cases of rabies in foxes were reported in the region of Kosovo near the border with the Republic of North Macedonia. Although no further rabies cases were reported in Kosovo since this time until 2012, strengthened rabies surveillance programmes in neighbouring countries that confirmed the presence of the disease in the Republic of North Macedonia, Serbia, Montenegro and Northern Albania (who-rabies-bulletin.org) suggest that fox rabies had likely been endemic also in Kosovo, at least until 2010. The most recently detected case of rabies in Kosovo was reported in one fox obtained from a village in Suhareke in September 2012 (Yakobson et al., 2014).

The most effective method to eliminate rabies in wildlife is oral rabies vaccination (ORV) (Cliquet and Aubert, 2004). Large parts of Western and Central Europe have been freed from fox mediated rabies using programmes relying on implementation of ORV (European Commission, 2002) and the boundaries of the disease-free area pushed towards the eastern and southern borders of the European Union (Cliquet and Aubert, 2004; Freuling et al., 2013; Müller et al., 2012a). The European Union recently shifted attention towards the elimination of rabies in neighbouring countries (Demetriou and Moynagh, 2011; Müller et al., 2012a). European Union funds to support ORV in the Western Balkans are channelled through the Instrument for Pre-Accession (IPA), which is used to procure supplies of rabies vaccine and to provide relevant technical assistance to support eradication programme activities (Demetriou and Moynagh, 2011).

Since 2010, multi-annual ORV programmes have been launched in all Western Balkan countries: Kosovo (Yakobson et al., 2014); Serbia (Lupulovic et al., 2015); Croatia (Bedeković et al., 2018); Montenegro (Henning et al., 2017); Greece (Papatheodorou et al., 2018); Bosnia-Herzegovina (Anonymous, 2017); North Macedonia and Albania (European Union, 2017).

The IPA programme supported control programmes for rabies in Kosovo. Details of the implementation of the ORV programme and associated challenges have been presented and discussed previously in a paper that covered ORV campaigns from spring 2010 to Autumn 2013 (Yakobson et al., 2014). Under the same project further campaigns were implemented in spring and autumn 2014 and autumn 2015.

Analysis of data collected to monitor the ORV campaigns up to autumn 2013 generally showed bait uptake and seropositivity in a lower proportion of the fox population sampled following the spring campaigns than after the autumn campaigns (Yakobson et al., 2014). A possible reason suggested for the observed lower proportions positive for bait uptake marker and seropositivity after spring campaigns is the seasonal turnover of the fox population, in which there are additions of young naive foxes to the population, and therefore to the hunted sample taken during the summer. The annual life cycle of the red fox (*Vulpes vulpes*) in Europe is broadly: mating during January and February; birthing (in dens) during March and April, with the cubs remaining dependent and close to the dens until July; dispersal of juveniles from their birth dens from July through to October (Vos, 2003). Turnover of

the population can be rapid, with foxes rarely living beyond two years in wild conditions (Larivière and Pasitschniak-Arts, 1996).

Baker et al. (2019) used a simulation model of rabies infection, spread and control to explore ORV strategies in a simulated fox population with seasonal birth pulses and relatively fast turnover. Their model reproduced a temporally dynamic herd immunity, broadly similar to that observed in the Kosovo data up to 2013. Their conclusions were that consecutive biannual ORV campaigns could rapidly control and eliminate rabies in the simulated population but they also noted that the autumn campaigns had the greater impact on probability of elimination.

The higher importance of the autumn campaign above the spring campaign is supported by knowledge of fox ecology, as described by Vos (2003), who noted that adult 'territory owners' play the key role in the spread and maintenance of rabies, with transmission peaking during the mating season (January and February). Vos further noted that there is no evidence that young foxes are responsible for any increase in rabies incidence during the dispersal season and therefore ORV campaigns specifically targeting the young fox population in spring have little effect on the overall aim of controlling and eliminating rabies. Vos suggests that countries lacking financial resources to support biannual ORV could implement vaccination once per year only, in late autumn or early winter. Although the recommendations of the European Commission (2002, 2015) have been to carry out ORV campaigns on a biannual basis in the spring and autumn, annual vaccination campaigns in USA (Texas) and Canada (Ontario) have been effective when baits were distributed in autumn or early winter (Fearneyhough, 1999; MacInnes et al., 2001).

This paper presents a further analysis of the campaign monitoring data covering all campaigns in Kosovo from 2010 to 2015, including multilevel, mixed effect logistic regression modelling. The major purpose of the analysis was to explore the seasonal variation in both bait uptake and seropositivity while taking account of other campaign-associated variables.

2. Materials and methods

Kosovo has an area of 10,908 square km and is situated between latitudes 41.8° and 43.3°N, and longitudes 20.0° and 21.7°E. Kosovo has land borders with Serbia to the north and east, Montenegro to the northwest, Albania to the southwest and North Macedonia to the south (Fig. 1). Geo-physically, Kosovo is surrounded by several mountain ranges with peaks as high as 2656 m. Within the territory, Kosovo is subdivided into two large plain areas lying at elevations between 400 m and 700 m above sea level, one to the north-east and one to the south-west. These plain areas are divided by central highland ranges rising to elevations of about 1000 m.

European Union support for the rabies control programmes in Kosovo, via the IPA, was provided through two distinct phases of a project managed by the European Union Office in Kosovo (EuropeAid/127852/D/SER/KOS). The project provided technical assistance to the responsible authorities, the Kosovo Food and Veterinary Agency (KFVA) and Kosovo Food and Veterinary Laboratory (KFVL). The first phase of the project established capacities for rabies diagnostic testing and provided technical assistance and support concerning implementation of rabies surveillance and operational activities for ORV campaigns.

Under the first project phase from 2010 to 2011, biannual ORV campaigns were carried out from spring 2010 to autumn 2011 (four campaigns). After a gap in spring 2012 an independent KFVA/KFVL-led campaign was carried out in autumn 2012 before the second phase of the project was implemented from 2013 to 2015. The second phase supported biannual ORV campaigns from spring 2013 to autumn 2014. When the IPA programme funding was due to end at the end of 2015, with funds for just one further ORV campaign, the decision was taken to implement the final campaign in autumn 2015, omitting a campaign in

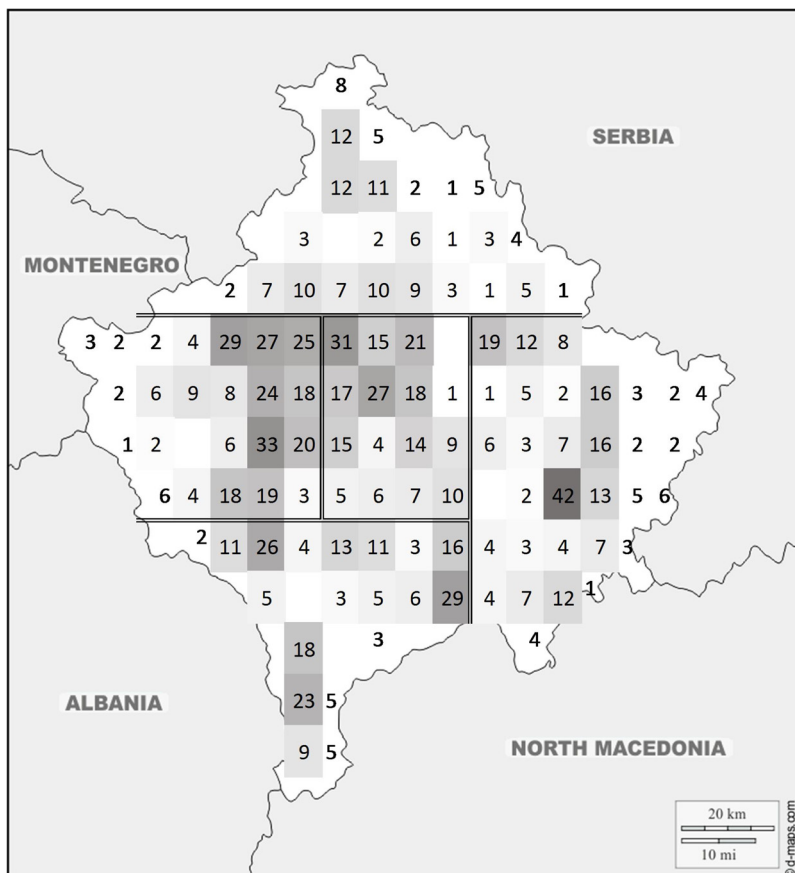


Fig. 1. Overview map of Kosovo showing all 1023 fox samples obtained by 0.1 degree latitude / longitude grid square. The vertical and horizontal lines indicate the division of the territory into five sectors, North, West, Centre, East and South. The numbers in shaded rectangles are the number of samples obtained from each 0.1 degree grid square during the entire ORV programme period.

the preceding spring. In total 10 ORV campaigns were carried out between spring 2010 and autumn 2015, of which six were in autumn and four in spring.

The vaccine used in all ORV campaigns conducted in Kosovo since 2010 was a registered SAD derived oral vaccine composed of two dominant subpopulations of virus, namely SAD Bern and SAD B19 “like”. Each bait contained a dose of 1.8 mL, containing a minimum of 10^6 TCID₅₀/mL (and maximum 10^8 TCID₅₀/mL) according to the manufacturers (Lysvulpen, <https://www.bioveta.eu/en/products/veterinary-products/lys-vulpen-por-ad-us-vet-1.html>). The bait casing contained 150 mg tetracycline (TET) as a marker for bait uptake in the target species. Maintenance of the cold chain throughout shipment from manufacturer, storage in Kosovo and distribution during ORV campaigns was confirmed using temperature data loggers that were shipped and stored together with the baits. Samples from the vaccine were randomly selected from the storage facility shortly before distribution and sent to the WHO Collaborating Centre for Rabies Surveillance and Research at the Friedrich-Loeffler-Institute (FLI), Germany, who provided an independent assessment of potency. Since it was not possible to re-order and acquire replacement stocks of vaccine in time for each ORV, in case the FLI reported sub-optimal titres, all vaccine purchased was distributed during the campaigns, regardless of the FLI assessment.

The chosen vaccine bait distribution strategy followed recommendations of the EU (European Commission, 2002) with bi-annual campaigns during spring (April/May) and autumn (October/November). The number of baits distributed was 250,000 for six of the ten campaigns, but varied from just below 250,000 to just over 350,000 for the others.

In all 10 ORV campaigns the whole territory of Kosovo was included for bait distribution. The duration of bait distribution activities varied from three to 10 days (median 5.5). Vaccine baits were mainly distributed by a private contractor using fixed-wing aircraft (Cessna 172)

equipped with the SURVIS-system for automatic dropping and registration of individual bait positions (for details see Müller et al., 2012b). In general, an average flight altitude of 300-500 m above ground and an average speed of 170–180 km/h were applied. For all campaigns, the flight sectors were adapted in advance according to natural or man-made landscape features. Densely populated urban areas were not included and the highest mountain terrain immediately adjacent to borders with Serbia, North Macedonia, Albania and Montenegro were excluded because low level flying was dangerous. Therefore an area of about 9200 km² was covered by aerial distribution flights in each of the 10 campaigns. Hand distribution of baits was a complementary measure in areas with a high density of settlements and in non-flying zones. During the first four ORV campaigns a flight line separation distance of 1000 m was used. During the second, third and fourth campaigns a modification was applied in a 5 km wide belt bordering neighbouring countries (totalling 2000 km²) in which the flight line distance was reduced to 500 m whilst the flight line distance in the other parts of Kosovo (7200 km²) remained unchanged at 1000 m, using a bait density of 42 and 30 baits/km² respectively. Bait density was above 25 baits/km² in all campaigns. From the fifth to the final campaign the flight line distance was set to 500 m across the whole baited territory (9200 km²) and 250,000 baits were distributed in each of these campaigns, with a density of 27.2 baits/km².

To verify bait acceptability, bait disappearance rates were estimated at the time of each campaign. Baits were hand placed in the field at several specific locations, the bait placements marked and re-visited after one, two and three days to observe and record bait presence or absence.

2.1. Fox sampling

Field sampling was organised to monitor the fox population during

the period of ORV campaigns, as described by [Yakobson et al. \(2014\)](#). Throughout the two phases of the technical assistance project foxes were actively hunted specifically to provide samples for monitoring the effects of campaigns. In line with the existing rabies surveillance protocol, any foxes, or other wild animals involved in human exposure or otherwise suspected of rabies infection were to be submitted for diagnostic investigation. In addition, any foxes and other wild animals that were found dead (usually 'road-kill') were also opportunistically collected and submitted to the KFVL. Collection of fox samples was for the purpose of ORV monitoring and during the whole period of the ORV programme no wild mammals were submitted because of suspicion of rabies infection. However, all foxes submitted for ORV monitoring purposes were also subjected to testing for rabies diagnosis.

2.2. The dataset used in the analyses reported here

Only data from fox samples are included in the dataset analysed for this paper, of which there were 1022 collected between the end of the first ORV in April 2010 and the end of December 2015. All fox samples were obtained from locations within the ORV distribution area ([Fig. 1](#)).

Species, date, location and method of acquisition (hunted or found dead) were recorded for each individual sample. Unfortunately, the age of the animals was not determined or recorded for the vast majority of submitted foxes thus precluding age from the analysis. For the purpose of descriptive summarisation and multilevel logistic regression analysis, each sample was assigned to a roughly quarter year period based on the date of sample acquisition relative to the final distribution date of the preceding ORV campaign. The first quarter (1Q) was for samples collected up to and including 90 days after the spring ORV, the second quarter (2Q) was for samples collected more than 90 days after the spring ORV but before the autumn ORV, the third quarter (3Q) was for samples collected up to and including 90 days after the autumn ORV and the fourth quarter (4Q) was for samples collected more than 90 days after the autumn ORV but before the following spring ORV. Samples obtained in 2Q and 4Q all fell within 91–180 days after the preceding ORV, except for samples collected in 2Q after ORV08, when a few samples were obtained up to 186 days after. Thus, these quarters roughly approximate to May, June, July (1Q: late spring/early summer), August, September, October (2Q: late summer/autumn), November, December, January (3Q: winter) and February, March, April (4Q: late winter/early spring), but it is stressed that samples were assigned to quarters based on timing relative to preceding campaign dates, not strictly according to calendar date.

Nine samples taken during the summer of 2015 were excluded from the univariate analysis and multilevel logistic regression analysis because these were collected in the year when the spring ORV was not carried out. However data from these nine samples are included in the descriptive tables and charts. Two samples collected after the 'un-supported' ORV in autumn 2012 (ORV05) were not included on charts or in the univariate analysis and multilevel logistic regression analysis because this number of samples is too small to be representative of a whole campaign.

Other details associated with each campaign were taken from the campaign completion reports, including flight line separation, total baits distributed, independently assessed vaccine titre (potency) and comments about the prevailing ambient temperatures at the time of distribution. The data on prevailing daytime temperature during the ORV distribution period was based on information recorded in the campaign completion report, so can only be a rough guide to daytime temperatures at the time of ORV. Unfortunately data available retrospectively from the three meteorological stations across Kosovo only provided monthly summary statistics, which were too imprecise to be meaningful for specific campaign periods. Nevertheless, using the campaign reports and contemporary records made by project staff it was possible to identify three campaigns when the prevailing temperatures were notably higher than during the other campaigns.

Nothing was recorded with respect to precipitation during campaigns.

It is conceivable that the biomarker positive or seropositive proportion of the population could be influenced by the number of previous sequential campaigns the population has been exposed to. Therefore a covariate was also created to account for the repeated baiting of the same areas consecutively. As mentioned above, the age of the animals sampled was not recorded. However, based on the average lifespan of red foxes in Kosovo being around 2 years, the first six months of which is spent close to the den, the number of ORV campaigns carried out during the 18 months preceding the sample date was derived as a variable to approximately represent possible previous repeat exposure to ORV. Previous ORV are counted for the 18 months back from the exact date of each individual sample collection, so this variable is specific to each sample. However, since the whole territory was covered at each campaign, this variable was similar for all samples collected after any campaign.

2.3. Laboratory tests

Vaccine titres for the randomly selected samples taken from the storage facility shortly before distribution and sent to the Friedrich-Loeffler-Institute were determined in three independent runs and geometric mean titres (in log 10 units of tissue culture infective dose (TCID₅₀) per ml) were reported for each batch of vaccine.

All foxes submitted for ORV monitoring purposes were also subjected to testing for rabies diagnosis: brain tissue (cerebellum, medulla oblongata, Ammon's horn) was examined for viral antigen using the standard fluorescent antibody test (FAT) ([Dean et al., 1996](#)). FAT-inconclusive results were confirmed using the Rabies Tissue Culture Infection Test (RTCIT; ([Webster and Casey, 1996](#))) or real time polymerase chain reaction (RT-qPCR) essentially as described ([Hoffmann et al., 2010](#)).

The presence of the biomarker (bait-uptake) in foxes collected post vaccination was detected by demonstration of tetracycline (TET)-induced fluorescence in bones (mandibula, tibia) or dentine of the canine tooth of animals using a method described elsewhere ([Johnston et al., 1999](#); [Linhart and Kenelly, 1967](#)). Provided that serum was obtainable with the sample (not always the case, particularly from opportunistic 'found dead' samples), ORV induced rabies virus specific antibodies were determined using a commercial blocking enzyme linked immunosorbent assay (ELISA, Biopro, Czech Republic) as described ([Wasniewski et al., 2013](#)). The interpretation of the ELISA test was based on information provided by the manufacturer indicating that a 40 % inhibition of the test serum compared to the negative controls is considered a cut-off for seropositivity and a 70 % inhibition considered equivalent to an antibody level of 0.5 IU/mL. For quality control, 10 % of serum samples of the first four ORV campaigns were tested at the Rabies Laboratory in the Kimron Veterinary Institute, Israel (accredited and EU approved laboratory for the pet travel scheme) on the presence of virus neutralising antibodies (VNA) using the rapid fluorescent focus inhibition test (RFFIT) ([Smith et al., 1973](#)), with modifications as described ([Cox and Schneider, 1976](#)).

2.4. Parameters derived from the results of the two laboratory tests

Since there was only one positive rabies case detected in a fox during the whole ORV programme period ([Yakobson et al., 2014](#)) it is not possible to use case reduction to measure ORV campaign effectiveness in this study. The analyses reported here therefore focus on parameters related to the measurable impact of the ORV campaigns in the fox population:

Bait uptake (%): calculated as the % of foxes sampled and tested that were positive for the tetracycline marker (TET), as in Eq. 1.

$$\text{Bait uptake} = \frac{\text{number TETpositive (vaccinated)}}{\text{total samples tested (TET)}} (\%) \quad (1)$$

Seropositive proportion (%): calculated as the % of foxes sampled and ELISA tested that had a positive ELISA result. This parameter was calculated using all the samples with a valid ELISA result, using Eq. 2.

$$\text{Seropositive rate} = \frac{\text{number ELISApositive}}{\text{total samples tested (ELISA)}} (\%) \quad (2)$$

Tetracycline and serology concordance (%): the % of tetracycline positive foxes that are also seropositive (i.e. concordance or agreement between the tetracycline result and the ELISA result). This parameter was calculated using only samples with BOTH a **positive** TET result AND a **valid** ELISA result using Eq. 3.

$$\text{Tetracycline serology concordance} = \frac{\text{number ELISApositive AND TETpositive}}{\text{total TETpositive samples tested by ELISA}} (\%) \quad (3)$$

2.5. Statistical analysis

In the descriptive and univariate results presented here 95 % confidence intervals for percentage estimates are calculated using the Wilson score intervals method (Wilson, 1927; Wallis, 2013) as provided in the statistical toolbox at *OpenEpi.com* (Dean et al., 2006). This method provides exact, non-symmetrical confidence intervals that are robust even when sample size is small and/or the percentages are close to 0% or 100 %. To test for differences in percentages the Fisher exact test was used as a test for difference between two groups and the Chi squared test was used as a test for homogeneity among multiple groups. Comments about statistical significance of differences are based on $p < 0.05$ as the criterion for significance.

Separate analyses using multilevel, mixed effect logistic regression modelling were carried out to estimate: (i) the probability of tested samples being positive for tetracycline marker (TET result); (ii) the probability of tested samples being seropositive (ELISA result), and; (iii) the probability of tetracycline-positive samples also being seropositive (concordance). Analyses used multilevel logistic regression models in which the outcome was binomial (negative or positive). The data structure is non-balanced with different numbers of samples from the periods of time between the ORV campaigns, also with some variables specific to particular campaigns and other variables specific to individual samples. Therefore to account for possible covariance in the response data at the level of individual campaigns, two-level mixed model analyses were used, treating ORV campaign as a random effect.

Statistical modelling was carried out using 'R' software (R version 3.6.3 (2020-02-29), Copyright 2020 The R Foundation for Statistical Computing). Models were fitted using a generalised linear mixed model, using the 'glmer' function in the lme4 package of 'R', which incorporates both fixed-effects parameters and random effects in a linear predictor, by maximum likelihood (Laplace Approximation). (Finch et al., 2014). The fitted model predicts the log-odds of an individual sample being positive. The main hypotheses to be tested were that the proportion of the population positive for bait uptake and proportion seropositive declines during summer, before the autumn ORV, regardless of a spring ORV being implemented. Thus the quarter of sample collection was an important potential explanatory variable. Other factors may have influenced the outcome alongside season, so these were included in the statistical modelling process. The potential influential variables available from the recorded data were assigned to categories for the purpose of testing in the analyses. The variables and categories are described in Table 1.

Model fitting followed a forward stepwise process. First, a null (intercept only) model was evaluated, after which models containing each potential explanatory variable singly were compared with the null model. These initial comparisons of single variable models with the null model made use of a likelihood ratio test with the anova function. The single variable that made the biggest and most statistically significant

reduction in deviance was retained and the remaining variables were each added to create a range of two-variable (fixed effects) models. These were compared with the first step (single fixed effect) model using several parameters: the anova p-value (likelihood ratio test), the Aikake Information Criterion (AIC) and the Bayesian Information Criterion (BIC). AIC and BIC are both measures of unexplained variations in the data with a penalty for model complexity. Models with lower values provide relatively better fit in the most parsimonious fashion. If appropriate, the variable that gave the biggest and most statistically significant reduction in deviance along with reductions in AIC and BIC was retained and the same process repeated until no further additions of variables improved the model fit. As more variables were added, the correlations between the fixed effects parameters were examined and variables omitted if high correlations were found. Because of the non-balanced nature of the data some combinations of variables resulted in a fitted mixed model that was singular, i.e. parameters were on the boundary of the feasible parameter space: variances of one or more linear combinations of effects were (close to) zero. When this happened, or if several variables produced similar changes in deviance, AIC and BIC, decisions on which model to settle on were based on the explanatory value of the variables in terms of existing knowledge of what factors could affect the outcome variable in question. The objective was to find the most parsimonious model that was at the same time meaningful and informative.

3. Results

Fig. 1 shows the spatial distribution of samples collected.

Table 2 gives an overview of the 'vital statistics' of the 10 ORV campaigns.

Table 2 shows that there were three campaigns during which the contemporary campaign reports noted daytime temperature being consistently above 17 °C (spring of 2010, spring and autumn of 2013). There were two campaigns where the independently assessed titre of the vaccine used was below $10^{6.5}$ TCID₅₀/mL (spring and autumn 2010) and three where the independently assessed titre was above $10^{7.5}$ TCID₅₀/mL (autumn 2013, spring and autumn 2014). Overall 90 % of fox samples were hunted but a general hunting ban in the summer of 2014 (for reasons not related to the ORV programme) severely reduced the proportion of hunted samples collected after the spring 2014 campaign.

The results of bait acceptance monitoring during the ORV campaigns gave bait disappearance percentages in all campaigns of around 80 % after 72 h in the field, ranging between 75 % (first campaign) and 95 % (fourth campaign).

Fig. 2 is a composite chart using data from all ORV campaigns aggregated by calendar month of sample collection. The total number of samples collected each month show a strongly seasonal pattern, broadly in line with the traditional hunting season in Kosovo, from autumn through winter to early spring. The proportions of tested samples that were positive for bait uptake (TET) and seropositive (ELISA) also follow a seasonal pattern: both being high (over 50 %) through winter and declining through spring and summer. Note that most (112/148) of the samples collected in the month of October were collected before the autumn ORV campaigns, so the effect of the autumn ORV is revealed by samples collected in November. Relatively few samples were collected between the spring and autumn ORV campaigns, but although 95 % confidence intervals are wide, bait uptake and seropositive 'rates' decline below 50 %, despite spring ORVs having been carried out.

Figs. 3 and 4 show bait uptake (TET, Fig. 3) and seropositive (ELISA, Fig. 4) 'rates' for samples collected in each of the approximately quarter-year periods following ORV campaigns. The data on which these figures are based are summarised in Table S1 (Supplementary material). The horizontal axis of these figures is a true timeline, with the exact dates of ORVs indicated by vertical lines.

Figs. 3 and 4 suggest that bait uptake and seropositivity were

Table 1
Categorisation of variables in the dataset for use in the logistic regression.

Outcome Variables	Category explanation
Tetracycline test result (bait uptake).	1: positive; 0: negative
ELISA test result (seropositivity).	1: positive; 0: negative
ELISA result for cases with a POSITIVE TET result.	1: positive; 0: negative
Random Effects	
ORV CAMPAIGN	Nine different campaigns (the only two data points associated with ORV05 were omitted)
Potential 'Explanatory' Variables (Fixed Effects)	
Campaign-associated variables	
Project phase: The main difference between the two phases was flight line separation	Phase 1: flight lines 1000 m apart; Phase 2: flight lines 500 m apart.
Number of baits distributed in the ORV campaign preceding sample date.	> 300 K (ONLY ORV02: 362 K) = 300 K (ONLY ORV03 & ORV04) < 300 K (the lowest number of bait distribution was 234.6 K in ORV01 (with 1 km lines); 250 K baits were distributed in ALL ORVs in project phase2 (with 500 m lines) < 6 (below manufacturer's published standard for the vaccine: ORV01 & ORV02) 6 to 7.5 (ORVs 03, 04, 06 & 10) > 7.5 (ORVs 07, 08, 09) > 17 °C (noted in the campaign reports for ORVs 01, 06 & 07) < = 17 °C (all other ORVs)
Titre (log 10) of vaccine distributed in the preceding ORV campaign (independent assessment).	
Prevailing daytime temperature during the preceding ORV distribution period.	
Individual sample variables	
'QUARTER': samples are split in 90 day periods (roughly quarter year) defined by ORV dates in each year.	1Q: < = 90 days after end date of SPRING ORV 2Q: > 90 days after end date of SPRING ORV 3Q: < = 90 days after end date of AUTUMN ORV 4Q: > 90 days after end date of AUTUMN ORV
Geographic area (sector) The location of sample acquisition was assigned to one of five blocks. These were delineated semi-arbitrarily with the intention of achieving roughly equal numbers of samples from each of the blocks.	North, South, East, West and Centre (see Fig. 1)
Source of the fox sample.	'Hunted' or 'found dead'
This was not expected to influence the probability of TET or ELISA positive, but since it was recorded it was included.	
Number of ORV in the 18 months preceding the sample date.	'1': for samples following ORV01 only (n = 40) '2': for samples following ORV02, 06 & 10 (06 & 10 were both after gaps in the sequence of ORVs) '3': for samples following ORVs 03, 04, 07, 08 & 09 '4': for some early samples after ORVs 04, 08 & 09

generally lower in the first project phase (ORV 1–4) compared with the second phase (except for ORV6). Bait uptake and seropositivity were generally lower in samples collected following spring campaigns than following autumn campaigns, often with a clear 'step down' in samples collected in the quarter immediately prior to the autumn campaign.

Table 2 shows that other factors were variable between campaigns that could also have influenced the outcome: e.g. flight line separation, number and density of baits distributed, vaccine potency (titre), environmental temperature. These are explored individually in Table 3 that shows bait uptake compared between different levels of each of the potential 'explanatory' variables described in Table 1, plus a binary time-frame variable referring to the season of campaign preceding sample collection.

Table 3 shows statistically significant associations between vaccine uptake and all potential 'explanatory' variables. Compared to project phase 1 (when flight line separation was 1 km) bait uptake was higher after campaigns in project phase 2 (using closer flight line separation (500 m). Bait uptake was different between campaigns where different numbers of baits were distributed; different between campaigns where the titre of vaccine was different, with the main difference being that uptake was higher when the titre was over $10^{7.5}$ TCID₅₀/mL; and higher after campaigns where the daytime temperatures were no higher than 17 °C. Bait uptake was higher in samples collected after autumn campaigns (coinciding with quarters 3 and 4) compared with after spring campaigns (coinciding with quarters 1 and 2). There was difference in bait uptake between samples taken at times with different numbers of recent (within 18 months) ORV campaigns. There were also differences in bait uptake between samples taken from different geographic sectors and samples obtained in different ways (hunted or found dead), although the chi-square and Fisher exact p-values for these associations were both close to 0.05.

Table 2 shows that several of these factors are not randomly

variable between campaigns: the number of baits distributed is highly associated with project phase (e.g. all phase 2 campaigns distributed 250,000 baits); two of the three campaigns carried out during hotter weather were in phase 2, and the other (ORV 01) coincided with distribution of the lowest number of baits; and all three campaigns carried out with vaccine with assessed titre over $10^{7.5}$ TID₅₀/mL were in phase 2. Multi-level mixed effect logistic regression analysis is therefore a necessary approach to account for covariance among these factors.

3.1. Multi-level mixed effect logistic regression

3.1.1. Bait uptake

Table S2 (Supplementary material) summarises the 'fit parameters' (AIC, BIC, deviance) and anova p-value for comparisons of all models examined during the forward stepwise model building process. Quarter (time period of sample collection relative to ORVs) was the single most significantly associated factor with odds of being positive for the tetracycline marker. The next most significant factor, when added to quarter, was the project phase (first or second). Model fit parameters suggested that prevalent temperature and the number of ORV campaigns in the 18 months preceding sample collection could also be significantly associated with bait uptake. Higher prevalent temperature was associated with lower odds of bait uptake, with a low p-value associated with this variable's coefficient in the model. The association between the number of recent ORV campaigns and odds of bait uptake was counterintuitive, suggesting lower odds of being positive for bait uptake in samples collected after more than one recent campaign, compared with those collected after one campaign only (in contrast to the relationship apparent in the univariate analysis: Table 3). The p-values associated with this variable's coefficients in the model were high except for samples collected after four recent campaigns. As these third and fourth variables were added to the model, 'singularity'

Table 2
Summary overview of the 'vital statistics' of the 10 ORV campaigns carried out in Kosovo between 2010 and 2015.

ORV round	distribution dates (duration in days)	n Baits distributed	vaccine batch	flight line (m) ¹	Bait density ²	Ave T ^o C ³	Vacc. Titre ⁴	n fox sampled (% hunted)	Days after ORV-samples obtained ⁵					
									Min	1 st Q	Med	3 rd Q	Max	
01 spring 2010	27/05/10 to 29/05/10	234,600	1017 & 1117	1000	25.5	24		40	(98 %)	86	120	134	142	158
02 autumn 2010	26/10/10 to 04/11/10	362,000	1017 & 1117	1000	39.3	15	5.6 & 5.4	38	(100 %)	2	47	57	94	172
03 spring 2011	27/04/11 to 02/05/11	300,000	4218	1000	32.6	15	7.2	94	(97 %)	3	47	120	159	171
04 autumn 2011	17/10/11 to 20/10/11	300,000	5617 & 5618	1000	32.6	17	?? & 6.8	157	(100 %)	1	16	39	53	100
05 autumn 2012	13/11/12 to 19/11/12	250,000	2219	500	27.2		??	2	(100 %)	27	111	133	149	159
06 spring 2013	06/05/13 to 10/05/13	250,000	3220	500	27.2	18	6.9	174	(82 %)	3	53	95.5	119	150
07 autumn 2013	18/10/13 to 21/10/13	250,000	8120	500	27.2	18	8.1	168	(89 %)	23	102	134	167	186
08 spring 2014	07/04/14 to 11/04/14	250,000	0721 & 0821	500	27.2	9	7.9 & 8.2	56	(59 %)	9	33	74	111	137
09 autumn 2014	15/10/14 to 22/10/14	250,000	3221	500	27.2	13	7.6	223	(93 %)	20	34	47	55	61
10 autumn 2015	20/10/15 to 28/10/15	250,000	5421	500	27.2	15	7.1	61	(0%) (98 %)	211 20	242 35	250 47	327 55	362 61
total								1022	(90 %)	1	45	90	127	362

1) General flight line separation (m) across the whole baited area. Flight lines could be different in some areas (e.g. border belt) as described in the text.

2) Average bait density (per km²) calculated as number of baits distributed / 9200 km² baited area (Kosovo area: 10,908 km² / baited area: 9200 km²).

3) Average environmental daytime temperature based on comments recorded in campaign reports.

4) Titre of vaccine (log 10 TID50/mL) as independently assessed by FLI. Note the titres for batches 1017 & 1117 which were distributed in ORV01 and ORV02 were obtained at the time of ORV02 distribution. '??' denotes 'not tested'.

5) Minimum, first quartile, median, 3rd quartile and maximum number of days between the end of the preceding ORV distribution and sample collection.

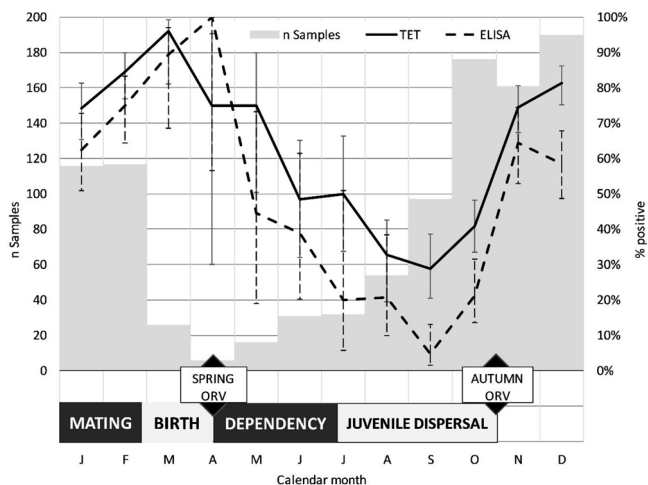


Fig. 2. Composite chart using data from all ORV campaigns aggregated by calendar month of collection. The grey bars show total number of samples (left axis) while the line plots show % bait uptake (TET) and % seropositive (ELISA) for each calendar month (right axis). The error bars are the 95 % confidence intervals. Approximate timing of biannual ORVs are shown along the horizontal axis with a representation of key periods in the red fox reproduction cycle. The timing of key periods in the red fox reproduction cycle is based on information from Vos (2003).

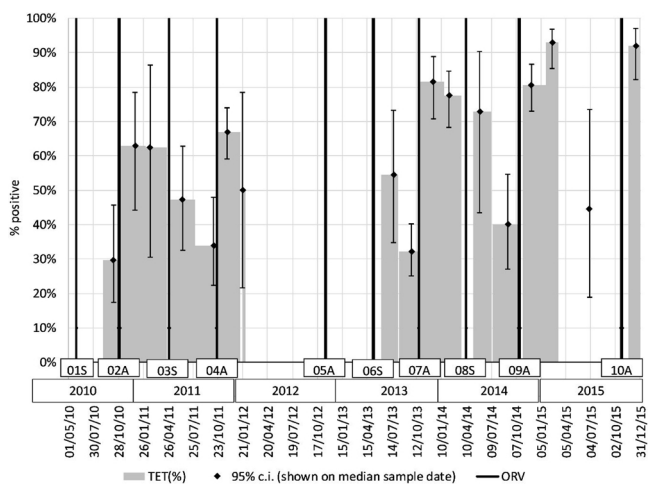


Fig. 3. The % bait uptake (TET positive) for foxes sampled in 90 day quarters following each individual campaign (except the autumn 2012 ORV, after which there were only 2 fox samples taken).

warnings were triggered, indicating that the data were too unbalanced to allow robust estimation of the model parameters. A likely explanation is that these variables are covariant with other factors. Therefore, the most robust model is the model incorporating ‘quarter’ and ‘project phase’. Table 4 shows the parameter estimates for this model.

The coefficients in the model were used to calculate the odds of bait uptake for different scenarios (quarter when sample collected, project phase). The odds was converted to probability (expressed as a decimal number between 0 and 1), which is easier to interpret, using the equation: $probability = odds / (1 + odds)$.

Fig. 5 shows the ‘expected’ bait uptake (as a proportion) for samples collected in different quarters after ORV campaigns and during each project phase. The result is consistent with the initial descriptive overview presented in Figs. 2 and 3, with expected bait uptake being high through winter and declining through spring and summer, and also with around an additional 15 % bait uptake in the second project phase, compared with the first.

Based on a model that also included prevalent temperature (details

not shown) a reduction of 7% (3Q and 4Q) to 11 % (1Q and 2Q) in bait uptake could be expected when environmental temperature at time of ORV distribution was reported as higher than usual.

3.1.2. Seropositivity

Table S3 (Supplementary material) summarises the ‘fit parameters’ (AIC, BIC, deviance) and anova p-value for comparisons of all models examined during the stepwise analysis process. Quarter (time period of sample collection relative to ORVs) was the single most significantly associated factor with odds of being ELISA positive. The next most significant factors, when added to quarter, were the assessed titre of the vaccine used in the preceding ORV (titre) and the number of ORV campaigns in the 18 months preceding sample collection (n ORV 18 m). These two had similar impacts on the fit of the model. As third variables were added to either [quarter + titre] or [quarter + n ORV 18 m] models ‘singularity’ warnings were triggered. The modelling suggested that vaccine with assessed titre over $10^{7.5}$ TID₅₀/mL was associated with higher odds of seropositivity, with a low p-value associated with this variable’s coefficient in the model. The association between the number of recent ORV campaigns and odds of seropositivity was inconsistent, suggesting lower odds of seropositivity in samples collected after two or four recent campaign, compared with those collected after one or three campaigns. The p-values associated with this variable in the model were high except for samples collected after four recent campaigns. It seems likely that the apparent associations with number of recent campaigns are confounded with other covariant factors. The most robust model would contain ‘quarter’ only, but to add information about the possible impact of ‘titre’, the model incorporating ‘quarter’ and ‘titre’ is also illustrated here. Table 5 shows the parameter estimates for these models.

Using the coefficients in the models, seropositivity probabilities were calculated as described for bait uptake. Fig. 6 shows the ‘expected’ proportion seropositive for samples collected in different quarters after ORV campaigns, with vaccines of differently assessed titre (the overall values are calculated from model (1) in Table 5 and the values for different titres from model (2)). On top of the seasonal pattern which follows the pattern in bait uptake, the possible impact of ‘titre’ estimated by model (2) is an additional 20 % seropositive when the vaccine titre had been assessed at over $10^{7.5}$ TID₅₀/mL, with no difference between vaccines of lower titres.

3.1.3. Concordance between bait uptake result and ELISA result

A similar analysis was carried out to estimate the probability that tetracycline positive samples only (i.e. those with evidence of bait uptake) were ELISA positive. Table S4 (Supplementary material) summarises the ‘fit parameters’ (AIC, BIC, deviance) and anova p-value for comparisons of models examined. The assessed titre of the vaccine used in the preceding ORV (titre) was the single most significantly associated factor with odds of tetracycline positive samples being ELISA positive.

The next most significant factor, when added to titre, was the geographic sector where the sample was obtained. At the third step, addition of quarter made a marginally significant reduction in deviance, reduction in AIC but increase in BIC. There was also a problem with singularity in this model. Further additions did not produce any more reliable information.

The modelling suggested that vaccine with assessed titre over $10^{7.5}$ TID₅₀/mL was associated with an increase in probability of seropositivity in tetracycline-positive samples from around 0.5 to 0.8, with a low p-value associated with this variable’s coefficient in the model. The apparent association between sector and seropositivity, suggested a higher probability (around 0.85) for samples obtained in North sector, compared to all other sectors (around 0.55).

4. Discussion

A positive ELISA result, indicating presence of rabies virus-specific

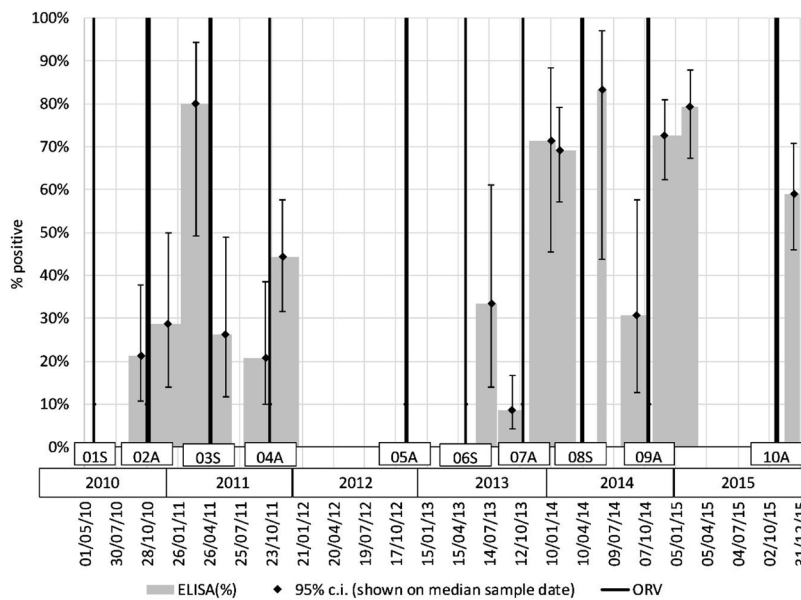


Fig. 4. The % seropositive (ELISA positive) for foxes sampled in 90 day quarters following each individual campaign (except the autumn 2012 ORV, after which there were only 2 fox samples taken). Explanatory notes for Figs. 3 and 4. The horizontal axis is a timeline. The % bait uptake or % seropositive each 90 day quarter period is charted as a bar, the width of which denotes the duration, between earliest and latest collected sample. The error bars are the 95 % confidence intervals, plotted around points denoting the median sample collection dates within each period. The full height black lines denote the date and duration (width) of each ORV campaign, labelled along the horizontal axis. ORV code signifies the sequence (first two digits) followed by 'S' for spring or 'A' for autumn.

antibodies, does not fully equate to effective immunity from infection, however it is a commonly used indicator of protection. For the same blocking ELISA as used to monitor the Kosovo ORV campaigns, an analysis of protection against lethal rabies infection in captive challenge settings found over 80 % probability of survival was obtained at > 40 % inhibition (the cut-off used for a positive ELISA result in the monitoring of ORV in Kosovo) and at > 70 % inhibition over 90 %

probability of survival was attained (Moore et al., 2017). Therefore, the seroprevalence is indicative of the proportion of foxes in the population that have a protective antibody level. A positive tetracycline result is a reliable indicator of bait uptake, detectable as soon as two days after consumption (Hanlon et al., 1989).

The population seroprevalence achieved following ORV campaigns depends on both the proportion of the population that consume vaccine

Table 3
Univariate measures of association between the possible explanatory variables and bait uptake.

Explanatory variables	n fox samples with TET results	Bait uptake (%) (95 % confidence intervals)	
Project phase			
Phase 1	317	54%	Fisher exact
Phase 2	676	69 %	p < 0.0001
Number of baits distributed			
< 300,000	714	67%	Chi sq (2df)
300,000	244	57 %	overall
> 300,000	35	63 %	p = 0.0197
Titre (log 10) of vaccine distributed in the preceding ORV campaign (independent assessment)			
< 6.5	73	45 %	Chi sq (2df)
6.5–7.5	473	53%	overall
> 7.5	447	78%	p < 0.0001
Prevailing daytime temperatures during campaign			
< = 17 °C	619	70 %	Fisher exact
> 17 °C	374	54%	p < 0.0001
Season of campaign preceding sample collection			
Autumn	643	78%	Fisher exact
Spring	350	37%	p < 0.0001
Quarter			
1Q	72	53%	Chi sq (3df)
2Q	278	33 %	overall
3Q	444	77 %	p < 0.0001
4Q	199	82 %	(77 %–87 %)
Sector			
North	129	60%	(51 %–68 %)
West	265	64 %	(58 %–69 %)
Centre	192	57 %	(48 %–64 %)
East	216	69 %	(63 %–75 %)
South	191	68 %	(61 %–74 %)
Hunted or found dead			
found dead	93	54%	Fisher exact
hunted	900	65 %	p = 0.0440
Number of ORV in preceding 18 months (relative to sample collection date)			
1	38	29%	Chi sq (3df)
2	264	52%	overall
3	590	72 %	p < 0.0001
4	101	60%	(51 %–69 %)

Table 4
Multi-level, mixed effects, logistic regression for ‘bait uptake’.

Random effects				
Groups	Name	Variance	Std.Dev.	
ORV campaign	(Intercept)	0.02883	0.1698	
Number of observations: 993. Groups: ORV campaign, 9				
Fixed effects				
	Estimate	Std.-Error	z-value	Pr(> z)
(Intercept)	-0.2082	0.2780	-0.749	0.453863
Quarter 2Q	-0.9023	0.2846	-3.171	0.001519
Quarter 3Q	1.0225	0.2969	3.444	0.000574
Quarter 4Q	1.1616	0.3419	3.398	0.000680
Project phase 2	0.6830	0.2047	3.337	0.000846

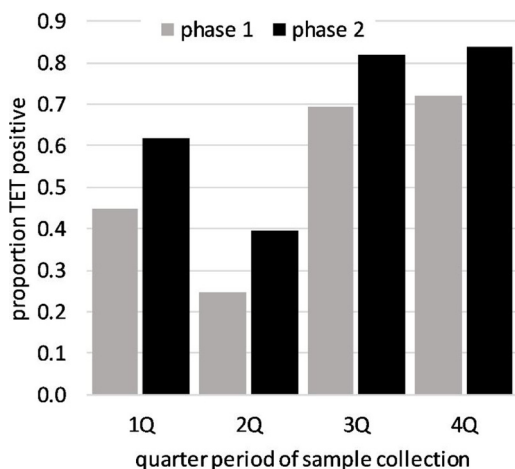


Fig. 5. The ‘expected’ population bait uptake (TET positive proportion) for foxes sampled in 90 day quarters following each ORV campaigns carried out in the two project phases.

Table 5
Multi-level, mixed effects, logistic regression for ‘seropositivity’.

1) Model including ‘quarter’ only				
Random effects				
Groups	Name	Variance	Std.Dev.	
ORV campaign	(Intercept)	0.167	0.4087	
Number of observations: 555. Groups: ORV campaign, 9				
Fixed effects				
	Estimate	Std.-Error	z-value	Pr(> z)
(Intercept)	-0.4438	0.4131	-1.074	0.28271
Quarter 2Q	-1.133	0.4254	-2.664	0.00773
Quarter 3Q	0.6916	0.4768	1.45	0.14696
Quarter 4Q	1.3001	0.515	2.525	0.01159

2) Model including ‘quarter’ + ‘titre’				
Random effects				
Groups	Name	Variance	Std.Dev.	
ORV campaign	(Intercept)	0	0	
Number of observations: 555. Groups: ORV campaign, 9				
Fixed effects				
	Estimate	Std.-Error	z-value	Pr(> z)
(Intercept)	-0.6644	0.4615	-1.44	0.14997
Quarter 2Q	-1.1229	0.4123	-2.724	0.00646
Quarter 3Q	0.6468	0.3747	1.726	0.08429
Quarter 4Q	0.8734	0.4407	1.982	0.04748
Titre > 7.5	0.9437	0.338	2.792	0.00524
Titre 6.5–7.5	-0.0417	0.3312	-0.126	0.89982

bait uptake) and the proportion of those bait consumptions that result in seroconversion (referred to as concordance in this analysis). Seroprevalence could logically be derived as bait uptake multiplied by concordance. These two metrics (uptake and concordance) may be influenced by various factors. Bait uptake could be influenced by: the availability of baits in the environment, which depends on bait density and distribution (flight line separation); feeding behaviour of foxes,

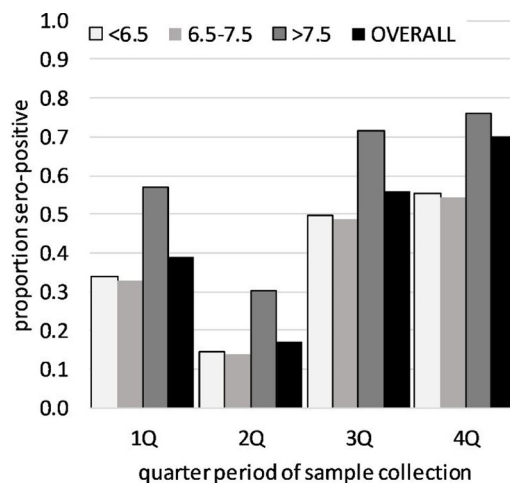


Fig. 6. The ‘expected’ population seroprevalence (ELISA positive proportion) for foxes sampled in 90 day quarters following each ORV campaigns carried out with vaccine assessed to have different titres.

which may be affected by seasonal life cycle factors such as competition and dominance, and; physical effects on the bait in the environment (e.g. high temperatures causing melting, or repeated freeze-thaw cycles). Concordance could be influenced by: quality and preservation of the vaccine (original titre and cold chain up to point/time of distribution); factors affecting vaccine stability in the environment (e.g. high temperatures), and; the frequency of partial bait consumption, where a fox may lick the carrier off the bait without breaking the capsule containing the vaccine, or swallow the capsule whole, so not exposing the lymphoid tissue in the oropharynx to the vaccine (Maki et al., 2017). There could also be a cumulative effect, on either or both uptake and concordance, of repeated exposure to sequential campaigns during the life of a fox.

Data available from samples obtained to monitor the Kosovo ORV campaigns showed seasonal patterns in the proportion of sampled foxes that were positive for bait uptake and in the seroprevalence. The data also showed a significant difference between campaigns carried out in the first and second phases of the technical support project. Among foxes sampled from mid-July to mid-October following a spring campaign, the proportion positive for tetracycline marker was below 30 % (first project phase) and below 40 % (second project phase), compared with around 70 % (first project phase) and 80 % (second project phase) of foxes sampled in the periods between autumn and the following spring campaigns. As could be expected, the percent of samples that were seropositive followed a similar seasonal pattern to bait uptake.

The multilevel models also suggested that reduced bait uptake could have been associated with higher environmental temperature at the time of the ORV distribution. Theoretically this could be explained by the possible effect of raised temperature on the integrity of the baits (due to melting). However, the information regarding environmental temperature was derived from comments recorded in the campaign completion records, rather than site- and date-specific meteorological data, which was not obtainable. In addition, the temperature variable was covariant with other factors making robust estimation of the model parameters problematic. Therefore, this finding on the possible impact of temperature should be treated cautiously.

The analyses have shown that bait uptake tended to be lower in the first project phase than in the second phase. The only difference between project phases that is accounted for in the available data is that the flight line separations were 1000 m in the first phase (with some modifications on border areas) and 500 m in the second phase. Because all the ORV with 500 m were in the second project phase and all with 1000 m were in the first phase, it is impossible to isolate the possible effects of flight line separation from any other unrecorded factors

associated with changes in practice between the project phases, including variation in numbers of baits distributed in each campaign. Linhart (1993) suggests that an ORV requires bait density that is 10–15 times the density of foxes. Fox density was estimated using camera surveys during the ORV programme in Kosovo and reported by Beatham et al. (2016) at 1.03 per km² (range 0.58–1.55), which is above the threshold suggested for maintenance of a rabies epidemic, 0.25 to 0.63 individuals per km² (Holmala and Kauhala, 2006). Bait densities in Kosovo ORVs were always above 25 baits/km² in all campaigns, which is greater than 15 times the estimated fox density. Practically, flight line separation could have had an effect on vaccine uptake because closer flight lines would result in more even distribution of a fixed number of baits. The relationship between the spatial arrangement of fox home-ranges and ORV flight-line separation has been highlighted as a key factor to consider in bait distribution strategy (Thulke et al., 2001). According to radio-tracking studies in Western Europe (Artois et al., 1990) the smallest home range of the red fox is 77 ha, which equates to a circle with diameter 900 m or a square a little less than 900 m each side. If vaccines are dropped along parallel lines separated by 500 m all such fox home ranges should be baited, but if the lines were 1,000 m apart some fox home ranges could fall entirely between bait lines and receive no bait at all.

Blancou et al. (1986) recommended that when delivering rabies vaccine to the red fox using a bait, the vaccine should be approximately 10 times more concentrated to obtain the same level of protection as the same vaccine given by direct oral instillation. The advertised content of the vaccine used throughout the ORV campaigns in Kosovo was from minimum 1.8×10^6 TCID₅₀ to maximum 1.8×10^8 TCID₅₀ (Lysvulpen <https://www.bioveta.eu/en/products/veterinary-products/lysvulpen-por-ad-us-vet-1.html>) but each batch of vaccine used was also independently assessed at the time of each campaign. There was some evidence in the analysis of Kosovo ORV data that use of vaccine batches with a titre over $10^{7.5}$ TID₅₀/mL could have been associated with higher seropositivity following those campaigns. Although, the titre variable was covariant with other factors, in a further analysis using only tetracycline positive samples, titre was strongly associated with concordance between tetracycline and serological positivity, providing evidence for a real effect of vaccine titre on probability of seroconversion. There was also an apparent association between the geographic sector where a sample was obtained and seropositivity of tetracycline positive samples (concordance), suggesting a higher probability for samples obtained in the North sector compared to all other sectors. There is no straightforward explanation for this apparent effect, although it could be speculated that higher altitudes with cooler temperatures in the North could be more favourable to vaccine stability when the baits are distributed.

The main, and robust, finding of the multilevel, mixed effect analyses was of a strong seasonal pattern in bait uptake and seropositivity. Both parameters were high through winter, following the autumn campaigns, and declined through spring and summer, with a low point in samples collected in the quarter year preceding the autumn campaigns. Concordance between tetracycline and serological positivity did not show a clear seasonal pattern. Therefore variation in concordance (partly influenced by random individual campaign factors and vaccine titre) is superimposed on the seasonally changing proportion of bait uptake positives in the population, producing a seasonal pattern in seroprevalence with some additional variation due to the different campaign-related factors.

The observed seasonal patterns are unlikely to be because foxes are less likely to take the bait during the ORV distribution campaign in spring, but because the population that is sampled in the months following the spring campaigns includes increasing numbers of 'new' juveniles that could not have been exposed to the ORV and would have lost any possible maternal immunity by late summer (Hostnik et al., 2003; Maki et al., 2017). According to Larivière and Pasitschniak-Arts (1996), turnover in a wild fox population is considerable: in the wild,

foxes rarely live more than two years and in areas where lethal fox control is applied (hunting), up to 80 % of a fox population is less than one year old.

Vos (2003) presented a detailed description of the ecology of the red fox in Europe, and explained how this is associated with the epidemiology of sylvatic rabies epidemics and the impact of ORV campaigns. Vos recommends that spring campaigns need not be done, because they do not vaccinate new foxes and also because the high risk time of year for rabies transmission is autumn/winter (based on fox behavioural ecology). Vos asserts that: "*The main target population of oral vaccination must be the animals that are actively involved in territory defence. During the mating season, practically the whole population is at risk of becoming infected. Thus, the optimal timing of oral vaccination campaigns is late autumn and/or early winter...*".

The experience from Kosovo, as reported here and previously (Yakobson et al., 2014), is that even with two campaigns per year, population seroprevalence dips once the emergent young foxes disperse in the population in summer. The usual timing of spring campaigns (as recommended by the EU, 2002 and 2015) is too early for the current year's crop of young foxes to take the baits. Conducting an ORV campaign later in the year to try to vaccinate the young foxes would be hampered by higher environmental temperatures in the Balkan region, which would likely have a negative effect on the efficacy of the vaccination campaign.

The data analysed here could not necessarily support the notion that spring campaigns have no significant impact. The only data available related to consecutive annual autumn campaigns with no intervening spring campaign are for the two autumn campaigns of 2014 and 2015. The bait uptake measured in the population after the autumn 2015 campaign (after a 'missed' spring campaign) was the highest of any campaign, but seroprevalence was lower than associated with the autumn 2014 campaign. That this lower seroprevalence could in any way be caused by missing a campaign in the preceding spring cannot be ruled out, but a contributory factor could have been that the independently measured titre for the vaccine distributed in 2015 was lower than in the previous three campaigns (Table 2). Fox cubs born to vaccinated vixens have been shown to have maternal antibodies for up to two and a half months after birth (Hostnik et al., 2003; Maki et al., 2017). It could therefore be argued that the spring campaigns may provide a 'boost' for pregnant vixens that would provide maternal immunity for the cubs, although the usual timing of spring campaigns falls towards the end of the fox birthing season.

The main finding of high levels of bait uptake and seroprevalence through winter, following the autumn campaigns, declining through summer despite the implementation of spring campaigns, supports the recommendation by Vos (2003) that countries lacking financial resources to support biannual campaigns should focus resources on once per year vaccination in late autumn or early winter. A further implication of the described fall off in seroprevalence each year, thought to be associated with rapid turnover of the fox population, is that after cessation of rabies vaccination programmes the fox population is likely to rapidly become naïve again. This means that unless rabies has been eradicated in wildlife of the region it could potentially re-establish rapidly. This in turn underlines the value of strongly coordinated regional programmes and good surveillance after cessation of vaccination.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.prevetmed.2020.105050>.

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Plan of infirmary care at patients with Angina Pectoris in the regional hospital in Peja

Pelaj B¹

¹The University of Prishtina, Faculty of Medicine, Prishtina, Kosovo

Abstract:

Angina is a pain the chest or a problem which occurs when the heart muscle is not sufficiently supplied with blood rich with oxygen. It can feel as a pressure or strain in the chest. The problem can also occur in the shoulders, asms neck, jaws and back. It is a symptom of the heart desease and it is usually a symptom of the heart coronary desease.

The main aim is the search of the infirmary tretmant and care for patients with angina Pectoris in the Regjional Hospital in Peja.Objectives: the information of patients during their stay in the Hospital regording his condicion. Education of patients regarding angina pectoris.The research of nurses knowledge about anginapectoris. The research of the engagement level of the nurses of the Coronary Units regarding the patients education during their stay in te Hospital. Analysis of data and proposal of dezereptive measures for the improvement of patient education about angina pectoris.

Methodology of work: For accomplishing this research the Hospital of Peja was chosen, the Coronary Unit. The research was carried out throught a semi- structured questionnaire, the approach was cuantitative.

The aimed group is Nurses. 16 questionnaires were given out designed for the nurses of the intern word and e Coronary Unid of Peja Hospital.

Collecting the data: the data collection was done with a semi- structured questionare. The questionare has\contains twenty for questions desinged to search this problem.

Data analysis: The gained data are grouped according to the questions and a statistical processing has been done by the programme for computing of statistical parameters of age: average age gemeder and the average time of stay in the hospital. Data presentation was done throught charts. The drawn conclusions will contribute in the proposol of measures for increasing of the staf nurse awareness in the management of infirmary care for patients with Angina Pectoris.

Keywords: Angina Pectoris, Regjional Hospital in Peja, Coronary Unit.

Angina is a pain the chest or a problem which occurs when the heart muscle is not sufficiently supplied with blood rich with oxygen. It can feel as a pressure or strain in the chest. The problem can also occur in the shoulders, asms neck, jaws and back. It is a symptom of the heart desease and it is usually a symptom of the heart coronary desease. There are many kinds of angina including the coronary acute syndrome, angina pectoris, chest pain, coronary artery spasm, microvascular angina, prinzmetol angina, inverse angina, stable angina, and the usual, nonstoble angina with variations. Angina occurs when the heart muscle does not reccieve the sufficient amount of blood to function normally. This usually happens When one or more coronary arteries are either narrowed or blocket which is otherwise called ischemia. The main risk factor fort heart desease dangers and coronary desease dangers SKM are:

Unhealthy levels of cholesterol, the high blood pressure, smoking, diabetes, overweight or obesity, metabolic syndrome, inactivity, unhealthy diets, old age (the danger increases for males after the age of 45 and for females after the age of 55), the family history with e arly heart deseases.

Plans of infirmary care

Nurse valuation: Gathering of information about the patient's symptoms and activities, the valuation of the factors for the dangers of coronary arteries deseases, then the patient's responses to angina, understanding of diagnosis by the patient and his family and keeping to the actual plan of treatment.[2]

Infermary diagnosis: the ineffective perfusion of Cardiac Tissues after SAK which is accompanied with pains in the chest and other prodromale symptoms, anxiety, tribulation, pain, gasping, change in vital signs etc. The lack of know ledge about the existing desease and about methods of evereoning ony complications.

The importance of a team work a Doctor- Nurse: Given that patients are of a different origin, age and different intellectual level, therefore nurses are required a great individual and team work for managing cases with angina. The mananging of the nurse care at patient's with angina pectoris can by only realizetd with on inter- colleague and inter- sectorial cooperation, making it easier for the patient who is threatened by potential complications during his stay in the hospital.

A nurse is always near the patient, does a careful observation (defecting the urgent symptoms and signs) and informs the doctor, in order that all necessary preventive measures be taken. She monitors all vital functions. All dependent and independent nursing activities are documentet on the right nurse lists.

Planning and aims: Aims include immediate treatment, reducing the pain, prevention of IAM, reducing of anxiety, awareness of the process of the illness, education about the illness in the self-care programme and prevention of complications.

Nursing priorities: During the nursing care there are also some priority activities or some activities of a more urgent nature, and they are:

1. Easing \ controlling the pain.
2. Prevention \ minimization of complications of Myocardium.
3. Providing the information regarding the process of illness \ prognosis and treatment.
4. Supporting the patients \ VS for starting the necessary changes in their lifestyle and behaviours.

Infrimary interventions

Treatment of Angina: Immediate actions be taken if the patient reports pains in the chest or when prodormale symptoms of a person indicate Anginale ischemia.

The patient is instructed to cease all the actevities and is advised to sit or he in bed in Folwer position. Vital sings are measured and the patient in observed if he has a breathing disorder, nitroglycerine is applied throught sublingual way and the patient's reaction is to be valuated (this can be repeated up to three doses). In case the patient's respiratory rate increases or in cose the level on oxygen saturation decreases, then on oxygen therapy must be applied.

If the pain is bigger and if it continues for more than 30 minutes even offer these applications, then the patient is valuatet for IAM and can be combe transferred to a unit for a more specific infrimary care.

Infrimary advice and the education of patients with angina pectoris: Patinent's are taught to recognize their symptoms, how to report on the changre of quality and intensity of symptoms during the stay in the hospital. They are also advised how and when to ask for help in case of reaccuring of symptoms fter their discharge from the hospital. This can imply the programme for quitting smoking, consultations about specific diets, the control of their body weight, the recommendations for a progressive increase of physical activities, participations in patient clubs etc.

The respect of time and protocol during the stuggle for reducing of possible compliations from counterindications of cardiovascular system, prevention of complications and their recogriton, as wellas the ability of action should be vived as team characteristics.

Having in mind the long term importance of patient education regarding the coronary disease, we have oriented ourselves to the research of informing nurses about the education of patients in the Coronary Units. Patients admitted with angina pectoris are vulnerable and frightened for the future. A good nurse must compile a consultative programme: The programme for consulting patients with angina is designed in that way that the patient and his family understand the disease, and identify the symptoms of myocardium ischemia. They are informed about the methods for prevention of chest pains and advancement of SAK are discussed.

In the self-care programme we must cooperate with the patient, his family and friends

Activities for minimization of angina episodes must be planned.

The patient must be advised that each pain that occurs after the application of these methods including the application of nitroglycerine must necessarily be treated at the nearest emergency centre.

Supplementary information with which patients must be advised on their discharge from hospital care and the plan of nursing care they must follow

Some of the information: They must be informed about the disease. What must be expected at home, activity, diet and life style.

The diet is by all means an inseparable part of the programme of care and the treatment of patients with Angina Pectoris and some of the instructions that the patients must be given to are: to reduce (restrict) the amount of alcohol they consume. To be advised not to smoke. They are supposed not to let anyone smoke in their homes. To instruct them to read and learn more about what they should and to have a healthier heart and healthier blood vessels. To avoid salty and fatty meals. You can direct them to a nutritionist who would help them plan healthy diet. Patients are advised about the best ways to avoid stressful situations. To also advise them about sexual activity. Patients must be aware and ask for professional medical help at the moment they are faced with: pain, pressure, strain, or squeezing in the chest, arms neck, or jaws, problems in breathing, hand numbness, perspiration or change in skin colours.

The main aim is the search of the inpatient treatment and care for patients with angina Pectoris in the Regional Hospital in Peja.

Objectives: the information of patients during their stay in the Hospital regarding his condition. Education of patients regarding angina pectoris.

The research of nurses knowledge about angina pectoris. The research of the engagement level of the nurses of the Coronary Units regarding the patients education during their stay in the Hospital. Analysis of data and proposal of derivative measures for the improvement of patient education about angina pectoris.

Methodology of work: For accomplishing this research the Hospital of Peja was chosen, the Coronary Unit. The research was carried out through a semi-structured questionnaire, the approach was quantitative.

The aimed group is Nurses. 16 questionnaires were given out designed for the nurses of the inpatient ward and the Coronary Unit of Peja Hospital.

Collecting the data: the data collection was done with a semi-structured questionnaire. The questionnaire has contains twenty for questions designed to search this problem.

Data analysis: The gained data are grouped according to the questions and a statistical processing has been done by the programme for computing of statistical parameters of age: average age gender and the average time of stay in the hospital. Data presentation was done through charts. The drawn conclusions will contribute in the proposal of measures for increasing of the staff nurse awareness in the management of inpatient care for patients with Angina Pectoris.

Results: In the course of their work preparation I have collected data regarding the number of patients hospitalized in the Coronary Unit of the Regional Hospital for the years 2014 and 2015.

Statistics for 2014		Statistics for 2015	
Patients with Angina Pectoris	54	68	
Male	34	40	
Female	20	28	
Average age	60	60	
Average stay in hospital	6 days	7 days	

The summary and analysis of responses given by nurses respondents of the questionnaire

Management of Infermary care in admission of patients in Hospital.

Nr	Question for Nurses	PO	%	JO	%
1.	Do you ask a patients about the time of his first symptoms?	10	62.5	6	37.5
2.	Do you log the time of patients admission?	12	75	4	25
3.	Do you log patient's symptoms an admission and during hospitalization?	7	45	9	55
4.	Do you log patient's vital signs on admission and during hospitalization?	16	100	0	0
5.	Do you ask patient's about his\her former illness?	10	62.5	6	37.5
6.	Do you ask patients about the factors of danger of the coronary disease?	4	25	12	75

Perception if nurses regarding the information and education of the patients in the coronary unit

Nr	Question for nurses	yes	%	No	%
7.	Do you think that the patient during his\ her stay in the Coronary Unit gets sufficient information about his\her illness?	8	50	8	50
8.	Do you think that the patients during his\ her stau in the Coronary Unit is sufficiently provided with the information about the nodification of danger factors ?	5	31.25	11	68.75
9.	Do you think that during his\ herstary in the Coronary Unit the Patient gets sufficient information about medications he\she is going to use ?	13	81	3	19
10.	Do you think that during his\ herstary in the Coronary Unit the Patients gets information for his everyday activities after discharging the hospital and about possible restrictions ?	9	67	7	43
11.	Do you think that during his\ herstary in the Coronary Unit the Patient gets sufficient information about diagnostinc examinations he Is submitted or that he night be submitted ?	10	62.5	6	37.5
12.	Do you think that during his\ herstary in the Coronary Unit the Patient gets sufficient information about the passibility of the care of the coronare disease ?	8	50	8	50

Questions for nurses and patients regarding the knowledge about the factors of danger (16 nurses and 58 patients).

Nr	Question	Nurse			Patient		
1	Who must give the information to patients about their illness	Answer response	Nr	%	Response	Nr	%
		Family member	3	20	Family member	5	8
		The Nurse	2	13	The Nurse	10	17
		The Doctor	10	77	The Doctor	43	75
		The Patient himself must be interested	0	0	The Patient himself must be interested	0	0
Nr	Question	Nurse			Patient		
2	General level of cholesterol in blood after the treatment from Angina Pectoris should be?	Response	Nr	%	Response	Nr	%
		< 4.2mmol/l	9	68	< 4.2mmol/l	12	21
		< 5.2mmol/l	3	19	< 5.2mmol/l	15	26
		< 6.2mmol/l	2	13	< 6.2mmol/l	6	11
		I don't have information	3		I don't have information	25	44
Nr	Question	Nurse			Patient		
3	What is good cholesterol?	Pergjegje	Nr	%	Pergjegje	Nr	%
		LDL	0	0	LDL	0	0
		HDL	2	12	HDL	0	0
		VLDL	13	82	VLDL	10	18
		I don't have information	1	6	I don't have information	48	82
Nr	Question	Nurse			Patient		
4	Which value of LDL cholesterol is preferred after Angina Pectoris?	Pergjegje	Nr	%	Pergjegje	Nr	%
		< 1.6mmol/l	2	13	< 1.6mmol/l	2	4
		< 2.6mmol/l	6	40	< 2.6mmol/l	4	7
		< 3.6mmol/l	8	50	< 3.6mmol/l	5	9
		I don't have information	0	0	I don't have information	47	80

Questions for Nurses and patients, regarding their knowledge about the factors of danger and the usual medications which are used after the infarct of myocardium

Nr	Question	Nurse			Patient		
5	The patient, after myocardial infarction should receive aspirin	Answer	Nr	%	Answer	Nr	%
		A year	1	7	A year	10	18
		Five years	3	19	Five years	5	9
		One month	0	0	One month	0	0
		All life	12	75	All life	38	64
		I have no information	0	0	I have no information	5	9
Nr	Question	Nurse			Patient		
6	Whenever nitrates can be applied during the	ANSWER	Nr	%	Answer	Nr	%

	day?	One time	0	0	One time	0	0
		Three times	4	25	Three times	12	21
		More than three times	11	75	More than three times	24	42
		I do not know	0	0	I do not know	22	78
Nr	Question	Nurse			Patient		
7	What are factorit coronary disease risk that can not be modified?	ANSWER	Nr	%	ANSWER	Nr	%
		Legacy	16	100	Legacy	34	59
		Gandr	10	63	Gander	16	28
		Age	11	65	Age	10	18
		I do not know	0	0	I do not know	24	42
Nr	Question	Nurse			Patient		
NR8	Which are factors of danger of the coronary disease that can be modified?	Answer	Nr	%	Answer	Nr	%
		Nurse			Patient		
		smoking	16	100	smoking	0	
		Hypertension	8	50	Hypertension	10	18
		Total of cholesterol	8	50	Total of cholesterol	5	9
		Low HDL	7	43	Low HDL	0	
		Obesity	8	50	Obesity	2	4
		Physical inactivity	13	73	Physical inactivity	10	18
		Diabetes	2	13	Diabetes	0	0
		I do not know	0	0	I do not know	31	51

Questions for Nurses and patients regarding the know ledge worth discussing during the education about the Coronary Desease.

Nr	Question	Nurse			Patient		
		Answer	Nr	%	Answer	Nr	%
9	Is physical activity after IAM forbidden?	In the first six weeks	1	7	In the first six weeks	5	6
		In the first week	2	13	In the first week	11	21
		In the first month	1	7	In the first month	8	14
		On the first day	12	73	On the first day	3	5
		I do not know	0	0	I do not know	31	54
Nr	Question	Nurse			Patient		
10	Which medicaments are to do with the secondary prevenrion?	Answer	Nr	%	Answer	Nr	%
		Nyroglycerine	0	0	Nyroglycerine	0	0
		Statins	4	25	Statins	12	21
		Diuretics	12	75	Diuretics	24	42
	I do not know	0	0	I do not know	22	37	
Nr	Question	Nurse			Patient		
11	Should the patient be vaccinated against e	Answer	Nr	%	Answer	Nr	%
		By no means	0	0	By no means	3	6

	seasonal infuelza after the infarct of myocardium?	Not important	0	0	Not important	4	7
		At will	9	60	At will	7	12
		Yes	7	40	Yes	16	28
		I do not know	0	0	I do not know	27	47
Nr	Question	Nurse			Patient		
		Answer	Nr	%	Answer	Nr	%
12	I s sexual activity allowed after Angina Pectoris?	Yes after six weeks	14	93	Yes after six weeks	11	18
		Yes after six month	2	7	Yes after six month	2	4
		Yes after a yers	0	0	Yes after a yers	0	0
		I do not know	0	0	I do not know	45	78

Discussion

INFIRMARY was not legally recognized as a separate and autonomas pfeession as are recognized medicine, stomatology, and pharmacy. The role of nurses was to assist doctors and implement their instructions. Problems with which were facet reflect on this research carried out aiming the nurse care for patients with Angina Pectoris.

The education of the citizens about the desease is a primary obligation of public of health workers. Profilized Nurses, as is the case with those working in the Coronary Unit, are obliged to inform patients about the illness, about the way how to care it and prevent complications, respectively are obliged to educate patients. To achieve this, nurses must always be in the process of professional education, which would enable security and accuracy in the information transmitted to patients.

Though the questionnaire a reflection has been drawn of the know ledge of nurses about the management of care cases focusing on education of patients when it cones to the illness, which is their professional concern. Therefore, there is a need for continuous, education and a need for a specialized nurse for education of patients, as well as the nurses who work in the Coronary Unit.

Educational programmes improve the care reduce rehospitalization and increase the quality of like and the functional condition of patients with Coronary deseases. The programmes effect survival and prevent reoccurring of Angina Pectoris and other illnesses [6]

Information of nurses about the danger factors is also. Other side it is clear that the infirmary care must include the evidenced education apart from interventions. This gives us the right to think that the nurse does not take an active role in diagnosing and in e patients therapy with Angina Pectoris.

The aim of the cardiac rehabilitation is to inprove the physical and psychical capacity of the patient stabilizing, stopping or reverzing the progress of a coronary desease. To achieve this the patient must clarify the questions about the desease. There must not be any doubts about physical activity and its importance. It is prudent that the nurse helps the patient to plan his physical activity. Consultations about diet are obligatory. The education and support for modification of the danger factors are also necessary (tobacco, arterial hypertension, diabetes, high cholesterol, obesity insufficient physical activity) [7, 8]

Education means also the information about medicaments, the way to take them, their benefits and possible ill-efeccts. [17]

It can be concluded that the results of this stady arouse some important issues and first of all an insufficient education for patients hospitalized regarding Angina Pectoris. At the some time, a current need in highlighted about the necessity of the existence of the protocol which wold include the

education of patients during this vulnerable period of their fight against the illness. Pointing out lack of an infirmiry documentation about nurses activites as also present in the research where infirmiry advice given to patients has not been logged. This is a result of the lack of graduate professionals at Infirmiry Faculties in the Regional Hospital in Peja because of not existing of a competition (advertisement) for graduate nurses.

Conclusion

According to the abovepointed data at the results of the research we can conclude that: From the collected data at the Coronary Unit of Peja Hospital emerges that most patients with Angina Pectoris are males. The average age of patients with Angina Pectoris was 61 which is complete conformity with the epidemiology of coronary disease. It is noticed that the patients are not given sufficient information about the illness and about the modification of the danger factors and that they do not have this necessary information during the stay in the Coronary Unit. This is a result of the lack of graduate professionals with a University degree at Peja Hospital.

It is proved a low level of necessary knowledge regarding the coronary disease, regarding the danger factors of this disease and regarding the activites that must be taken to fight the disease.

During their stay in the hospital, patients are not provided with sufficient information about the element of secondary prevention of the coronary disease, as a more effective and a cheaper way for preventing possible complications, controlling the disease, lowering mortality and lowering disability.

Proposal of measures

If there is not anadequat Infirmiry management in managing of cases and if there is no health aducation of patients by nurses, then an action plan be developed for improvement and that plan must contain issues suchas: the education of infirmiry stoffin managing of the infirmiry care in Coronary Units; the increase of intellectual level of the infirmiry staff with people with the Faculty of Infirmiry; the increase of knowledge of nurses about the coronary disease and the necessity of patient education; a general engagement of the management staff in all levels; the continuous training of health workers for a better care for patients; it is important to increase hygienic standarts in public health institutions; to increase the awarenness in a better communication Doctor-Nurse-patient; the ethical code of civility to be part of health workers; to accomplish an increase in technological capacities, the training of health staff of all levels and their continuous education; a general engagement of the management in advancing the infirmiry staff on all levels; Patients satisfaction with health service to be an important indicator that reflects the results of the staff and the Hospital; the creation of protocols for patient compensation or their relatives (family member) in cases of problems or anybconsequences caused by professional carelessness of Hospital staff; to create recreative spaces within the hospital for patients with a longer stay in the hospital; to enrich the patients meny with dietary food according to the illness natyre of the patients; the insistence to document the activities and aperiodical valuation of all the activites done by nurses; appointment of a specialized nurse for education within the hospital; there is a need to found clubs for patients after the period with Angina Pectoris; and the protocol for education.

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Smoking in the families of students and among students in the student center in Pristina, and damages caused by smoking

Pelaj B

¹The University of Prishtina, Faculty of Medicine, Prishtina, Kosovo

Abstract:

Tobacco is a plant of the genus *Nicotiana* of different types, modified or unmodified genetically, with all its parts. What is worrying that smoking harms not only the person who consumes, but also a person who is near him. The aim of this research is data collection on the number of students and their family members who smoke and negative impacts caused by smoking on health as well as in their budget.

The research is based on the use of data from the survey consisting of 300 surveyed students. 150 females and 150 males, the total number of members of their families is 1843 people.

Smoking is fastening to persons who consume without thinking at all the consequences that come from smoking jointly with death. This negative phenomenon (smoking) is responsible for the so-called "epidemic" of lung cancer and other diseases associated.

Key words: Smoking, damage caused by smoking, students, families.

Introduction

Tobacco is a plant, a part of the genus *Nicotiana* and of the *Solanaceae* (nightshade) family, geographic origin is South America, while in our countries has reached from Turkey. [1]

While burning tobacco releases 4000 materials from nicotine and carbon dioxide up to tars, substances with benzopyrene compound, radioactive cobalt that are among the main causes of lung cancer, especially in men. [1,2]

One cigarette lifts 5.5 minutes life, but this causes damage to the human body, but is a paucity of those tobacco smokers that this really can not accept, and to suspend the consumption of you, it is because of the psychological addiction to smoking as and due to the form of smoke reflex smokers. [2,3]

One cigarette consists of: paper, filter and some additional material, Cigars smoke contains 4000 chemicals of which 2000 are poisons. [1.3].

Smoking causes chronic disease, impaired cardiovascular, cerebrovascular, pulmonary emphysema, as well as various cancers and narrowing of blood vessels, etc. [4]

According to Law No. 9636 dated. 06. 11. 2006. "For the protection of health from tobacco products", quitting in work environments, health institutions.

In Kosovo was adopted anti-smoking law in local institutions and in March 2013.

The aim of this study

The aim of this study is to collect data on the number of students in dormitories who smoke and members of their families also consume tobacco. Tobacco appearance as negative factor in the human body for the appearance of many diseases.

Material and methods

This is a descriptive study conducted in the Student Center in Prishtina (student dormitories). Research is based on a survey consisting of 12 questions which interviewed 300 students at their center 150 females and 150 males and total number of their members, 1843, during the period October 2015 to February 2016.

The data gathered through questionnaires completed by students. Material is taken from the literature, brochures obtained from the National Institute of Public Health, Pharmaceutical Society of Kosovo and the Internet.

Results

In the Student Center statistics show a large percentage of smoking.

Table.1. Presentation of the number of dormitories, the respondents, the number and percentage of those who consume tobacco

<i>Number of dormitories</i>	<i>The number of respondents</i>	No. of people who smoke	The percentage of smokers
Dormitory No. 1	60	9	15%
Dormitory No. 2	60	12	20%
Dormitory No. 3	60	27	45%
Dormitory No. 4	60	8	13%
Dormitory No. 5	60	15	25%
In total	300	71	100%

According table shows that have 300 respondents where 71 of them are active smokers. Of these 300 respondents 150 were women and 150 men in the dormitory No. 1 and 2 are women surveyed in dormitory NR 3 and 5 are male respondents and respondents in dormitory No. 4 are 30 females and 30 males.

Table. 2. Number and percentage of women and men who consume tobacco

Gender	<i>The number of respondents</i>	No. of people who smoke	The percentage of non smokers
females	150	24	16%
males	150	47	31.33%

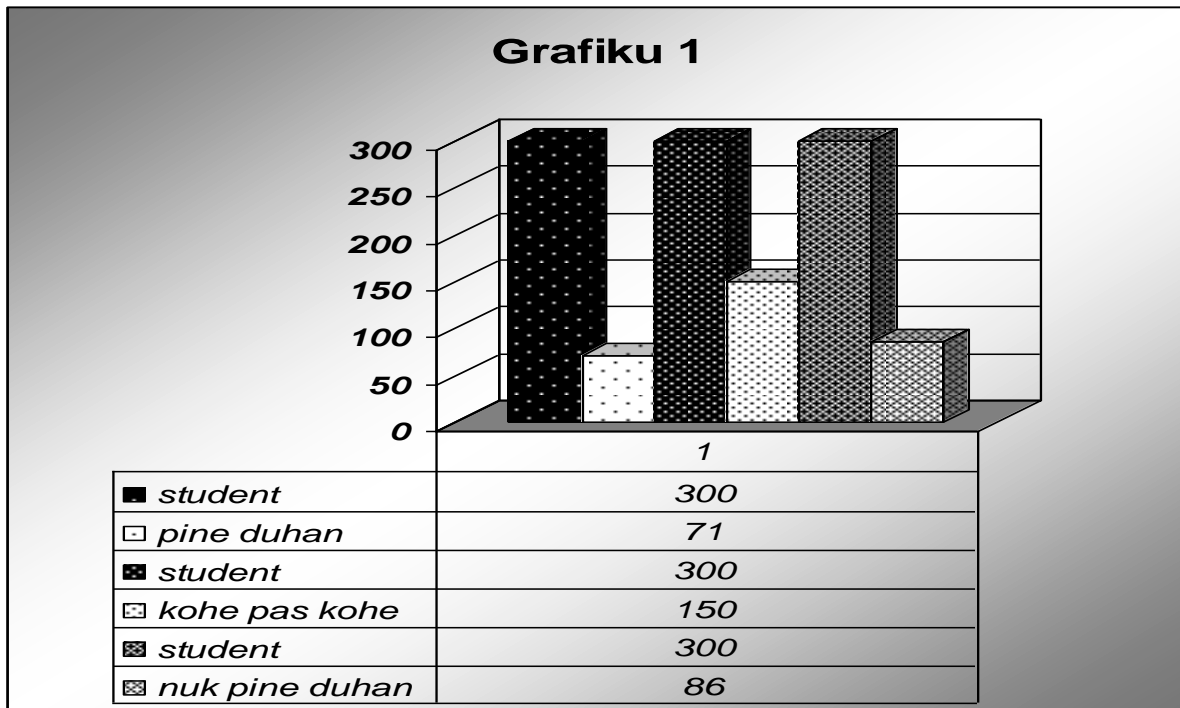
According to this table clearly seen than anything the 6:25 femera consume tobacco and each of 3 male consumes tobacco.

Table 3. Number and percentage of students who don't smoke

<i>Number of dormitories</i>	<i>The number of respondents</i>	No. of people who smoke	The percentage of non smokers
Dormitory No. 1	60	51	85%
Dormitory No. 2	60	48	80%
Dormitory No. 3	60	33	55%
Dormitory No. 4	60	52	86.6%
Dormitory No. 5	60	45	75%

This table shows that in the dormitory No. 4 smoking consumed less than in other dormitories, while the dormitory 3 most consumed smoking. Of all respondents turns out that 93% of students have knowledge of the damage caused by smoking and 7% have less knowledge for the damages caused by this negative factor.

All students who consume smoke of this research turns out that we spend 0.50 € minimum for 1 day and 0.50 € maximum 2.

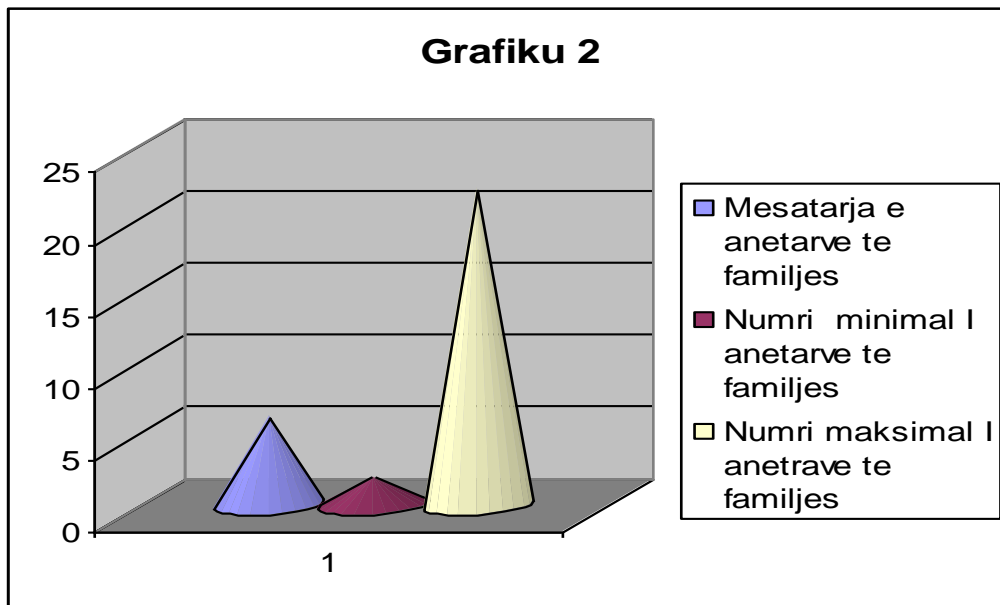


Graf.1. The students who consume tobacco

Also from this research we come to the conclusion that 24% of students consume tobacco regularly, 50% occasionally (sometimes in society), and 26% do not consume smoke at all, although living in the environment where is use smoking.

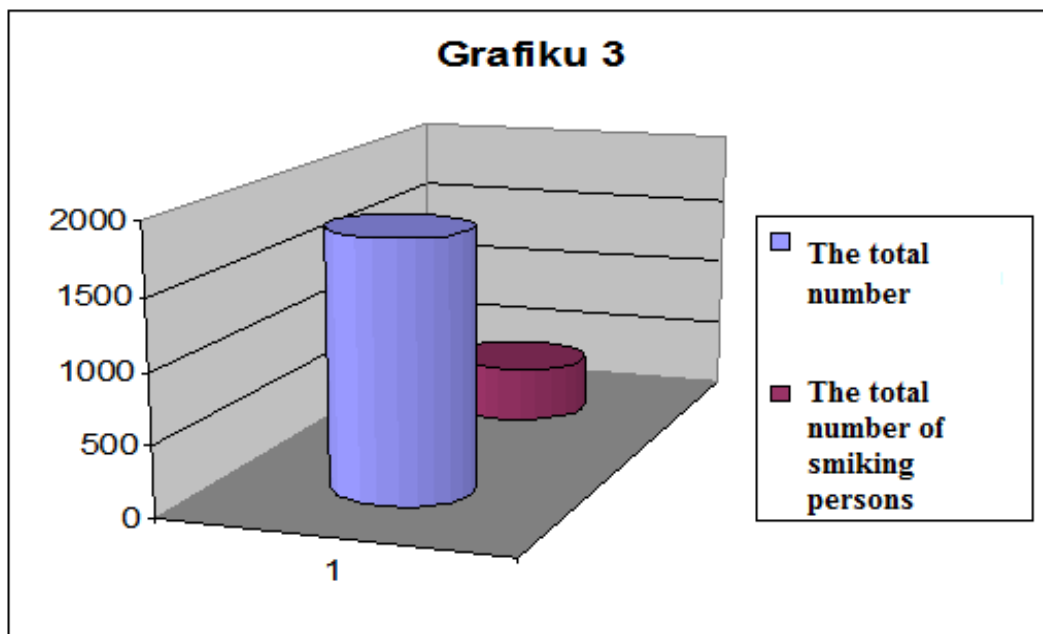
All respondents prefer the smoking consumed in the external environment, although smokers do not practice.

Of these 300 student respondents, the overall number of their family members is 1843 persons.



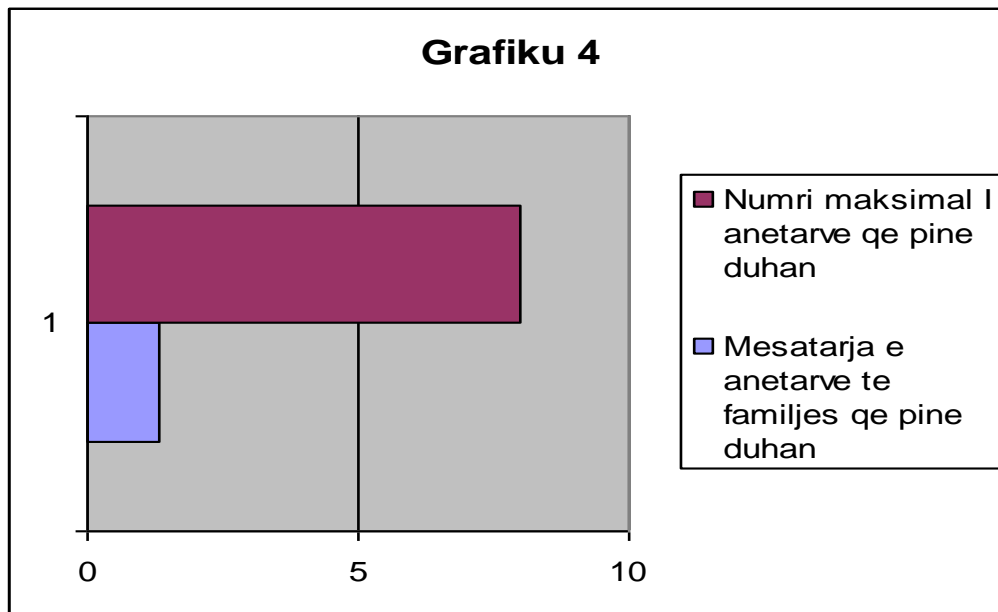
Graf. 2. The number of members in the family

Average members per one family is 6.23. Minimum number for one family is 2 persons. The maximum number for a family is 22 persons.



Graf. 3. The total number of those who smoke

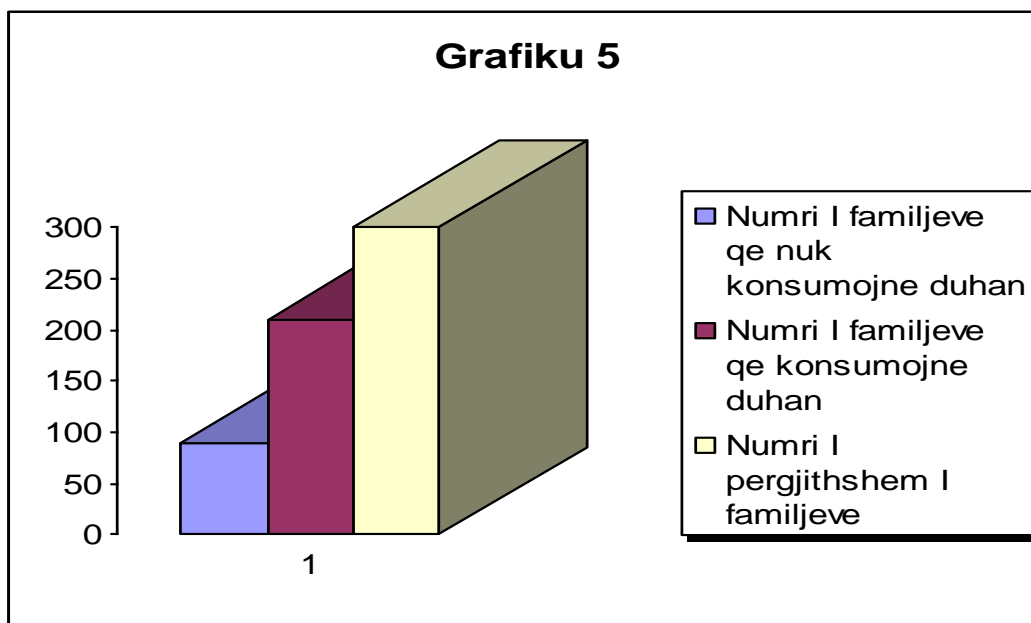
Number of students and their family members who smoke is a total of 394 persons or 21.38.



Graf.4. Smoking persons in one family

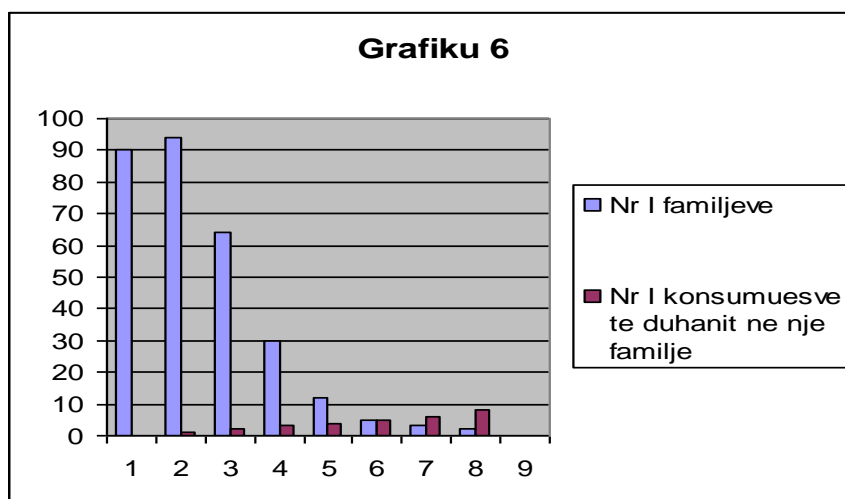
Overall members who smoke is 1.33 persons for one family.

The maximum number of tobacco consumers persons in a household is 8 by 16 family members (every second).



Graf. 5. Number of households consume and not consume tobacco

The number of families who don't smoke is 90 families out of 300 or 30% (Chart 5). Number of households consumed smoking is 210 by 300 or 70% (Chart 5).



Graf. 6. The number of tobacco consumers in a family

90 families do not smoke, 94 families from 1 consumer of tobacco, 64 families from 2 consumer of tobacco, 30 families have 3 tobacco consumers. 12 families from consuming tobacco 6, 5 families have 5 tw tobacco consumers, 3 families have consumers of tobacco 6 2 8 families have consumers of tobacco. Number of family members on respiratory diseases is 38 or 2,06% by 1843. Average user with respiratory disease is 0.13 persons per family. As any student of respondents did not suffer from any illness caused by smoking or respiratory disease, because it is a long time not to consume tobacco.

Discussion

Smoking is the number one problem of the emergence of many diseases that today facing our country and many other countries of the world. According to these statistics the survey each of 3 male (student) consumes tobacco and each of 6.25 a woman (student).

While according to a survey conducted by Peace of students every third male consumes tobacco and each of seven female and generally consumes tobacco in Kosovo within the day spent around 450 000 to € 500 000. [5]

According to an in searching made by non-governmental organizations about 65% of young people aged 20 to 35 years old are tobacco consumers. According to this research we have concluded that in the Student Center, 24% of smoking regularly consume 50% occasionally and 26% are sucking passive (sitting in the area where smoking is consumed).

While generally taking their families are consumers of tobacco 68.6%. It turns out that the statistics obtained from this research are different from those obtained by the previous research and literature.

Smoking is a cause 30% for respiratory diseases, stomach, pancreas, kidney, urinary bladder and uterus of women and 90% of deaths from lung cancer. [4.5]

People who smoke immediately wake up in the morning as the first work they do start coughing and sputum rattle in average quantities.

The people who do not smoke lungs through the ciliary system may self cleaned throughout the day, which smokers can not do when their ciliary system is out of function and the lungs are not able to extract mucus.

Conclusion

From the analyzed data it can be concluded that 68.6% of Kosovo families is exposed to smoking.

These persons are exposed to a very high risk to gain cardiovascular disease, respiratory cancer of the stomach or lung abscess of many other diseases caused by smoking.

Other than smoking causes damage to those who consume affects the health of those who are close to where the most vulnerable are children.

We must try to prevent smoking, changing the behavior of tobacco smokers, changing views on smoking. Inculcating obedience to smoking as a habit of harmful and dangerous habit, not as necessary. Fining those who consume tobacco in places forbidden by law.

Changing the thought of not smoking as the forced norm of behavior in society.

To be effective the fight against smoking that need the whole society to join in this great activities, this should complete most desire in order to have health and healthy offspring.

So should prophylactic fought in faculties and schools where young people still smoking habit is not devastating.

“The greatest success in the fight against smoking is personal behavioural change”

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Emergency Care Sick Palliative and Problems Oncology in Emergency Department during the COVID-19 Pandemic

Basri Lenjani¹, Blerim Krasniqi^{1,2*}, Gani Shabani¹, Premtim Rashiti¹, Arber Demiri¹, Besarta Pelaj², Filanza Spahiu² and Dardan Lenjan²

¹Emergency Clinic, University Clinical Centre of Kosova, Pristina, Republic of Kosovo

²College Medical of Sciences Resonan, Republic of Kosovo

Abstract

Emergency medical care in palliative patients during the COVID-19 pandemic, it is important to provide a consistent treatment for stable patients that should be consistent with the goals and benefits, the perspective of these patients, but avoiding palliative patients with a poor prognosis that is unlikely to survive. Cancer is the second leading cause of death in the world around 8.8 million deaths a year. Worldwide, about 7 million to 10 million patients are diagnosed with cancer each year, recently there has been a significant increase in the number of cases diagnosed with cancer. About 70% of cancer deaths are in low- and middle-income countries. The goals of emergency medical care based on the criteria of BLS and ACLS, that is should be done "Do not do resuscitation, do not intubate but continue medical treatment excluding end tracheal intubation without prospects for the patient, but offering BLS only treatment concentrated symptomatic. ED is often the only place that can provide the necessary medical interventions (e.g., intravenous fluids or pain management medications). Medications as well as immediate access to advanced diagnostic tests when needed such as CT, RM and other diagnostic and treatment procedures.

Keywords: Palliative care; Emergency department; Emergency medical support; Training

Introduction

Emergency medical care for palliative patients during the COVID-19 pandemic, it is important that in ED to provide a consistent treatment for stable patients that should be consistent with the goals and benefits, the patient's perspective and avoid patient's palliative with a poor prognosis that is unlikely to survive [1]. Palliative care includes for people suffering from life-threatening diseases, aiming to improve the quality of life by often attempting to positively progress the disease. Palliative care addresses not only the issue of physical suffering from the disease, but also issues related to in all aspects: Physiological, social, psychological and spiritual. Palliative care is essentially full ongoing active care in patients with life-threatening illnesses. Palliative care should provide relief of signs and symptoms by improving the best possible quality of life. The five types of cancer that cause the highest number of deaths at the system level are carcinomas: Pulmonary 1.69 million deaths, hepatic 788,000 deaths, colorectal 774,000 deaths, gastric 754,000 deaths, and thoracic cortex 571,000 deaths. In most countries there may be changes to a country's health care system, but they can be well-organized units with home medical teams, primary and secondary care, hospital consultants and health care providers in emergency department [2]. ED is often the only place that can provide life expectancy for possible and necessary medical interventions (e.g., intravenous fluids or painkillers. Medications as well as immediate access to advanced diagnostic tests (e.g., computed tomography or magnetic resonance imaging) as well as other diagnostic and treatment procedures and ACLS, how to proceed "Do not resuscitate, do not intubate but continue medical treatment, knowing the meningitis in the population? Excluding benign benefit intratracheal intubation, but offering BLS only concentrated symptomatic treatment. Palliative addresses not only the issue of physical suffering from the disease, but also issues related to in all aspects: physiological, social, psychological of spiritual [3]. EMS must to provide complete, continuous, active emergency medical care to patients with life-threatening illnesses and by providing relief from signs and symptoms by improving the best possible quality of life.

Purpose of the Work

The purpose is to show the number of malignant patients seeking ED, the reason for coming for emergency medical care and aiming to improve the quality of life and the course of the

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*Correspondence:

Blerim Krasniqi, College Medical of Sciences Resonance, Pristine 10000, Republic of Kosovo, Tel: +381 38 500 600 23 36; Mobile: +377 44 165 770; E-mail: blerim.h.krasniqi@gmail.com

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disease occupying pain, monitoring and treatment of side effects of cytostatics, radiotherapy and patients affected by COVID-19 in the Emergency Clinic.

Material and Methods

The study is of retrospective observational type which included 105 patients with oncological problems, palliative as well as concomitant diseases for the period March to August 2020 during the pandemic COVID-19 [4]. The data were taken from the documentation of patients in the ED archive as anesthetic data (gender, age, and place of residence, status of life parameters, results of objective examination, results of laboratory diagnostic research, radiological data. The data are presented in tables and graphs.

Working methodology: These patients were treated by emergency physicians and consulting services with the emergency medical care team for assistance with any need related to palliative care and we reviewed the clinical characteristics and outcomes of patients who received medical interventions that did not match the expected prognosis [5-7]. Palliative care intervention focused on observation, diagnostics, specialist consulting, moderate or advanced treatment, and systematization. Data were collected from the medical register. For the period March - August 2020 during the time of the COVID-19 pandemic, such as age, gender, location, evaluation of vital signs, monitoring, complications and hospitalization.

Results

The study is of retrospective type realized for the period March to August 2020 treated 1 to 5 patients with oncological and palliative problems, with concomitant diseases and identified with the PR-CPR COVID-19 test at the Emergency Clinic of UCCK in Prishtina. Patients by age <65 years were 8 cases or 7.6%, 65 to 74 years were 16 cases or 15.2%, 75 to 84 years were 45 cases or 42.8%, 85 to 94 years were 29 cases or 27.6% and 95 to 104 years were 7 cases or 6.6% (Tables 1-11) [8,9].

According to the place of residence in the city there were 66 cases or 62.8%, while in the village there were 49 cases or 37.2%. According to the vital signs stable were 28 cases or 26.4%, while unstable 77 cases or 73.6%. According to the support provision Blishin 79 cases or 75.2%, while with ACLS there were 26 cases or 25.0%. According to the stabilization of the stabilized health condition were 77 or 77.3%, while unsterilized were 53 cases or 22.7%. There were 78 cases or 77.3% admitted to the hospital, while 37 cases or 25.7 were not admitted to the hospital (Table 12).

Table 1: Age, median (range), y.

Age	No. (%)
<65	8 (7.6)
65-74	16 (15.2)
75-84	45 (42.8)
85-94	29 (27.6)
95-104	7 (6.6)

Table 2: Confirmed with COVID19 PT-CPR.

Positive	66 (62.8)
Not confirmed COVID PT-CPR	
Negative, for COVID-19	29 (27.6)
Unknown, not tested suspected COVID-19	6 (5.5)
Negative, treated for a medical condition other than COVID-19	4 (7.6)

Table 3: Sex/Ethnicity/race.

Sex	
Male	58 (55.2)
Female	47 (44.7)
Ethnicity	
Albanien	98 (93.3)
Other	17 (16.1)

Table 4: Comorbidities.

Comorbidities	No. (%)
Hypertension	32 (30.4)
Cardiovascular disease Comorbidities	19 (18.0)
Diabetes mellitus	8 (7.6)
Chronic kidney disease	8 (7.6)
Documented history of dementia	7 (6.6)
Obesity (BMI ≥ 30) ^a	5 (4.7)
Chronic lung condition	4 (3.8)
Neurologic disease and/or history of neurosurgery	10 (9.5)
End-stage renal failure on hemodialysis	6 (5.7)
Immunosuppression	2 (1.9)
Active cancer	2 (1.9)
Liver disease	2 (1.9)

Table 5: By Location.

Place	No. (%)
City	66 (62.8)
Village	49 (37.2)

Table 6: Signs and symptoms.

Signs and symptoms	No. (%)
Pain	57 (54.2)
Vomitus	9 (8.5)
Anemia	7 (6.6)
Cardiac dysrhythmia	9 (8.5)
Dyspnea	10 (52.7)
Conscious	4 (3.8)
Unconscious	9 (8.5)

Table 7: By vital signs.

Vital sign	No. (%)
Stabl	28 (26.4)
No stabl	77 (73.6)

Table 8: By CPR.

CPR	No. (%)
BLS	79 (75.2)
ACLS	26 (25.0)

Patients with moderate complications were 48 cases or 47.5% and those with severe complications were 57 cases or 52.5%. Oncology patients after the presentation of signs and symptoms with pain were 57 cases or 54.2%, with vomiting were 9 cases or 8.5, with anemia 7 cases or 6.6%, with cardiac dissection were 9 cases or 8.5%, with dyspnea were 10 cases or 9.52%, conscious 4 cases or 3.8% and unconscious were 9 cases or 8.5%.

Table 9: By stabilization ED.

Stabilization ED	No. (%)
Stabilization	77 (77.3)
No stabilization	38 (22.7)

Table 10: By admissions.

Admissions	No. (%)
Hospitalization	78 (74.2)
No hospitalization	37 (25.8)

Table 11: By complications.

Complications	No. (%)
Moderate	48 (47.5)
Serious	57 (52.5)

Table 12: Possible problems.

Possible Problems	No. (%)
Stay for a long time in ED	21 (20.0)
They often come to ED	18 (17.1)
Other clinics do not want to accept them	15 (14.2)
Unbearable family members waiting	13 (12.3)
Unnecessary examinations	10 (9.5)
Pain management is difficult	9 (8.5)
Relatives of the patient with anyone seeking hospitalization	9 (8.5)
They hardly agree with reality	10 (9.5)

It is a necessity of the time that in ED there is a palliative care space, but it is necessary that emergency physicians should receive official training on how to manage palliative patients; this will contribute and help reduce ED overcrowding. Patients with oncological and palliative problems in ED, after receiving, monitoring, observation, laboratory diagnosis, radiology and medical consultations, with decision-making were systematized according to the diagnosis and treatment unit for further treatment according to the pathology of the disease. After the palliative care intervention the criteria of BLS were determined, ACLS "Do not do resuscitation alone" (follow all the principles of palliative care except CPR), do not incubate, continue the symptomatic medical treatment "(follow all the steps of palliative care) excluding intubation and CPR); and taking care directed only at relieving pain and psychological and social problems.

Discussion

It is very important that the emergency physician should make an early identification of signs and symptoms, based on primary criteria (life-threatening patients, frequent hospitalizations due to worsening vital signs, complex care requirements) and secondary (Indicators). Specific needs, long-term care hospitalization, advanced age, pathological fractures, metastases, necessary oxygen therapy, outpatient cardiac arrest and limited social support (e.g., family stress, chronic mental illness) to be admitted ED.

Conclusion

Implemented medical interventions of patients with malignant diseases are a small part of the total number of interventions and

treatment. The most common medical care was emergency medical care, pain management, monitoring and treatment of side effects of cytostatics and radiotherapy. Based on the research, it is clear that we have an increase in medical visits with oncological problems, the emergency doctor, very quickly manages to identify the obstacles and showing the optimal care in ED, as well as the use of valuable resources of health care.

But a very important thing for these patients with oncological problems diagnosed with COVID-19 their transport to the respective clinics, was noticed the creation of medical teams for transport by ambulance ACLS with emergency doctor because other clinics which dealt with the treatment of patients with oncological problems verified or suspected of having COVID-19 did not know how to transport them. The establishment of medical teams with emergency transport physicians will enable a correct professional transport and in this way can avoid possible complications during transport. ED special spaces should be created, human resources educated and trained for palliative care treatment. A comprehensive national program should be established for a specific integrated program in emergency palliative care training, guidelines and protocols designed for emergency medical service providers at three levels of health care.

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Research Article

Emergency care sick palliative and problems oncology in emergency department during the COVID-19 pandemic

Basri Lenjani^{1*}, Nehat Baftiu^{1*}, Premtim Rashiti¹, Gani Shabani¹, Blerim Krasniqi², Arber Demiri², Besarta Pelaj², Fllanza Spahiu², Arban Demi² and Dardan Lenjani²

¹Emergency Clinic, University Clinical Centre of Kosova, Pristina, Kosova

²College Medical of Sciences Resonanca, Kosova

Abstract

Emergency medical care in palliative patients during the COVID-19 pandemic, it is important to provide a consistent treatment for stable patients that should be consistent with the goals and benefits, the perspective of these patients, but avoiding palliative patients with a poor prognosis that is unlikely to survive. Cancer is the second leading cause of death in the world around 8.8 million deaths a year. Worldwide, about 7-10 million patients are diagnosed with cancer each year, recently there has been a significant increase in the number of cases diagnosed with cancer. About 70% of cancer deaths are in low- and middle-income countries. The goals of emergency medical care based on the criteria of BLS and ACLS, that is should be done "Do not do resuscitation, do not intubate but continue medical treatment excluding endotracheal intubation without prospects for the patient, but offering BLS only treatment concentrated symptomatic. ED is often the only place that can provide the necessary medical interventions (e.g., intravenous fluids or pain management medications. Medications as well as immediate access to advanced diagnostic tests when needed such as CT, RM and other diagnostic and treatment procedures.

Introduction

Emergency medical care for palliative patients during the COVID-19 pandemic, it is important that in ED to provide a consistent treatment for stable patients that should be consistent with the goals and benefits, the patient's perspective and avoid patients palliative with a poor prognosis that is unlikely to survive. Palliative care includes for people suffering from life-threatening diseases, aiming to improve the quality of life by often attempting to positively progress the disease. Palliative care addresses not only the issue of physical suffering from the disease, but also issues related to in all aspects: physiological, social, psychological and spiritual. Palliative care is essentially full ongoing active care in patients with life-threatening illnesses. Palliative care should provide relief of signs and symptoms by improving the best possible quality of life. The five types of cancer that cause the highest number of deaths at the system level are carcinomas: Pulmonary 1.69 million deaths, hepatic 788,000 deaths, colorectal 774,000 deaths, gastric 754,000 deaths, thoracic cortex 571,000 deaths [1].

More Information

*Address for Correspondence: Basri Lenjani, Emergency Clinic, University Clinical Centre of Kosova, Pristina, Kosova, Email: basri.lenjani@yahoo.com; blerim.h.krasniqi@gmail.com

Nehat Baftiu, Emergency Clinic, University Clinical Centre of Kosova, Pristina, Kosova, Email: nehatbaftiu@hotmail.com

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Keywords: Palliative care; Emergency; Department; Resuscitation; Medical support; Medical treatment; Education and training



In most countries there may be changes to a country's health care system, but they can be well-organized units with home medical teams, primary and secondary care, hospital consultants and health care providers in emergency department. ED is often the only place that can provide life expectancy for possible and necessary medical interventions (eg, intravenous fluids or painkillers. Medications as well as immediate access to advanced diagnostic tests (e.g. computed tomography or magnetic resonance imaging) as well as other diagnostic and treatment procedures, and ACLS, how to proceed "Do not resuscitate, do not intubate but continue medical treatment, knowing the meningitis in the population? Excluding benign benefit intratracheal intubation, but offering BLS only concentrated symptomatic treatment. Palliative addresses not only the issue of physical suffering from the disease, but also issues related to in all aspects: physiological, social, psychological of spiritual [2]. EMS must to provide complete, continuous, active emergency medical care to patients with life-threatening illnesses and by providing relief from signs and symptoms by improving the best possible quality of life.



Purpose of the work

The purpose is to show the number of malignant patients seeking ED, the reason for coming for emergency medical care and aiming to improve the quality of life and the course of the disease. Occupying pain, monitoring and treatment of side effects of cytostatics, radiotherapy and patients affected by COVID-19 in the Emergency Clinic.

Material and methods

The study is of retrospective observational type which included 105 patients with oncological problems, palliative as well as concomitant diseases for the period March - August 2020 during the pandemic COVID-19. The data were taken from the documentation of patients in the ED archive as anamnestic data (gender, age, place of residence, status of life parameters, results of objective examination, results of laboratory diagnostic research, radiological data). The data are presented in tables and graphs [3]. Working methodology. These patients were treated by emergency physicians and consulting services with the emergency medical care team for assistance with any need related to palliative care and we reviewed the clinical characteristics and outcomes of patients who received medical interventions that did not match the expected prognosis. Palliative care intervention focused on observation, diagnostics, specialist consulting, moderate or advanced treatment, and systematization [4,5]. Data were collected from the medical register. For the period March - August 2020 during the time of the COVID-19 pandemic, such as age, gender, location, evaluation of vital signs, monitoring, complications and hospitalization.

Results

The study is of retrospective type realized for the period March-August 2020 treated 105 patients with oncological and palliative problems, with concomitant diseases and identified with the PR-CPR COVID-19 test at the Emergency Clinic of UCCK in Prishtina. (Tables 1-12). Patients by age < 65 years were 8 cases or 7.6%, 65-74 years were 16 cases or 15.2%, 75-84 years were 45 cases or 42.8%, 85-94 years were 29 cases or 27.6% and 95-104 years were 7 cases or 6.6% [6].

According to the place of residence in the city there were 66 cases or 62.8%, while in the village there were 49 cases or 37.2%. According to the vital signs stable were 28 cases or 26.4%, while unstable 77 cases or 73.6%. According to the support provision BLishin 79 cases or 75.2%, while with ACLS

Table 1: Age, median (range), y.

Age, median (range), y	No. (%)
< 65	8 (7.6)
65-74	16 (15.2)
75-84	45 (42.8)
85-94	29 (27.6)
95-104	7 (6.6)

Table 2: Confirmed with COVID19 PT-CPR.

Positive	66 (62.8)
Not confirmed covid PT-CPR	
Negative, for COVID-19	29 (27.6)
Unknown, not tested suspected COVID-19	6 (5.5)
Negative, treated for a medical condition other than COVID-19	4 (7.6)

Table 3: Sex/Ethnicity/race.

Sex	
Male	58 (55.2)
Female	47 (44.7)
Ethnicity	
Albanien	98 (93.3)
Other	17 (16.1)

Table 4: Comorbidities.

No. (%)	
32 (30.4)	Hypertension
19 (18.0)	Cardiovascular disease
	Comorbidities
8 (7.6)	Diabetes mellitus
8 (7.6)	Chronic kidney disease
7 (6.6)	Documented history of dementia
5 (4.7)	Obesity (BMI \geq 30) ^a
4 (3.8)	Chronic lung condition
10 (9.5)	Neurologic disease and/or history of neurosurgery
6 (5.7)	End-stage renal failure on hemodialysis
2 (1.9)	Immunosuppression
2 (1.9)	Active cancer
2 (1.9)	Liver disease

Table 5: By Location.

Place	No (%)
City	66 (62.8)
Village	49(37.2)

Table 6: Signs and symptoms.

Signs and symptoms	No. (%)
Pain	57 (54.2)
Vomitus	9 (8.5)
Anemi	7 (6.6)
Cardiac dysrhythmia	9 (8.5)
Dyspne	10 (52.7)
Conscious	4 (3.8)
Unconscious	9 (8.5)

Table 7: By Vital Signs.

Vital sign	No. (%)
Stabl	28 (26.4)
No stabl	77 (73.6)

Table 8: By CPR.

CPR	No. (%)
BLS	79 (75.2)
ACLS	26 (25.0)

Table 9: By Stabilization ED.

Stabilization ED	No. (%)
Stabilization	77 (77.3)
No stabilization	38(22.7)

**Table 10:** By Admissions.

Admissions	No. (%)
Hospitalization	78 (74,2)
No hospitalization	37(25.8)

Table 11: By Complications.

Complications	No. (%)
moderate	48 (47,5)
Serious	57(52.5)

Table 12: Possible problems.

No. (%)	
21 (20.0)	Stay for a long time in ED
18 (17.1)	They often come to ED
15 (14.2)	Other clinics do not want to accept them
13(12.3)	Unbearable family members waiting
10 (9.5)	Unnecessary examinations
9 (8.5)	Pain management is difficult
9 (8.5)	Relatives of the patient with anyone seeking hospitalization
10 (9.5)	They hardly agree with reality

there were 26 cases or 25.0%. According to the stabilization of the stabilized health condition were 77 or 77.3%, while unstabilized were 53 cases or 22.7%. There were 78 cases or 77.3% admitted to the hospital, while 37 cases or 25.7 were not admitted to the hospital.

Patients with moderate complications were 48 cases or 47.5% and those with severe complications were 57 cases or 52.5%. Oncology patients after the presentation of signs and symptoms with pain were 57 cases or 54.2%, with vomiting were 9 cases or 8.5, with anemia 7 cases or 6.6%, with cardiac dissection were 9 cases or 8.5%, with dyspnea were 10 cases or 9.52%, conscious 4 cases or 3.8% and unconscious were 9 cases or 8.5%.

It is a necessity of the time that in ED there is a palliative care space, but it is necessary that emergency physicians should receive official training on how to manage palliative patients, this will contribute and help reduce ED overcrowding. Patients with oncological and palliative problems in ED, after receiving, monitoring, observation, laboratory diagnosis, radiology and medical consultations, with decision-making were systematized according to the diagnosis and treatment unit for further treatment according to the pathology of the disease [7,8]. After the palliative care intervention the criteria of BLS were determined, ACLS "Do not do resuscitation alone" (follow all the principles of palliative care except CPR), do not intubate, continue the symptomatic medical treatment "(follow all the steps of palliative care) excluding intubation and CPR); and taking care directed only at relieving pain and psychological and social problems [9].

Discussion

It is very important that the emergency physician should make an early identification of signs and symptoms, based

on primary criteria (life-threatening patients, frequent hospitalizations due to worsening vital signs, complex care requirements) and secondary (Indicators), specific needs, long-term care hospitalization, advanced age, pathological fractures, metastases, necessary oxygen therapy, outpatient cardiac arrest and limited social support (eg, family stress, chronic mental illness) to be admitted Emergency Department (ED).

Conclusion

Implemented medical interventions of patients with malignant diseases are a small part of the total number of interventions and treatment. The most common medical care was emergency medical care, pain management, monitoring and treatment of side effects of cytostatics and radiotherapy. Based on the research, it is clear that we have an increase in medical visits with oncological problems, the emergency doctor, very quickly manages to identify the obstacles and showing the optimal care in ED, as well as the use of valuable resources of health care.

But a very important thing for these patients with oncological problems diagnosed with COVID-19 their transport to the respective clinics, was noticed the creation of medical teams for transport by ambulance ACLS with emergency doctor because other clinics which dealt with the treatment of patients with oncological problems verified or suspected of having COVID-19 did not know how to transport them. The establishment of medical teams with emergency transport physicians will enable a correct professional transport and in this way can avoid possible complications during transport. ED special spaces should be created, human resources educated and trained for palliative care treatment.

A comprehensive national program should be established for a specific integrated program in emergency palliative care training, guidelines and protocols designed for emergency medical service providers at three levels of health care.

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Research Article

Emergency Medical Care and Management of Sports Injuries on the Football Court

Basri Lenjani, Premtim Rashiti*, Gani Shabani, Arber Demiri, Besarta Pelaj, Arban Demi, Rilind Sylaj, Erza Voca Mulaj and Dardan Lenjani

Emergency Clinic, University Clinical Centre of Kosova, Kosova

Abstract

Entry: Sports medicine is a clinical subspecialty that deals with the examination, monitoring, diagnosis, treatment and prevention of injuries that occur during sports events, training and physical activities in pre-hospital settings. Managing dramatic situations with minor and multiple injuries is a challenge that requires a quick approach to a dramatic event in managing minor and multiple injuries on the football field and in other sports in support of SHME in pre-hospital and hospital level.

Purpose of the paper: Providing emergency medical care at all basic stages of managing minor and multiple injuries on the football field and in other sports in order to implement BLS, ACLS, BTLs, PTLs, ATLS care measures reducing morbidity, disability and mortality.

Working methodology: The research is of retrospective, descriptive, qualitative type. The material was taken from the archive of the Emergency Clinic of UCCK for the period January to December 2019. Only the sick or injured in sports matches were taken in the research; Age, gender, type of illness and injury and type of medical care, equipment available and training and education.

Result: Sports injuries are very costly, and according to the pathology with diseases were 15 cases or 21.4%, injuries were 55 cases or 78/6%. Injured by age The largest number of injured with injuries in the field of football sports the most affected age was the age of 21-25 years with 28 cases or 40.00%, over 25 years were 27 cases or 38.58% and with a smaller number were aged 15-20 years 15 cases or 21.42%.

Discussion and conclusions: A very important factor in sports injuries is the provision of optimal medical care for footballers and other sports in head, neck, spine, chest, abdomen, and pelvis and limb injuries and with a joint communication with the cooperation of health care professionals in selection of priority cases. Education of medical staff, nurses, paramedics with courses, use of medical equipment, BLS, ACLS, BTLs, PTLs, ATLS as well as standard procedures for providing and transporting medical care to the hospital.

Keywords: Illness; Injury; Sports; Health care; Collision football; Medical care, EMS

Introduction

Key points

Advancing and empowering EMS through emergency medical care law, sports law and possible national intervention strategy is a very important and priority modality to identify intervention needs for the prevention of sports injuries. Research on the mandatory organization of change, education, training and education to prepare and train health care professionals as well as EMS and after training to be able to provide care and emergency assistance at the right time and moment and this approach can provide new research opportunities aimed at preventing injury and disability and morbidity in sports. Our research was sensitizing to create the awareness of sports clubs should always have a medical team during sports events by invoking the law on medical care, the Law on Sports and the national strategy for injury management in sports. From the research Uncontrolled (random) evidence has been used extensively in sports injury

prevention studies, and to evaluate, modify policy interventions in order to function better.

Entry

Sports medicine is a clinical subspecialty undertaken in providing emergency medical care to improve their performance of athletes, in recovering from injuries and to prevent further injuries occurring during sporting events, training and physical activities. Emergency sports medicine in mass gatherings provides emergency medical care in minor and mass incidents, sympathizers grouped more than 1000 fans in a football match as well as in other sports. The first priority of any dramatic scene where people's lives are threatened, EMS are obliged to do the assessment of the scene or the scene to be is safe to enter and can continue assessment, triage and treatment according to priorities. Multiple disaster management which mainly as its factors of origin of disaster are directly related to the behavior of sympathizers in sports fields [1]. Acute and life-threatening illnesses, injuries and poisonings and require attention to initially stabilize the victims before reaching the SHME. Managing dramatic situations with multiple victims is a challenge that requires ongoing study in theoretical, practical and experiential terms in order to provide clues to an event or disaster. EMS should be present at a dramatic event, but of course unlikely to control the situation but should be prepared and provided in advance, EMS in managing multiple victims in football as well as in other sports when the need arises [2]. Managing dramatic situations with multiple and minor casualties in sports fields requires the provision of human resources, EMS medical staff, other support services in the management of minor disasters, and sympathizers [3].

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***Corresponding author:** Premtim Rashiti, Emergency Clinic, University Clinical Centre of Kosova, Pristine 10000, Republic of Kosovo. E-mail: premtim.r@gmail.com

Factors influencing the management of mass incidents at such sporting events are: Availability, failure to separate sympathizers, failure of match organizers to adhere to guidelines, presence of shutters around the field, EMS access to the field, non-categorization inadequate "risk level" during the match, inadequate access system, insufficient EMS, insufficient barriers, inadequate functioning of the Operations Center, inadequate access system, insufficient trained personnel, inadequate match locations large, the use of tear gas, pre-sale of tickets, matches with large crowds and unreserved seats [1].

The escalation of minor and major risk, based on risk classification is usually based on: mass gathering, the nature of the expected crowd, rivalry of sympathizers, the timing of the football match, and the forecast of weather conditions are prerequisites for the occurrence of diseases or injuries can be expected [1].

Moderate risk escalation expected crowds of more than; 50% of stadium capacity, sports match time day, week, night, meteorological conditions, hot, cold, snow and humidity, nature of sports match, importance, resentment, known rivalry, expected chaos, alcohol present wet conditions/expected rain, sale of regular or black tickets, seats, reserved seats are prerequisites that can generate obvious and very serious problems [1].

Also other factors that may increase the risk are; Insufficient food and drink for spectators, lack of additional capacity, lack of inadequate EMS, lack of emergency exits, lack of adequate signage for basic services in the stadium, provision and intentional use of pyrotechnic devices or tear gas are prerequisites to control the crowd. The number of people seeking integrated EMS during mass gatherings depends on the venue and the legislation of that country and may vary depending on the mass present from 1,000 to 25,000 people or more [3].

EMS are obliged within the security perimeter of the stadium and provide medical care including in all areas within the safety zone, scheduled match, lack of previous information, lack of space for action or dysfunctional, lack of adequate communication in the stadium, insufficient education and training of administrators and security staff, insufficient food and drink for spectators. Mandatory requirements for the football match (obligations from UEFA). The host club is responsible for providing; EMS staff (emergency medicine specialist), at least one stretch for the medical team to arrive at the stadium/hall and until evacuation of the injured player [2].

EMS components to the mass gathered at the scene requires; adequately educated and trained staff, support staff, physician with appropriate equipment and supported by infrastructure, logistics and mobilization of the integrated system of EMS [4].

Medical monitoring and diagnostic equipment which are necessary for the management of diseases and injuries in sports fields which are mandatory and mandatory standards by the home of UEFA and FIFA.

Airway, neck and spinal cord management equipment; manual aspirator, respirators, respirator, lubricant, rigid cervical neck support set [3].

Respiratory management equipment; stethoscope, pulse oximeter, oxygen, trauma masks, tubes, and pocket masks. Mask with bag and valve, separating device for bronchodilators, portable oxygen cylinders [1].

Circulation management equipment; infusion equipment for IV,

External Automatic Defibrillator (AED), equipment for measuring blood pressure with cuffs of suitable sizes, glucometer/ test strip [1].

Other (small) equipment; tourniquets, adhesive fixing materials, adhesive fixing materials, light for pupil examinations, IV cannulas of different sizes, solid scissors, disinfectants, disposable gloves, sharp tool boxes, goggles [1].

Emergency medicine bag; adrenaline 1:10,000 injection, antihistamines, hydrocortisone, benzodiazepines, cardiac life-saving drugs, epipen or anapen auto-injector, bronchodilators, glycerol trinitrate spray, glucose tablets/gel, emergency diabetic drugs and antiemetic [1].

Major (mandatory) equipment; spinal trauma carrier, vacuum spoon or mattress stretcher and sufficient fixation equipment, complete set of splints, spinal trauma carrier, vacuum spoon or mattress stretcher and sufficient fixation equipment and complete set of splints [1].

Recommended

The following items should be available; cricothyrotomy set with disposable scalpel equipment for intubation, anti-hypertensive drugs, basket stretcher or stove, external pacing defibrillator and capnography for CO2 monitoring [3].

Emergency medical care Injuries ABC and per seumdje CAB. Victim assessment and primary examination 1 minute. CPR and stabilization 5 minutes, Immobilization and transport to hospital 4 minutes. Manage the airways. Control sprains and immobilize head and neck spine and transport. Treatment of sports injuries as follows; Bubbles - impact broken bone break, ice, ligament, elevation. In open and closed fractures and in DRABC ligament injuries and after the stabilization of the vital signs of the injured, emergency transport with medical care to the hospital. In DRABC sprains and after stabilization of vital signs of the injured, emergency transport with medical care to the hospital [3,5].

In a football stadium is an environment where a large number of people gather, to watch sports matches, medical emergencies often occur on football fields as well as on sports fields and this component must be managed quickly, effectively and efficiently by EMS. Must be adequate and appropriate medical professionals, cooperative and available to provide necessary medical care. All medical professionals must have education, training and education in a football event [6].

Purpose of the Research

Providing emergency medical care, prevention, treatment, is necessary to ensure full recovery in the prevention of other injuries in all the basic stages of managing minor and multiple injuries in football and other sports. BLS, ACLS, BTLs, PTLs, ATLS reducing morbidity, disability and mortality. EMS helps prevent, treat, and speed recovery, reduce pain, and promote movement of all injured muscles, restoring normal function, and preventing any recurrent damage or discomfort to the affected tissue. The purpose is also to coordinate medical actions with experienced multidisciplinary medical teams that include surgeons/emergency physicians, orthopedists, cardiologists, nurses.

Material and Methods

Participants

The research material was taken from the archive of the Emergency Clinic of UCKK. The research is of retrospective, descriptive, qualitative type, for the period January-December 2019. Only injuries

in the field of football sports were taken into research. In the research were taken 70 cases only sick or injured in sports matches; age, gender, type of illness and injury, medical care, available equipment and education. The average age of males was 18-30 years, while according to gender 58 cases for males and 12 cases for females.

Variables

Age and gender were chosen as variables to obtain data on sports injuries. Simple demographics for subjects. Types of Injuries was used as a variable to obtain data based on time seasons time series and injuries were categorized into echoes, neck, spinal cord injuries to head and neck, chest, abdomen, upper and lower limbs and pelvis, as a variable for the study were the type of system level injuries.

Statistical analysis

Data were analyzed using the IBM SPSS program. Simple descriptive statistics, tables, diagrams, which were used to explain injury patterns between systems.

Results

The research material was taken from the archive of the Emergency Clinic of UCCK only from sports injuries on the football field. The research is of retrospective, descriptive, qualitative type, for the period January-December 2019. (Tables 1-6) (Graph 1).

Table 1: Pathology according to diseases and injuries.

Pathology	No. (%)
Diseases	15 (21.4)
Injuries	55 (78.6)
Total	70 (100)

Table 2: Number of sick and injured by gender.

Age	No. (%)
15-20 year	15 (21.2)
21-25 year	28 (40.0)
Over 26 year	27 (38.8)
Total	70 (100)

Table 3: Most common diseases.

Ilnes	i No. (%)
Convulsive seizures	4/26.6
Heart attack	3/20.0
Brain aattack	1/6.6
Hipoglicemia	4/26.6
Cardiac arrest	1/6.6
Asthma	1/6.6
Total	15/

Table 4: Number of educated and trained medical staff managing sports clubs.

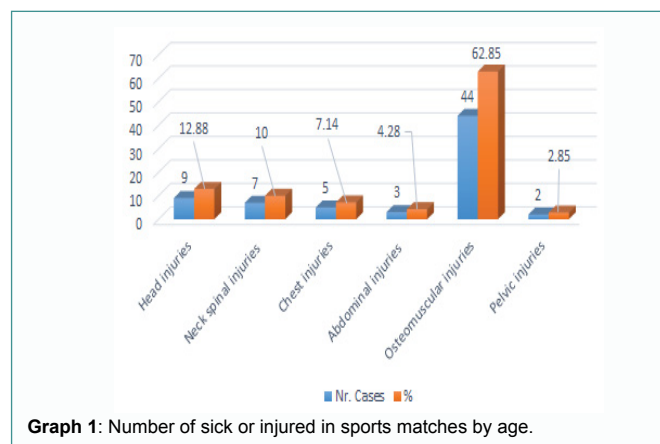
Compulsory courses	No. (%)
BLS	24
ACLS	4
BTLS	/
ATL	/
PHTLS	/
Total	28

Table 5: Equipment ambulance EMS.

Equipment Ambulance EMS	
Ambulance BLS	75/78.9
Ambulance ACLS	5/5.2
Ambulance no equipment	15/15.9
Total	95/100

Table 6: EMS Tranport privat and EMS.

EMS Tranport	No. (%)
Privat	15 (21.4)
EMS	55 (78.6)
Total	70/100



Graph 1: Number of sick or injured in sports matches by age.

Discussion

The results of our study showed that problems appear in different steps during the management of diseases and injuries in sports fields in the absence of mobile teams, capacities, human resources, medical equipment, triage areas and their system. Lack of coordination of actions was the main cause, delays in the management of the sick and injured on sports fields. Injured by age The largest number of injured with injuries in the field of football sports the most affected age was the age of 21-25 years with 28 cases or 40.00%, over 25 years were 27 cases or 38.58% and with a smaller number were aged 15-20 years 15 cases or 21.42%.

Injured according to pathology with osteomuscular injuries were 44 cases or 62.8%, with head injuries were 9 cases or 12.8%, with neck and spinal injuries were 7 cases or 10.0%, with chest injuries were 5 cases 7.1%, with abdominal injuries were 3 cases or 4.2% and with pelvic injuries were 2 cases or 2.8%.

The most common diseases according to frequency with Convulsive seizures 4 cases or 26.6% with heart attack 3 cases or 20.0%, brain attack 1 cases or 6.6%, hypoglycemia 4 cases or 26.6%, cardiac arrest 1 case or 6.6% and with asthma 1 case or 6.6%. These cases were among the sympathizers. All these cases were hospitalized after admission, monitoring, diagnostic observation and treatment were placed in the appropriate clinics according to the nature of the disease.

All cases were transported by private cars without care and medical transport, because EMS was insufficient. EMS ambulances with BLS medical equipment were 75 ambulances or 78.9% with ACLS 5 ambulances or 5.2% and EMS ambulances not equipped with medical equipment were 15 ambulances or 15.9%. It is required that EMS should be modernized respecting the standards of medical care transport based on the law of medical care as in education, education and training with basic courses for outpatient service, but this component is not developed, so we must review the current laws that regulate EMS. Medical treatment at the scene was low-key because the medical teams did not have any medical training and most of them were uneducated, educated and trained with compulsory courses and the triage system was deficient in assessing the condition of the patients and the injured. As for the national medical team, they have been trained by the UEFA Medical Committee, but among them are not professional medical teams and in their composition are a pediatric surgeon and two physiotherapists, but bypassed the emergency doctor, orthopedic, anesthesiologist, cardiologist, nurses who have been eliminated, not respecting the recommendations of UEFA and FIFA.

This issue should be regulated by the National Football Federation of Kosovo. A very important factor in sports injuries is the provision of optimal medical care for footballers and in other sports in head, neck, spine, chest, abdomen, pelvis and limb injuries as well as joint communication in collaboration with health care professionals in selection of priority cases [1]. National Sports Federations of Kosovo based on the rules written by UEFA, FIFA national sports teams must manage doctors professional multidisciplinary medical teams such as emergency physicians, anesthesiologists, sports doctors, traumatologists, cardiologists, nurses, educated physiotherapists, trained and certified with the required courses BLS-D, PHTLS, ACLS, ATLS, T-triage.

Education of medical staff, nurses, paramedics with courses, use of medical equipment, BLS, ACLS, BTLs, PTLs, ATLS as well as standard procedures for providing and transporting medical care to the hospital. The Sports Law of the Republic of Kosovo, No. 2003/24 - XIV, must be supplemented and amended to meet the recommendations of FIFA and UEFA for emergency medical care in sports, including Article 60 of this Law on Health Protection and the use of stimulant substances and methods in sports [7-10].

The results of this research work suggest that the National Federation of Kosovo should oblige sports clubs to provide EMS during training and during the organization of sports games, to prevent treatment and wanting to achieve an optimal recovery. The establishment of the national center and the national strategy is immediate is a valuable reflection of the scope of our research in the prevention of sports injuries [11-17].

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Research Article

Leadership Development and Functioning in Primary Health Care in Peja

Besarta Pelaj¹, Basri Lenjani^{1*}, Haxhi Kamberi², Eqrem Gara², Milazim Gjocaj² and Fellanza Spahiu²

¹Emergency Clinic, University Clinical Centre of Kosova, Kosova

²Faculty of Medicine, University of Gjakova, Kosova

Abstract

Leadership development is seen as central to the modernization agenda of the NHS. The purpose is to evaluate the managerial behaviors of managers of health institutions (MF), which refer to the following skills: motivating others, delegating tasks, planning, communicating, encouraging and decision-making. This paper reviews the evidence concerning leadership development in nursing. Two major concepts at the core of these evolving roles are innovation and interdependency. Reflective practice, continuing education, and other educational efforts can target these concepts as a way of augmenting knowledge, skills, and abilities in care coordination and clinical leadership the conclusion reached is that leadership is only one element in the changes that need to occur in health care.

Aim: The purpose is to evaluate the managerial behaviors of managers of health institutions (MF), which refer to the following skills: motivating others, delegating tasks, planning, communicating, encouraging and decision making.

Material and method: The research is of a quantitative type. Through this research, we will reflect the real state of leadership being explored through the Health Personnel Survey, where this type of observation is structured on the basis of certain criteria for leadership in primary care. Data collection technique - evaluation of closed and open anonymous type questionnaire. Data analysis techniques involve collecting numerical information using frequency, percentage statistical methods. The target group was MFMC health personnel in Peja. The total number of subjects in the research was 40. Activities undertaken to increase the knowledge of the PC nurses.

The Center for Continuing Vocational Nursing Education, established by the Ministry of Health, is tasked with developing and organizing continuing education for nurses, which represents another achievement of the Health Ministry (HM) towards the development of human resources in the health sector.

Purpose of the research: The main purpose of this research is to identify the causes of injuries caused by firearms, assessment of vital signs, severity of injuries at the system level, geographical distribution, emergency medical care in pre-hospital and hospital settings, increasing the survival of victims of transport, the accident site to the hospital and reducing the morbidity, disability and mortality of victims.

Keywords: Leadership; Nursing leadership; Development; Primary care; Health; Institutions

Introduction

Primary Health Care principles internationally; the development of the progressive Primary Health Care movement; and experimentation with new models of health service delivery and primary care. Since 1999, Kosovo has made significant progress in developing health services. The latest report "Tracking Universal Health Coverage: The First Monitoring Report" (WHO and World Bank Group 2015) shows that we are far from achieving it [1]. The report, which is the first of its kind to measure health coverage and financial protection to assess countries' progress towards Universal Health Coverage (UHC), looked at the global approach to basic health services in 2013 including family planning, antenatal care, children, immunization of children, antiretroviral therapy, treatment of tuberculosis and access to clean water and sanitation.

WHO and the World Bank Group (2015) "recommend that countries pursuing UHC should aim to achieve a minimum of 80% population coverage with basic health services and that everyone everywhere should be protected from catastrophic and depleting payments health [1].

Health worker shortages and inequities in resource distribution; shortcomings of political, public sector and medical /health leadership; and a complex and protracted health transition. The Action Plan (2010-2014) is a strategic document that informs all health institutions, donors and other partners in the health sector on the key priorities of the MF. Strong management and leadership competencies have been identified as critical in enhancing health system performance [2,3].

The AP is an operational document aimed at detailing the strategic priorities, results and actions, through which the SSH will positively impact the development of the health system and health status of the population of Kosovo Findings suggest that the sub-district is located within a hierarchical governance context, with performance monitored through the use of multiple accountability mechanisms including standard operating procedures, facility audits and target setting processes [3,4].

While our data suggest that gains in leadership were emerging, our experience is of a system struggling to shift from a hierarchical to a more relational understanding of how to enable improvements in performance, and to implement these changes in practice [5-7].

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***Corresponding author:** Basri Lenjani Krasniqi, Emergency Clinic, University Clinical Centre of Kosova, Pristina, Kosova, E-mail: basri.lenjani@yahoo.com

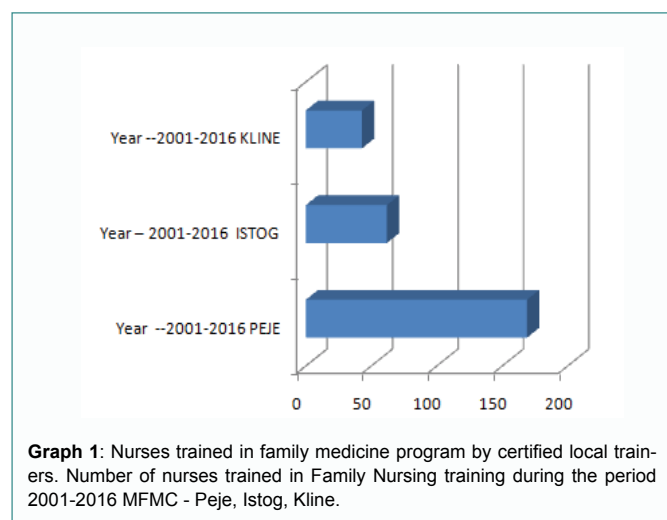
Material and Methods

The research is of a quantitative type. Through this research, we will reflect the real state of leadership being explored through the Health Personnel Survey, where this type of observation is structured on the basis of certain criteria for leadership in primary care. Data collection technique-evaluation of closed and open anonymous type questionnaire. Data analysis techniques involve collecting numerical information using frequency, percentage statistical methods [8].

The target group was MFMC health personnel in Peja. The total number of subjects in the research was 40. Activities undertaken to increase the knowledge of the PC nurses. The Center for Continuing Vocational Nursing Education, established by the Ministry of Health, is tasked with developing and organizing continuing education for nurses, which represents another achievement of the Healthy Ministry (HM) towards the development of human resources in the health sector.

Results

The data are taken from the sources of the UCCK archive - Primary care Peja (Graph 1).



So far in 2016, in Peja this training is held 2 hours a year for 6 months and they have the right to be sent to training for 10-12 nurses per year which means sending maximum 5-6 nurses to training for each organization. 6 months training. These and other initiatives taken by the central level demonstrate the commitment of the Health Ministry (MSH) to meet one of the objectives of the Kosovo health strategy.

We can freely say that Kosovo today is not starting from the zero point, as significant progress has been made in recent years. Providing quality and accessible health services based on the needs of the population will be a challenge (Figure 1) (Graph 2).

Results of Questionnaire No.1

Leadership Development and Functioning at MFMC Peja Out of 40 (100%) of the research subjects give the following results from the answers to the questionnaire: From the first question, - To what extent do you know about the concept of management, 23 (46%) of the participants answered that they have a lot of knowledge about management, whereas 10 (34%) have little knowledge of the concept of management, and 7 (20%) have no knowledge of management.

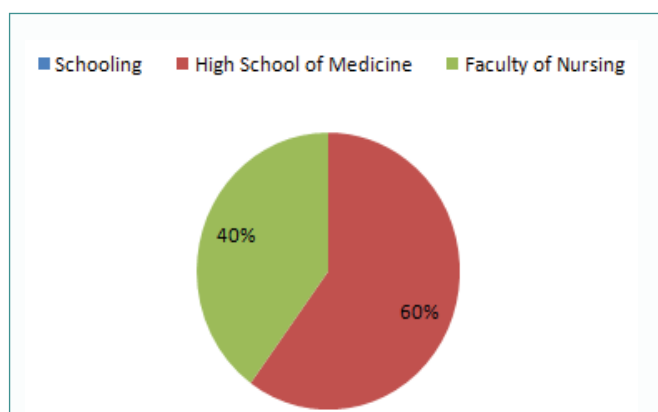
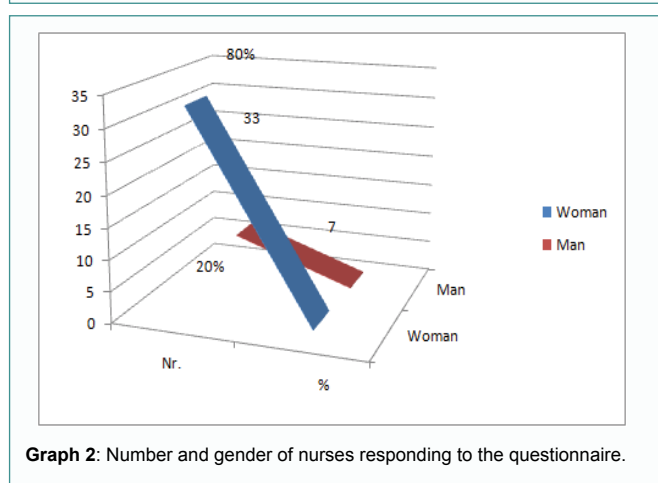


Figure 1: School qualification. Of the 40 (100%) subjects, 24 (65%) were in medical high school, and 16 (35%) were in the faculty of nursing.



How motivated are you by managers?

Of the 40 respondents 10 (25%) agree that they are highly motivated by managers, 10 (25%) responded that they are slightly motivated by managers, while 8 (20%) think that they do not sufficiently motivate health workers. Whereas, only 12 (30%) of the subjects answered that managers do not motivate health workers.

How satisfied are you with the working conditions? Out of 40 respondents, 20 (50%) are not very satisfied with working conditions, 9 (21%) are slightly satisfied with working conditions, 7 (18%) are satisfied with working conditions, 4 (11%) are not satisfied with working conditions at all.

How much are senior management nurses trained on?

Of the total number of respondents 17 (42%) of them answered that they have no management training, whereas 11 (34%) of the respondents think that there is little management training, and 6 (12%) think that are not trained enough to manage, and only 6 (12%) say that many are trained to manage.

How many nurses participate in decision making?

From this question it results that 16 respondents (39%) answered that few participate in decision making, 12 (35.6%) are not very involved in decision making, 8 (14.4%) are not involved in decision making, while 6 (11%) of the subjects think that they are very involved in decision making.

Do you collaborate with staff in decision making?

Only 10 respondents (25%) from no. Overall, 40 (respondents) say that few cooperate in decision making, 10 (25%) do not cooperate much in decision making, 10 (25%) do not cooperate in decision making, while 10 (25%) of the subjects think that many cooperate with staff in decision making.

Question 2: Developing and Functioning Leadership in Primary Health Care - Peja.

Question: 1. What do you think would contribute to the effectiveness of being a family nursing leader?

This chart shows that 50% of respondents respond that discipline and awareness at work contributes to the efficiency of being a family nursing leader and 20% to working with colleagues.

What are the problems you encounter most when exercising your position as a nursing leader?

Chart 2 shows that 54% of respondents say that the problem when exercising their position as Head of Nursing is the lack of medicines, and 46% say the problem lies because we do not have access to decision-making.

How will you overcome the problems that may arise during work?

From this graph. It turns out that 60% of the respondents think that problems at work are overcome by professional development in the workplace and 40% think that they are overcome by proper cooperation with colleagues.

What should be done from your position to raise the level of quality of health care services in Primary Care?

From this graph. We see that the results of the question: What to do from your position to raise the level of quality of health services in Primary Care, 29% of respondents think Extra Training, 25% Management Motivation and Salary high 46% of participants think. Is management an innate or acquired quality? Respondents think that management is a quality born 25% and gained 53%.

What is the difference between a manager and a leader?

We see that 74% of respondents see managers as leaders and 26% as leaders.

Do you think wage increases affect labor productivity?

As a result, 80% of respondents think that wage increases affect labor productivity and 20% do not.

Do you think a good manager can detect and eliminate service vulnerabilities?

From this answer we see that 86.60% of respondents think that a good manager can detect and eliminate service weaknesses while 13.40% disagree.

Should managers participate in the development of human resources policy?

From this answer we see that 72% of the respondents think that managers should participate in the development of human resources policy while 28% do not have this opinion.

Do you think that the manager's work affects the achievement of the planned objectives?

From graph. We see that 80% of the respondents think that the manager's work affects the achievement of the planned objectives, while 14% say that it does not.

Discussion

Relational leadership-associated with practices such as mentoring/coaching others, and enabling the relationships and commitment needed to work together to achieve common purpose-has been identified as important in strengthening the performance of health systems.

The First Monitoring Report" (WHO and World Bank Group 2015) shows that we are far from achieving it [1]. The report, which is the first of its kind to measure health coverage and financial protection to assess countries' progress towards Universal Health Coverage (UHC), looked at the global approach to basic health services in 2013 including family planning, antenatal care, children, immunization of children, antiretroviral therapy, treatment of tuberculosis and access to clean water and sanitation [9-11].

In our experience, the ethos of action learning, on the other hand, is able to generate the rich, context specific lessons not only about what may be needed to strengthen leadership in similar settings but also what sort of effects the LD response might be generating [8,12-16].

Conclusion

Family medicine leadership should be provided with institutional support (either at central or local level) regarding the services they provide to the population. Of course, by improving the conditions of health workers, health services to patients in general will also improve. But very important is the development of an employee performance appraisal system. Throughout this research we understand that health workers have the feeling that their evaluation should be done by their peers, rather than related to the management action of an organization.

From this research we also understand that improving the management of health systems requires cross-sectoral efforts by all stakeholders at all levels. Nurses, who provide most healthcare services in collaboration with colleagues in both the health and non-health sectors, have an important role to play in this process. It must be ensured that nursing is continually changing in response to the demands of the time in order to improve the quality and quality of care provided through professional management through proper management, access to health policy and decision making. From the results obtained from this research we also understand that more should be invested in motivating nurses for superior education and job classification and pay by education grade.

Recommendation

Proper management by the nurses as well as the performance of duties with moral, ethical and professional responsibility having in mind the clear job description and the delegation of responsibilities. Work harder on schooling and salary levels. Provide better working conditions, do not miss Emergency and elementary health services. Work more on the part of the Ministry of Health for health insurance. Provide funds from Municipalities and Government for setting up the Principal Center of Family Medical Care Etc.

Improving the health status of the population and the quality of health care services improving management and leadership at all levels of the health system. Improvement of existing resource management

and quality of services related to quality of care improvement and Strategic Goals.

Functional reorganization and completion of existing infrastructure in a comprehensive priority focusing on improving key health services, emphasizing prevention and having a positive impact on the health status of the population of Kosovo.

The healthcare network and its infrastructure should function according to the prioritized needs of the population, Strengthen capacities in GPs of family medicine and maternal and child health. Improve the healthy lifestyle of the most vulnerable groups of the population. Reduce the burden of chronic non-communicable diseases and life-threatening diseases, reduced infectious disease burden. Development and implementation of the Health Information System Develop a modern, quality, transparent and accountable public health sector that responds to the needs and demands of the population and able to manage citizens' contributions efficiently, delivering optimal health outcomes that can be regionally comparable and consistent with the requirements for European integration.

In conclusion, this experience suggests that processes of relational LD can promote the relationships necessary for effective team engagements, can encourage actors to trust each other to exercise productive discretion and can enhance the ability of managers to engage with their colleagues in a more supportive way.

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Nursing Care and Health Care Plan with Chest Pain

Besarta Pelaj^{*1}, Basri Lenjani^{*1}

¹The university of Prishtina, Faculty of Medicine, Prishtina, Kosovo.

²Emergency Clinic, University Clinical Centre of Kosova, Pristina, Republic of Kosovo.

***Corresponding author:** Basri Lenjani, MD. PhD, University Clinical Centre of Kosova, Emergency Clinic, College Medical of Sciences Resonance, Republic of Kosovo. E Mail: basrilenjani@yahoo.com

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Abstract

Fire Angina is a pain the chest or a problem which occurs when the heart muscle is not sufficiently supplied with blood rich with oxygen. It can feel as a pressure or strain in the chest. The problem can also occur in the shoulders, arms neck, jaws and back. It is a symptom of the heart disease and it is usually a symptom of the heart coronary disease. There are many kinds of angina including the coronary acute syndrome, angina pectoris, chest pain, coronary artery spasm, microvascular angina, Prinzmetal angina, inverse angina, stable angina, and the usual, nonstable angina with variations. Nursing Care Plans. The goals of treatment for Angina pectoris are to relieve chest pain, stabilize heart rhythm, reduce cardiac workload, revascularize the coronary artery, and preserve myocardial tissue. Angina occurs when the heart muscle does not receive the sufficient amount of blood to function normally. This usually happens when one or more coronary arteries are either narrowed or blocked which is otherwise called ischemia. The main risk factor for heart disease dangers and coronary disease dangers SKM are: Unhealthy levels of cholesterol, the high blood pressure, smoking, diabetes, overweight or obesity, metabolic syndrome, inactivity, unhealthy diets, old age (the danger increases for males after the age of 45 and for females after the age of 55), the family history with early heart diseases. The goals of treatment for Angina pectoris are to relieve chest pain, stabilize heart rhythm, reduce cardiac workload, revascularize the coronary artery, and preserve myocardial tissue.

Keywords: Nursing care, angina pectoris, nonstable, heart, coronary disease, nursing plan.

INTRODUCTION

Nursing care and health care plan with chest pain - Plans of nursing care - Nurse valuation:

Gathering of information about the patient's symptoms and activities, the valuation of the factors for the dangers of coronary arteries diseases, then the patient's responses to angina, understanding of diagnosis by the patient and his family and keeping to the actual plan of treatment. [1, 2].

Nursing Diagnosis:

The ineffective perfusion of Cardiac Tissues after SAK which is accompanied with pains in the chest and other prodromal symptoms, anxiety, tribulation, pain, gasping, change in vital signs etc. The lack of knowledge about the existing disease and about methods of averting any complications. [3,4].

The importance of a team works a Doctor- Nurse:

Given that patients are of a different origin, age and different intellectual level, therefore nurses are required a great individual and team work for managing cases with angina. The managing of the nurse care at patient's with angina pectoris can be only realized with an inter- colleague and inter- sectorial cooperation, making it easier for the patient who is threatened by potential complications during his stay in the hospital. A nurse is always near the patient, does a careful observation (detecting the urgent symptoms and signs) and informs the doctor, in order that all necessary preventive measures be taken. She monitors all vital functions. All dependent and independent nursing activities are documented on the right nurse lists. [5,6].

Planning and aims:

Aims include immediate treatment, reducing the pain, prevention of IAM, reducing of anxiety, awareness of the process of the illness, education about the illness in the self- care program and prevention of complications.

Nursing priorities:

During the nursing care there are also some priority activities or some activities of a more urgent nature, and they are:

1. Easing \ controlling the pain.
2. Prevention \ minimization of complications of Myocardium.
3. Providing the information regarding the process of illness \ prognosis and treatment.
4. Supporting the patients \ VS for starting the necessary changes in their lifestyle and behaviours.

Nursing interventions - Treatment of Angina:

Immediate actions be taken if the patient reports pains in the chest or when prodromal symptoms of a person indicate Anginal ischemia. The patient is instructed to cease all the activities and is advised to sit or lie in bed in Fowler position. Vital signs are measured and the patient is observed if he has a breathing disorder, nitroglycerine is applied through the sublingual way and the patient's reaction is to be evaluated (this can be repeated up to three doses). In case the patient's respiratory rate increases or in case the level of oxygen saturation decreases, then oxygen therapy must be applied. If the pain is bigger and if it continues for more than 30 minutes even after these applications, then the patient is evaluated for IAM and can be transferred to a unit for a more specific infirmary care. [3,7,8].

Nursing advice and the education of patients with angina pectoris:

Patients are taught to recognize their symptoms, how to report on the change of quality and intensity of symptoms during the stay in the hospital.

They are also advised how and when to ask for help in case of reoccurring of symptoms after their discharge from the hospital. This can imply the program for quitting smoking, consultations about specific diets, the control of their body

weight, the recommendations for a progressive increase of physical activities, participations in patient clubs etc. The respect of time and protocol during the struggle for reducing of possible complications from counter indications of cardiovascular system, prevention of complications and their recognition, as well as the ability of action should be viewed as team characteristics. Having in mind the long term importance of patient education regarding the coronary disease, we have oriented ourselves to the research of informing nurses about the education of patient's in the Coronary Units. Patients admitted with angina pectoris are vulnerable and frightened for the future. [1. 9. 4, 6.10].

A good nurse must compile a consultary program: The program for consulting patients with angina is designed in that way that the patient and his family understand the disease, and identify the symptoms of myocardium ischemia. They are informed about the methods for prevention of chest pains and advancement of SAK are discussed.

In the self-care program we must cooperate with the patient, his family and friends:

Activities for minimization of angina episodes must be planned. The patient must be advised that each pain that occurs after the application of these methods including the application of nitroglycerine must necessarily be treated at the nearest emergency centre. [11. 12,13].

Supplementary information with which patients must be advised on their discharge from hospital care and the plan of nursery care they must follow Some of the information:

They must be informed about the disease. What must be expected at home, activity, diet and life style. The diet is by all means an inseparable part of the program of care and the treatment of patients with Angina Pectoris and some for the instructions that the patients must be given to are: to reduce (restrict) the amount of alcohol they consume [4,7]. To be advised must to smoke. They are supposed not to let anyone smoke in their homes. To instruct them to read and learn more about what they should and to have a healthier heart and healthier blood vessels. To avoid salty and fatty meals. You can direct them to a nutritionist who would help them plan healthy diet. Patients are advised about the best ways to avoid stressful situations. To also advise them about sexual activity. Patients must be aware and ask for professional medical help at the moment they are faced with: pain, pressure, strain, or squeezing in the chest, arms neck, or jaws, problems in breathing, hand numbness, perspiration or change in skin colours. [2,12,14, 14].

The main aim:

The main aim is the search of the infirmity treatment and care for patients with angina Pectoris in the Regional Hospital in Peja [9].

PURPOSE OF THE WORK

Purpose and Objectives of the research the information of patients during their stay in the Hospital regarding his condition. Education of patients regarding angina pectoris. The research of nurses knowledge about angina pectoris. The research of the engagement level of the nurses of the Coronary Units regarding the patient's education during their stay in the Hospital.

Analysis of data and proposal of descriptive measures for the improvement of patient education about angina pectoris. The goals of treatment for Angina pectoris are to relieve chest pain, stabilize heart rhythm, reduce cardiac workload, revascularize the coronary artery, and preserve myocardial tissue.

MATERIAL AND METHODS

Methodology of work:

For accomplishing this research the Hospital of Peja was chosen, the Coronary Unit. The research was carried out through a semi- structured questionnaire, the approach was quantitative.

The aimed group is Nurses:

16 questionnaires were given out designed for the nurses of the intern ward and e Coronary Unid of Peja Hospital.

Collecting the data:

The data collection was done with a semi- structured questionare. The questionare has\contains twenty for questions designed to search this problem.

Data analysis:

The gained data are grouped according to the questions and a statistical processing has been done by the program for computing of statistical parameters of age: average age gemeder and the average time of stay in the hospital. Data presentation was done through charts. The drawn conclusions will contribute in the proposal of measures for increasing of the staff nurse awareness in the management of infirmary care for patients with Angina Pectoris.

RESULTS

In the course of thir work preparation I have collected data regarding the number of patients hospitalized in the Coronary Unit of the Regional Hospital for the years 2018 and 2019 (**Table 1: Table 6**).

Statistics for 2018		Statistics for 2019
Patients with Angina Pectoris	54	68
Male	34	40
Female	20	28
Average age	60	60
Average stay in hospital	6 day	7 day

Table 1. The summary and analysis of responses given by nurses respondents of the questionnaire.

Nr.	Question for Nurses	Yes	%	No	%
1.	Do you ask a patients about the time of his first symptoms?	10	63	6	38
2.	Do you log the time of patients admission?	12	75	4	25
3.	Do you log patient's symptoms an admission and during hospitalization?	7	45	9	55
4.	Do you log patient's vital signs on admission and during hospitalization?	16	100	0	0
5.	Do you ask patient's about his\her former illness?	10	63	6	38
6.	Do you ask patients about the factors of danger of the coronary disease?	4	25	12	75

Table 2. Management of Infermary care in admission of patients in Hospital.

Nr. Question for nurses	Yes	%	No	%
7. Do you think that the patient during his\ her stay in the Coronary Unit gets sufficient information about his\her illness?	8	50	8	50
8. Do you think that the patients during his\ her stay in the Coronary Unit is sufficiently provided with the information about the notification of danger factors ?	5	31.25	11	68.75
9. Do you think that during his\ her stay in the Coronary Unit the Patient gets sufficient information about medications he\she is going to use ?	13	81	3	19
10. Do you think that during his\ her stay in the Coronary Unit the Patients gets information for his everyday activities after discharging the hospital and about possible restrictions ?	9	67	7	43
11. Do you think that during his\ her stay in the Coronary Unit the Patient gets sufficient information about diagnostic examinations he Is submitted or that he might be submitted ?	10	62.5	6	37.5
12. Do you think that during his\ her stay in the Coronary Unit the Patient gets sufficient information about the possibility of the care of the coronare disease ?	8	50	8	50

Table 3. Perception if nurses regarding the information and education of the patients in the coronary unit.

Nr	Question	Nurse			Patient		
1	Who must give the information to patients about their illness	Answer response	Nr	%	Response	Nr	%
		Family number	3	20	Family number	5	8
		The Nurse	2	13	The Nurse	10	17
		The Doctor	10	77	The Doctor	43	75
		The Patient him selt must be interested	0	0	The Patient him selt must be interested	0	0
Nr	Question	Nurse			Patient		
2	General level of cholesterol in blood after the tretmant from Angina Pectoris shoulde?	Response	Nr	%	Response	Nr	%
		< 4.2mmol/ l	9	68	< 4.2mmol/ l	12	21
		< 5.2mmol/ l	3	19	< 5.2mmol/ l	15	26
		< 6.2mmol/ l	2	13	< 6.2mmol/ l	6	11
		I don't have information	3		I don' t have information	25	44
Nr	Question	Nurse			Patient		
3	What is good cholesterol?	Pergjegje	Nr	%	Pergjegje	Nr	%
		LDL	0	0	LDL	0	0
		HDL	2	12	HDL	0	0
		VLDL	13	82	VLDL	10	18
		I don't have information	1	6	I don't have information	48	82
Nr	Question	Nurse			Patient		
4	Whiq value of LDL cholesterol is preferred after Angina Pectoris?	Pergjegje	Nr	%	Pergjegje	Nr	%
		< 1.6mmol/ l	2	13	< 1.6mmol/ l	2	4
		< 2.6mmol/ l	6	40	< 2.6mmol/ l	4	7
		< 3.6mmol/ l	8	50	< 3.6mmol/ l	5	9

		I don't have information	0	0	I don't have information	47	80

Table 4. Questions for nurses and patients regarding the knowledge about the factors of danger (16 nurses and 58 patients).

Nr	Question	Nurse			Patient		
		Answer	Nr	%	Answer	Nr	%
5	The patient, after myocardial infarction should receive aspirin	A year	1	7	A year	10	18
		Five years	3	19	Five years	5	9
		One month	0	0	One month	0	0
		All life	12	75	All life	38	64
		I have no information	0	0	I have no information	5	9
		Nr	Question	Nurse			Patient
6	Whenever nitrates can be applied during the day?	Answer	Nr	%	Answer	Nr	%
		One time	0	0	One time	0	0
		Three times	4	25	Three times	12	21
		More than three times	11	75	More than three times	24	42
		I do not know	0	0	I do not know	22	78
		Nr	Question	Nurse			Patient
7	What are factor it coronary disease risk that cannot be modified?	Answer	Nr	%	Answer	Nr	%
		Legacy	16	100	Legacy	34	59
		Gandr	10	63	Gander	16	28
		Age	11	65	Age	10	18
		I do not know	0	0	I do not know	24	42
		Nr	Question	Nurse			Patient
8	Which are factors of danger of the coronary	Answer	Nr	%	Answer	Nr	%
		Nurse			Patient		

disease that can be modified?							
	smoking	16	100	smoking	0		
	Hypertension	8	50	Hypertension	10	18	
	Total of cholesterol	8	50	Total of cholesterol	5	9	
	Low HDL	7	43	Low HDL	0		
	Obesity	8	50	Obesity	2	4	
	Physical inactivity	13	73	Physical inactivity	10	18	
	Diabetes	2	13	Diabetes	0	0	
	I do not know	0	0	I do not know	31	51	

Table. 5. Questions for Nurses and patients, regarding their knowledge about the factors of danger and the usual medicaments which are used after the infarct of myocardium.

Nr	Question	Nurse			Patient		
		Answer	Nr	%	Answer	Nr	%
9	Is physical activity after IAM forbidden?	In the first six weeks	1	7	In the first six weeks	5	6
		In the first week	2	13	In the first week	11	21
		In the first month	1	7	In the first month	8	14
		On the first day	12	73	On the first day	3	5
		I do not know	0	0	I do not know	31	54
		Question	Nurse			Patient	
Nr							
10	Which medicaments are to do with the secondary prevention?	Answer	Nr	%	Answer	Nr	%
		Nitroglycerine	0	0	Nitroglycerine	0	0
		Statins	4	25	Statins	12	21
		Diuretics	12	75	Diuretics	24	42
		I do not know	0	0	I do not know	22	37
Nr	Question	Nurse			Patient		

Nr	Question	Nurses			Patients		
		Answer	Nr	%	Answer	Nr	%
11	Should the patient be vaccinated against seasonal influenza after the infarct of myocardium?	By no means	0	0	By no means	3	6
		Not important	0	0	Not important	4	7
		At will	9	60	At will	7	12
		Yes	7	40	Yes	16	28
		I do not know	0	0	I do not know	27	47
Nr	Question						
12	Is sexual activity allowed after Angina Pectoris?	Answer	Nr	%	Answer	Nr	%
		Yes after six weeks	14	93	Yes after six weeks	11	18
		Yes after six month	2	7	Yes after six month	2	4
		Yes after a yers	0	0	Yes after a yers	0	0
		I do not know	0	0	I do not know	45	78

Table 6. Questions for Nurses and patients regarding the knowledge worth discussing during the education about the Coronary Disease.

DISCUSSION

Infirmity was not legally recognized as a separate and autonomous profession as are recognized medicine, stomatology, and pharmacy. The role of nurses was to assist doctors and implement their instructions. Problems with which were faced reflect on this research carried out aiming the nurse care for patients with Angina Pectoris.

The education of the citizens about the disease is a primary obligation of public health workers. Profilled Nurses, as is the case with those working in the Coronary Unit, are obliged to inform patients about the illness, about the way how to care it and prevent complications, respectively are obliged to educate patients. To achieve this, nurses must always be in the process of professional education, which would enable security and accuracy in the information transmitted to patients.

Though the questionnaire a reflection has been drawn of the knowledge of nurses about the management of care cases focusing on education of patients when it comes to the illness, which is their professional concern.

Therefore, there is a need for continuous education and a need for a specialized nurse for education of patients, as well as the nurses who work in the Coronary Unit. Educational programs improve the care reduce rehospitalization and increase the quality of life and the functional condition of patients with Coronary diseases. The programs effect survival and prevent reoccurring of Angina Pectoris and other illnesses [9]

Information of nurses about the danger factors is also. Other side it is clear that the infirmity care must include the evidenced education apart from interventions. This gives us the right to think that the nurse does not take an active role in diagnosing and in the patients therapy with Angina Pectoris.

The aim of the cardiac rehabilitation is to improve the physical and psychical capacity of the patient stabilizing, stopping or reversing the progress of a coronary disease. To achieve this the patient must clarify the questions about the disease. There must not be any doubts about physical activity and its importance. It is prudent that the nurse helps the patient to plan his physical activity. Consultations about diet are obligatory. The education and support for modification of the danger factors are also necessary (tobacco, arterial hypertension, diabetes, high cholesterol, obesity insufficient physical activity) [13, 14]

Education means also the information about medicaments, the way to take them, their benefits and possible ill-effects. [16] It can be concluded that the results of this study arouse some important issues and first of all an insufficient education for patients hospitalized regarding Angina Pectoris. At the same time, a current need is highlighted about the necessity of the existence of the protocol which would include the education of patients during this vulnerable period of their fight against the illness. Pointing out lack of an infirmary documentation about nurses activities as also present in the research where infirmary advice given to patients has not been logged. This is a result of the lack of graduate professionals at Infirmary Faculties in the Regional Hospital in Peja because of not existing of a competition (advertisement) for graduate nurses. [1.9.4,6].

CONCLUSIONS

According to the abovepointed data at the results of the research we can conclude that: From the collected data at the Coronary Unit of Peja Hospital emerges that most patients with Angina Pectoris are males. The average age of patients with Angina Pectoris was 61 which is complete conformity with the epidemiology of coronary disease. It is noticed that the patients are not given sufficient information about the illness and about the modification of the danger factors and that they do not have this necessary information during the stay in the Coronary Unit. This is a result of the lack of graduate professionals with a University degree at Peja Hospital.

It is proved a low level of necessary knowledge regarding the coronary disease, regarding the danger factors of this disease and regarding the activities that must be taken to fight the disease.

During their stay in the hospital, patients are not provided with sufficient information about the element of secondary prevention of the coronary disease, as a more effective and a cheaper way for preventing possible complications, controlling the disease, lowering mortality and lowering disability.

Proposal of measures: If there is not inadequate Infirmary management in managing of cases and if there is no health education of patients by nurses, then an action plan be developed for improvement and that plan must contain issues such as: the education of infirmary staff managing of the infirmary care in Coronary Units; the increase of intellectual level of the infirmary staff with people with the Faculty of Infirmary; the increase of knowledge of nurses about the coronary disease and the necessity of patient education; a general engagement of the management staff in all levels; the continuous training of health workers for a better care for patients; it is important to increase hygienic standards in public health institutions; to increase the awareness in a better communication Doctor-Nurse-patient; the ethical code of civility to be part of health workers; to accomplish an increase in technological capacities, the training of health staff of all levels and their continuous education; a general engagement of the management in advancing the infirmary staff on all levels; Patients satisfaction with health service to be an important indicator that reflects the results of the staff and the Hospital; the creation of protocols for patient compensation or their relatives (family member) in cases of problems or any consequences caused by professional carelessness of Hospital staff; to create recreative spaces within the hospital for patients with a longer stay in the hospital; to

enrich the patients many with dietary food according to the illness nature of the patients; the insistence to document the activities and a periodical valuation of all the activities done by nurses; appointment of a specialized nurse for education within the hospital; there is a need to found clubs for patients after the period with Angina Pectoris; and the protocol for education. The heart failure outpatient clinics represent one of the possible applications of this work in the field of nursing; these are sectors that require monitoring, evaluation, teaching and research to support the well-being of patients with this clinical disorder, and the actions of the nurse can significantly improve the quality of life of patients and reduce the cost of readmissions. [2.17-20].

DISCLOSURE

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Emergency Care Sick Palliative and Problems Oncology in Emergency Department during the COVID-19 Pandemic

Basri Lenjani¹, Blerim Krasniqi^{1,2*}, Gani Shabani¹, Premtim Rashiti¹, Arber Demiri¹, Besarta Pelaj², Filanza Spahiu² and Dardan Lenjan²

¹Emergency Clinic, University Clinical Centre of Kosova, Pristina, Republic of Kosovo

²College Medical of Sciences Resonan, Republic of Kosovo

Abstract

Emergency medical care in palliative patients during the COVID-19 pandemic, it is important to provide a consistent treatment for stable patients that should be consistent with the goals and benefits, the perspective of these patients, but avoiding palliative patients with a poor prognosis that is unlikely to survive. Cancer is the second leading cause of death in the world around 8.8 million deaths a year. Worldwide, about 7 million to 10 million patients are diagnosed with cancer each year, recently there has been a significant increase in the number of cases diagnosed with cancer. About 70% of cancer deaths are in low- and middle-income countries. The goals of emergency medical care based on the criteria of BLS and ACLS, that is should be done "Do not do resuscitation, do not intubate but continue medical treatment excluding end tracheal intubation without prospects for the patient, but offering BLS only treatment concentrated symptomatic. ED is often the only place that can provide the necessary medical interventions (e.g., intravenous fluids or pain management medications). Medications as well as immediate access to advanced diagnostic tests when needed such as CT, RM and other diagnostic and treatment procedures.

Keywords: Palliative care; Emergency department; Emergency medical support; Training

Introduction

Emergency medical care for palliative patients during the COVID-19 pandemic, it is important that in ED to provide a consistent treatment for stable patients that should be consistent with the goals and benefits, the patient's perspective and avoid patient's palliative with a poor prognosis that is unlikely to survive [1]. Palliative care includes for people suffering from life-threatening diseases, aiming to improve the quality of life by often attempting to positively progress the disease. Palliative care addresses not only the issue of physical suffering from the disease, but also issues related to in all aspects: Physiological, social, psychological and spiritual. Palliative care is essentially full ongoing active care in patients with life-threatening illnesses. Palliative care should provide relief of signs and symptoms by improving the best possible quality of life. The five types of cancer that cause the highest number of deaths at the system level are carcinomas: Pulmonary 1.69 million deaths, hepatic 788,000 deaths, colorectal 774,000 deaths, gastric 754,000 deaths, and thoracic cortex 571,000 deaths. In most countries there may be changes to a country's health care system, but they can be well-organized units with home medical teams, primary and secondary care, hospital consultants and health care providers in emergency department [2]. ED is often the only place that can provide life expectancy for possible and necessary medical interventions (e.g., intravenous fluids or painkillers. Medications as well as immediate access to advanced diagnostic tests (e.g., computed tomography or magnetic resonance imaging) as well as other diagnostic and treatment procedures and ACLS, how to proceed "Do not resuscitate, do not intubate but continue medical treatment, knowing the meningitis in the population? Excluding benign benefit intratracheal intubation, but offering BLS only concentrated symptomatic treatment. Palliative addresses not only the issue of physical suffering from the disease, but also issues related to in all aspects: physiological, social, psychological of spiritual [3]. EMS must to provide complete, continuous, active emergency medical care to patients with life-threatening illnesses and by providing relief from signs and symptoms by improving the best possible quality of life.

Purpose of the Work

The purpose is to show the number of malignant patients seeking ED, the reason for coming for emergency medical care and aiming to improve the quality of life and the course of the

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*Correspondence:

Blerim Krasniqi, College Medical of Sciences Resonance, Pristine 10000, Republic of Kosovo, Tel: +381 38 500 600 23 36; Mobile: +377 44 165 770; E-mail: blerim.h.krasniqi@gmail.com

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disease occupying pain, monitoring and treatment of side effects of cytostatics, radiotherapy and patients affected by COVID-19 in the Emergency Clinic.

Material and Methods

The study is of retrospective observational type which included 105 patients with oncological problems, palliative as well as concomitant diseases for the period March to August 2020 during the pandemic COVID-19 [4]. The data were taken from the documentation of patients in the ED archive as anesthetic data (gender, age, and place of residence, status of life parameters, results of objective examination, results of laboratory diagnostic research, radiological data. The data are presented in tables and graphs.

Working methodology: These patients were treated by emergency physicians and consulting services with the emergency medical care team for assistance with any need related to palliative care and we reviewed the clinical characteristics and outcomes of patients who received medical interventions that did not match the expected prognosis [5-7]. Palliative care intervention focused on observation, diagnostics, specialist consulting, moderate or advanced treatment, and systematization. Data were collected from the medical register. For the period March - August 2020 during the time of the COVID-19 pandemic, such as age, gender, location, evaluation of vital signs, monitoring, complications and hospitalization.

Results

The study is of retrospective type realized for the period March to August 2020 treated 1 to 5 patients with oncological and palliative problems, with concomitant diseases and identified with the PR-CPR COVID-19 test at the Emergency Clinic of UCCK in Prishtina. Patients by age <65 years were 8 cases or 7.6%, 65 to 74 years were 16 cases or 15.2%, 75 to 84 years were 45 cases or 42.8%, 85 to 94 years were 29 cases or 27.6% and 95 to 104 years were 7 cases or 6.6% (Tables 1-11) [8,9].

According to the place of residence in the city there were 66 cases or 62.8%, while in the village there were 49 cases or 37.2%. According to the vital signs stable were 28 cases or 26.4%, while unstable 77 cases or 73.6%. According to the support provision BLS there were 79 cases or 75.2%, while with ACLS there were 26 cases or 25.0%. According to the stabilization of the stabilized health condition were 77 or 77.3%, while unsterilized were 53 cases or 22.7%. There were 78 cases or 77.3% admitted to the hospital, while 37 cases or 25.7 were not admitted to the hospital (Table 12).

Table 1: Age, median (range), y.

Age	No. (%)
<65	8 (7.6)
65-74	16 (15.2)
75-84	45 (42.8)
85-94	29 (27.6)
95-104	7 (6.6)

Table 2: Confirmed with COVID19 PT-CPR.

Positive	66 (62.8)
Not confirmed COVID PT-CPR	
Negative, for COVID-19	29 (27.6)
Unknown, not tested suspected COVID-19	6 (5.5)
Negative, treated for a medical condition other than COVID-19	4 (7.6)

Table 3: Sex/Ethnicity/race.

Sex	
Male	58 (55.2)
Female	47 (44.7)
Ethnicity	
Albanien	98 (93.3)
Other	17 (16.1)

Table 4: Comorbidities.

Comorbidities	No. (%)
Hypertension	32 (30.4)
Cardiovascular disease Comorbidities	19 (18.0)
Diabetes mellitus	8 (7.6)
Chronic kidney disease	8 (7.6)
Documented history of dementia	7 (6.6)
Obesity (BMI ≥ 30) ^a	5 (4.7)
Chronic lung condition	4 (3.8)
Neurologic disease and/or history of neurosurgery	10 (9.5)
End-stage renal failure on hemodialysis	6 (5.7)
Immunosuppression	2 (1.9)
Active cancer	2 (1.9)
Liver disease	2 (1.9)

Table 5: By Location.

Place	No. (%)
City	66 (62.8)
Village	49 (37.2)

Table 6: Signs and symptoms.

Signs and symptoms	No. (%)
Pain	57 (54.2)
Vomitus	9 (8.5)
Anemia	7 (6.6)
Cardiac dysrhythmia	9 (8.5)
Dyspnea	10 (52.7)
Conscious	4 (3.8)
Unconscious	9 (8.5)

Table 7: By vital signs.

Vital sign	No. (%)
Stabl	28 (26.4)
No stabl	77 (73.6)

Table 8: By CPR.

CPR	No. (%)
BLS	79 (75.2)
ACLS	26 (25.0)

Patients with moderate complications were 48 cases or 47.5% and those with severe complications were 57 cases or 52.5%. Oncology patients after the presentation of signs and symptoms with pain were 57 cases or 54.2%, with vomiting were 9 cases or 8.5, with anemia 7 cases or 6.6%, with cardiac dissection were 9 cases or 8.5%, with dyspnea were 10 cases or 9.52%, conscious 4 cases or 3.8% and unconscious were 9 cases or 8.5%.

Table 9: By stabilization ED.

Stabilization ED	No. (%)
Stabilization	77 (77.3)
No stabilization	38 (22.7)

Table 10: By admissions.

Admissions	No. (%)
Hospitalization	78 (74.2)
No hospitalization	37 (25.8)

Table 11: By complications.

Complications	No. (%)
Moderate	48 (47.5)
Serious	57 (52.5)

Table 12: Possible problems.

Possible Problems	No. (%)
Stay for a long time in ED	21 (20.0)
They often come to ED	18 (17.1)
Other clinics do not want to accept them	15 (14.2)
Unbearable family members waiting	13 (12.3)
Unnecessary examinations	10 (9.5)
Pain management is difficult	9 (8.5)
Relatives of the patient with anyone seeking hospitalization	9 (8.5)
They hardly agree with reality	10 (9.5)

It is a necessity of the time that in ED there is a palliative care space, but it is necessary that emergency physicians should receive official training on how to manage palliative patients; this will contribute and help reduce ED overcrowding. Patients with oncological and palliative problems in ED, after receiving, monitoring, observation, laboratory diagnosis, radiology and medical consultations, with decision-making were systematized according to the diagnosis and treatment unit for further treatment according to the pathology of the disease. After the palliative care intervention the criteria of BLS were determined, ACLS "Do not do resuscitation alone" (follow all the principles of palliative care except CPR), do not incubate, continue the symptomatic medical treatment "(follow all the steps of palliative care) excluding intubation and CPR); and taking care directed only at relieving pain and psychological and social problems.

Discussion

It is very important that the emergency physician should make an early identification of signs and symptoms, based on primary criteria (life-threatening patients, frequent hospitalizations due to worsening vital signs, complex care requirements) and secondary (Indicators). Specific needs, long-term care hospitalization, advanced age, pathological fractures, metastases, necessary oxygen therapy, outpatient cardiac arrest and limited social support (e.g., family stress, chronic mental illness) to be admitted ED.

Conclusion

Implemented medical interventions of patients with malignant diseases are a small part of the total number of interventions and

treatment. The most common medical care was emergency medical care, pain management, monitoring and treatment of side effects of cytostatics and radiotherapy. Based on the research, it is clear that we have an increase in medical visits with oncological problems, the emergency doctor, very quickly manages to identify the obstacles and showing the optimal care in ED, as well as the use of valuable resources of health care.

But a very important thing for these patients with oncological problems diagnosed with COVID-19 their transport to the respective clinics, was noticed the creation of medical teams for transport by ambulance ACLS with emergency doctor because other clinics which dealt with the treatment of patients with oncological problems verified or suspected of having COVID-19 did not know how to transport them. The establishment of medical teams with emergency transport physicians will enable a correct professional transport and in this way can avoid possible complications during transport. ED special spaces should be created, human resources educated and trained for palliative care treatment. A comprehensive national program should be established for a specific integrated program in emergency palliative care training, guidelines and protocols designed for emergency medical service providers at three levels of health care.

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Adiponektini. Roli i interleukinit-6 (IL-6) në mekanizmin e sëmundjes së sheqerit dhe rezistencës insulinore dhe roli i faktorit nekrotizues-tumoroz (TNF- α) në mekanizmin e trashësis, rezistencës insulinore dhe paradiabetit

Besim Memedi, Selim Qerkezi, Bekim Ismaili, Zamira Bexheti

Universiteti i Tetovës

Rrezyme

Hyrje: Sëmundja e sheqerit (Diabeti) është sëmundje e rëndësishme sociale, e cila kaplon përafërsisht 5 – 8% të popullatës (Kodama et al.,2013). Llojet më të shpeshta të diabetit janë: diabeti insulino vartës, tip-1 ose tipi i të rinjëve dhe diabeti insulino-vartës, tip-2 ose diabeti në moshë të pjekur.

Qëllimi: Studimi i efektit të fruktozës në metabolizmin e karbohidrateve, adiponektinës, insulinës dhe parametrave metabolitikë. Studimi i efektit të fruktozës në metabolizmin e karbohidrateve, adiponektinës, insulinës dhe parametrave metabolitikë

Roli i Interleukinit-6 (IL-6) në mekanizmin e sëmundjes së sheqerit dhe rezistencës insulinore. Roli i faktorit tumor-nekrotik (TNF- α) në mekanizmin e trashësis, rezistencës insulinore dhe paradiabetit

Materiali dhe metoda: Minjët të moshës së pjekur madhore, të gjinis mashkullore dhe femrore të llojit Wistar, (3 muajshe) 128 me peshë 160-180 g dhe minjtë e bardhë të moshës më të re (të moshës madhore të papjekur 10-25 ditë). Numri i përgjithshëm i minjëve ishte 65 dhe pesha fillestare prej 45-60 g, të gjitha minjtë u përdorën për qëllimet dhe detyrat e hulumtimit. Numri i kafshëve eksperimental në grupet individuale është midis 5-8 dhe detajet janë dhënë në seksionet përkatëse të seksionit të rezultateve. **Vaj palme**, aplikimi i të cilit bëheshte me sondë speciale me rrugë orale drejt sistemit gastro-intestinal

Rezultatet: Në figurën 1 janë paraqitura ndryshimet në TNF- α nën ndikimin e vajit të palmës. Statistikisht e rëndësishme është niveli i rritur i këtyre citokineve në gjakë të minjëve të rritur dhe të mitur në krahsim me grupet kontrolluese. Figura 2. Efekti i vajit të palmës (30 dhe 60 ditë) ndaj niveleve të gjakut të IL-6 te minjtë e vegjël (juvenile) dhe te të mëdhejt (adult) minjtë mashkull. Figura 3. Ndikimi i vajit të palmës, e aplikuar 30 dhe 60 ditë ndaj GTT të minjëve të mëdhenj të gjinis mashkullore. Rezultatet nga hulumtimet ndaj minjëve të vogël (jo të pjekur) nëpërmes GTT janë prezentuar në figurën e 4. Ata tregojn ndryshimet në GTT, të cilat janë me statistikë të rëndësishme në ditën e 60, ndaj 30 ditë nga trajtimi me vaj palme.

Përfundimi: Administrimi i zgjatur 60-ditor i vajit të palmës, te minjtë eksperimental të moshës së pjekur dhe të moshës minore rrisin nivelet e TNF- α dhe IL-6 në gjak, duke ndryshuar metabolizmin e karbohidrateve me të dhëna për zhvillimin e gjendjeve paradiabetike.

Fjalë kyçe : adiponektin, vaj palme, citokine.

Adiponektini. Roli i interleukinit-6 (IL-6) në mekanizmin e sëmundjes së sheqerit dhe rezistencës insulinore dhe roli i faktorit nekrotizues-tumoroz (TNF- α) në mekanizmin e trashësis, rezistencës insulinore dhe paradiabetit

Sëmundja e sheqerit (Diabeti) është sëmundje e rëndësishme sociale, e cila kaplon përafërsisht 5-8 % të popullatës (Kodama et al., 2013). Llojet më të shpeshta të Diabetit janë: diabeti insulino-vartës, tip-1 ose tipi i të rinjëve dhe diabeti insulino-vartës, tip-2 ose diabeti në moshë të pjekur. Patogjeneza e sëmundjes së sheqerit është e zgjeruar dhe kyç faktorë të ndryshëm si gjenetik, ushqyes, infeksione, stres, ndotësit e ambientit dhe tjerë, ku në shumicën e rasteve janë kombinimi i dy dhe më tepër faktorve. Udhëheqës i patogjenezës është çregullimi i funksionit të beta-qelizave të pankreasit, e cila prodhon dhe sekretion hormonin-insulin, i rëndësishëm për ndryshimet e karbohidrateve, metabolizmin te njeriu dhe te një numër i kafshëve eksperimental. Diabeti karakterizohet me çregullim të metabolizmit të karbohidrateve, proteineve dhe lipideve dhe është ndërlidhur me rrezikun e shumë komplikimeve.

Me të gjitha rëndësit shkencore dhe klinike ndaj diabetit të sheqerit bëjnë pjesë faktorë të shumtë të pasqaruar. Si p.sh : obeziteti, radikalet e lira të ritur, disa citokine dhe tjerë mundet të jenë shkaktarë për zhvillimin e diabetit të sheqerit. Analizat në literaturë ndaj rolit të adiponektinës janë publikuar nga ne (Gateva P, Besim M, Boyadjieva N, 2012). Janë dokumentuar këta fakte për adiponektinën: Adiponektina shprehet dhe sekretohet nga adipocitet. Ai është nga grupa e adipociteve kundërinfektiv dhe ka të dhëna se gjatë trashësis zvoglohet prodhimi dhe sekretimi saj. Rezultatet bindëse nga hulumtimet tregojn, se zvoglimi i sekretimit të adiponektinit pozitivisht korelon me rezistencën insulinore, ateriosklerozën dhe dislipidemin (Kadowaki, 2005). Adiponektina paraqitet nga citokinet e ndryshme, të cilat luajn rol gjatë ndezjeve. Është vërtetuar, se TNF- α dhe PPAR- γ zvoglojnë ekspresionin e genit për adiponektinën. Hulumtimet me substanca, të cilat rrisin ndjeshmërinë të receptorëve insulinor për insulin, tregojn rritjen e niveleve të adiponektinës te minjët dhe te njerzit. Te pacientët me trashësi të rëndësishme pas humbjes së peshës me ndikimin e shërimit me Orlistat, gjithahstu vërtetohen rritje të niveleve të adiponektinës dhe përmisimin e ndjeshmërisë insulinore (Despres et al., 2005).

Marur në përgjithësi, të gjitha faktet e publikuara tregojn rolin e prodhimit nga adipocitet të citokineve dhe adiponektineve në patogjenezën e diabetit tip 2. Të pakta janë hulumtimet e substancave farmakologjike, duke kyçur substancat me aplikim në kombinimet ushqimore, ndaj adiponektinës dhe diabetit. Përdorimi i gjërë i fruktozës si zëvendësues i glukozës parashtron

pyetjen për ndikimin ndaj adiponektinës. Pjesë nga eksperimentet shkencore fokusojnë efektin e fruktozës ndaj treguesve të ndryshimeve të karbohidrateve dhe adiponektinës.

Adiponektina rritet në proces të diferencimit të adipociteve (Carbone et al., 2012). Më të njohura janë tre izoforme të adiponektinës : me peshë të vogël molekulare, me peshë të mesme molekulare dhe me peshë të madhe molekulare (Magkos et al., 2007). Adiponektina- HMW është forma aktive më-biologjike dhe përcakton ndryshimet në adiponektinin total gjatë obezitetit (Almeda-Valdes et al., 2010). Adiponektina mundet të rris oksidimin në qeliza dhe të përmisoj ndjeshmërinë insulinor (Carbone et al., 2012), si dhe të nxisë glikogenogenezën në mëlqin e zezë, e cila fiziologjikisht reflekton në zvoglimin e nivelit të glukozës në gjakë. Adiponektina mundet të rrit kapjen e glikozës në muskuj nëpërmes rritjes së GLUT 4 (Utzschneider et al., 2006). Adiponektina është me aktivitet antinflamatorë dhe kjo me rolin e saj korelon në regullimin insulinor të T-qelizave (Carbone et al., 2012). L-6 kyçet në mekanizmat, nëpërmes të cilës trashësia aktivizon rezistencën insulinore. Autor të shumt përcaktojnë më me rëndësi rolin e TNF- α dhe të asaj në IL-6 në shkallë të aktivizimit të rezistencës insulinore. Njëra nga hipotezat është, se gjatë rritjes së indit të bardhë yndyror rritet hipoksia në adipocitet, e cila shprehet ndaj faktorit transkripcional hypoxia-inducible faktor-1 (HIF) me ndryshime të fundit në aktivizimin e NF- κ B dhe kjo çon deri te ndryshimet në funksionin e adipociteve dhe deri te sekretimi i TNF- α u IL-6 (Bruning et al., 2012).

Roli i faktorit tumor-nekrotik (TNF- α) në mekanizmin e trashësis, rezistencës insulinore dhe paradiabetit

TNF- α është citokin antiinflamator, e cila prodhohet nga qelizat e llojeve të ndryshme por kryesisht nga makrofaget dhe limfocitet. Ka të dhëna, se prodhohen nga qelizat yndyrore dhe gjatë trashësis prodhimi dhe sekretimi tyre rritet (Codoñer-Franch P et al., 2012). Hulumtimet ndaj prodhimit të TNF- α dhe trashësis tregojnë ndryshime në mes individëve me dhe pa rritje të masës yndyrore. Ka kundërshtime në literaturë , që tregojnë se jo çdo herë gjindet korelacion në mes rritjes së masës yndyrore dhe rritjes së sasisë së TNF- α në gjakë (Codoñer-Franch P et al., 2012). Kjo shkaktoj që hulumtuesit të qëndrojnë pas hipotezës, se faktorë të rëndësishëm të metabolizmit si leptini dhe adipokine tjera regullojnë sekrecionin e TNF- α . Shumica e hulumtuesve të bashkuar në përfundimin se janë të domosdoshme më shumë hulumtime, për tu prezentuar roli i TNF- α gjatë obezitetit tek njerzit, sepse më shumë fakte janë nga hulumtimet eksperimentale.

Në përfundim TNF- α është citokin i rëndësishëm me rolin e saj ndaj ndezjeve, lëshohet roli i saj në zhvillimin e trashësis. Faktet eksperimentale dhe klinike tregojn se TNF- α rregullojnë ndjeshmërin insulinore nëpërmes ndikimit ndaj receptorëve insulinor. Studimet ndaj substancave të ndryshme ushqyese dhe substancave farmakologjike për ndikimin ndaj TNF- α dhe rezikun e diabetit janë më të reja dhe sjellin fakte në diskutimin respektivisht roli i këtyre citokineve në mekanizmin e ndjeshmëris insulinore.

Roli i interleukin-6 (IL-6) në mekanizmin e sëmundjes e sheqerit dhe rezistencës insulinore

Më lartë theksuam, se IL-6 prodhohet dhe sekretohet nga indi yndyror. Hulumtimet e Curat et al (2004) tregojn, se adipocitet sekretojnë IL-6 dhe ai mer pjesë në rregullimin e homeostazës glukozare dhe indirore. Studimet e Chung et al (2006) dokumentojn reducimin e glukozë transportin GLUT4. Interesi në lidhje me studimin në punimin e radhës me fakte, se para-adipocitet sekretojnë konsiderueshëm më shumë sasi të IL-6, se sa adipocitet (Mack et al., 2009). Gjithashtu bindëse janë faktet nga një numër i madh i mësimëve të citokineve pro-inflamatore, pjesmarës në patogjenezën e sëmundjes së sheqerit (Calle MC et al., 2012). Hulumtimet (Bahceci M et al., 2007) ndaj vullnetarve të shëndoshë me ose pa obezitet dhe pacientët me sëmundje së sheqerit me ose pa obezitet tregojn nivele të rritura të IL-6 në shkallë të ndryshme, më të rritura gjatë obezitetit të shprehur. Me rëndësi është korelimi në mes rritjes së nivelit të IL-6 dhe rritja e nivelit të proteinës C-reaktive te pacientët me obezitet me ose pa diabet. Më shumë rritje vërehen gjatë rasteve ku njëkohsisht te pacientët shprehen 2 faktorë – diabeti dhe obeziteti. Gjithashtu ka të dhëna se IL-6 stimulon sekrecionin insulinor nëpërmes rritjes së GLP-1 ekspresionit në qelizat pankreatike (Ellingsgaard et al., 2011).

Rritja e niveleve të IL-6 ndërlidhen me dëmtimin e funksionit të mëlqisë së zezë, çregullim i ndryshimeve të karbohidrateve. Analizimi i fakteve tregon, se rritja e prodhimit dhe sekretimit të IL-6 gjatë obezitetit mund të sjell deri të vazhdimësisë së rritjes së prodhimit të insulinës dhe me këtë – deri të zvogëlimi i aktivitetit të funksionit të β -qelizave. Marur të gjithë faktet në literaturë, jepet bazë të rrumbullaksohet përfundimi se IL-6 të cilat sekretohen nga WAT, muskujt skeletor dhe mëlqisë së zezë (Wieckowska et al., 2008), kanë rolin në mekanizmin e ndjeshmëris insulinore dhe zhvillimin e diabetit tip 2.

Qëllimi

Studimi i efektit të fruktozës në metabolizmin e karbohidrateve, adiponektinës, insulinës dhe parametrave metabolitike

Roli i Interleukinit-6 (IL-6) në mekanizmin e sëmundjes së sheqerit dhe rezistencës insulinore.

Roli i faktorit tumor-nekrotik (TNF- α) në mekanizmin e trashësisë, rezistencës insulinore dhe paradiabetit

Materiali dhe metoda

Minjët të moshës së pjekur madhore, të gjinis mashkullore dhe femrore të llojit Wistar, (3 muajshe) 128 me peshë 160-180 g dhe minjtë e bardhë të moshës më të re (të moshës madhore të papjekur 10-25 ditë). Numri i përgjithshëm i minjëve ishte 65 dhe pesha fillestare prej 45-60 g, të gjitha minjët u përdorën për qëllimet dhe detyrat e hulumtimit. Numri i kafshëve eksperimental në grupet individuale është midis 5-8 dhe detajet janë dhënë në seksionet përkatëse të seksionit të rezultateve.

Kujdesi ndaj kafshëve eksperimental u zhvillua nën kushte standarde të temperaturës dhe kërkesa përkatëse për dhomë. Ushqimi i mijëve eksperimental përfshin të dy grupet (kryesisht për grupet e kontrollit) dhe regjimet e dietës të pasuruara me Vaj palme ose vaj derri (për grupet eksperimentale). Të gjithë minjët eksperimental kishin asje në ujë të llojit Ad Libitum. Gjatë hulumtimit të fruktozës të minjët që kishin qasje të tretësirave të fruktozës, e cila më mirë është përshkruar në rezultatet

Rezultatet

Rezultatet janë të paraqitura në figurat më poshtë. Në figurën 1 janë paraqitura ndryshimet në TNF- α nën ndikimin e vajit të palmës. Statistikisht e rëndësishme është niveli i rritur i këtyre citokineve në gjakë të minjëve të rritur dhe të mitur në krahasim me grupet kontrolluese (* $p < 0.05$; ** $p < 0.001$).

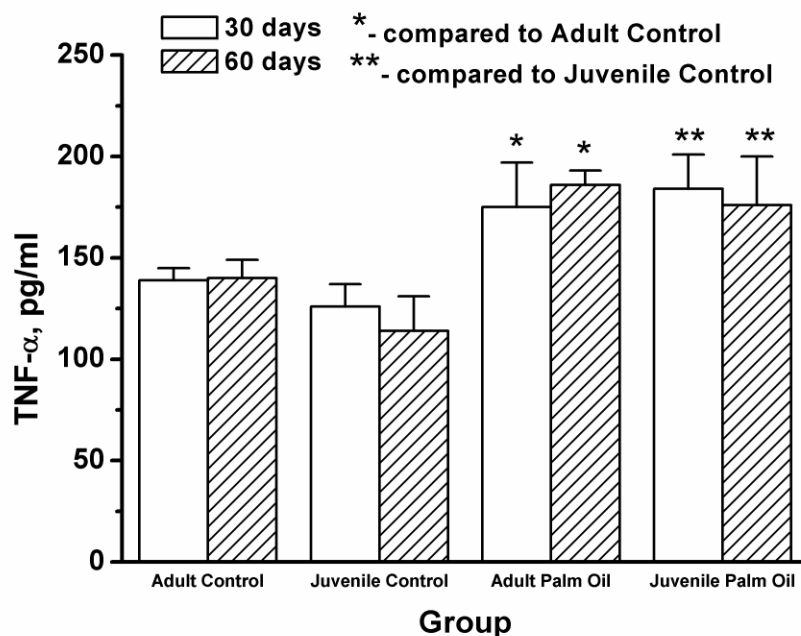


Figura 1. Nivelet e TNF- α në serum të gjakut të minjët e rritur dhe të vegjël të linjës Wistar me ose pa aplikim të vajit të palmës në vazhdimsi të 30 dhe 60 ditëve.

Analizat statistikore :

One way ANOVA: $P < 0.0001$: the variances among column means are significantly greater than expected by chance.

Bartlett: The SD's belong to the same population.

Bonferoni: $t_{cr} = 2.697$; H_0 : if $t > t_{cr}$, then $P < 0.05$

Diskusioni

Në kornizën e secilit grup të rritur aplikimi i vajit të palmës sjell deri te statistikat e rëndësishme (për minjët e vegjël $P < 0.001$, kurse për minjët e mëdhenj $P < 0.01$) nivelet e rritura të TNF- α në serum të gjakut. Fuqia e këtij efekti nuk ndikohet nga mosha, e as nga periudha e aplikimit të vajit të palmës. Rezultatet e ndryshimive në TNF- α nën ndikimin e vaji të palmës dokumentojn statistika të rëndësishme të rritjes së këtyre citokineve në gjakë, si të kafshëve të mëdhenj ashtu edhe kafshëve të vegjël eksperimental, krahsuar me grupet kontrolluese. Është e njohur se TNF- α është citokin pro-inflamator, për herë të parë rezultatet e dokumentuara flasin se ndodh rritja e saj nën ndikimin e aplikimit të vazhdueshëm të vajit të palmës te kafshët eksperimental të moshave të

ndryshme. Rezultatet japin bazë për të lejuar rezik të rritur nga ndezja si në moshën e re, poashtu edhe në moshë më të rritur nën ndikimin e vajit të palmës.

Hulumtimet i vazhduan me përcaktimin edhe të citokinit të dytë IL-6. Në figurën 2 prezentohen ndryshimet të IL-6 nën ndikimin e vajit të palmës. Janë raportuar ndryshime të rëndësishme të minjët e vegjël në dallim me minjët e moshës madhore, si dhe te të dy grupet dallimet janë statistikisht të rëndësishme në mes kafshëve të grupit kontrollues dhe të kafshëve të grupit eksperimental (hulumtues).

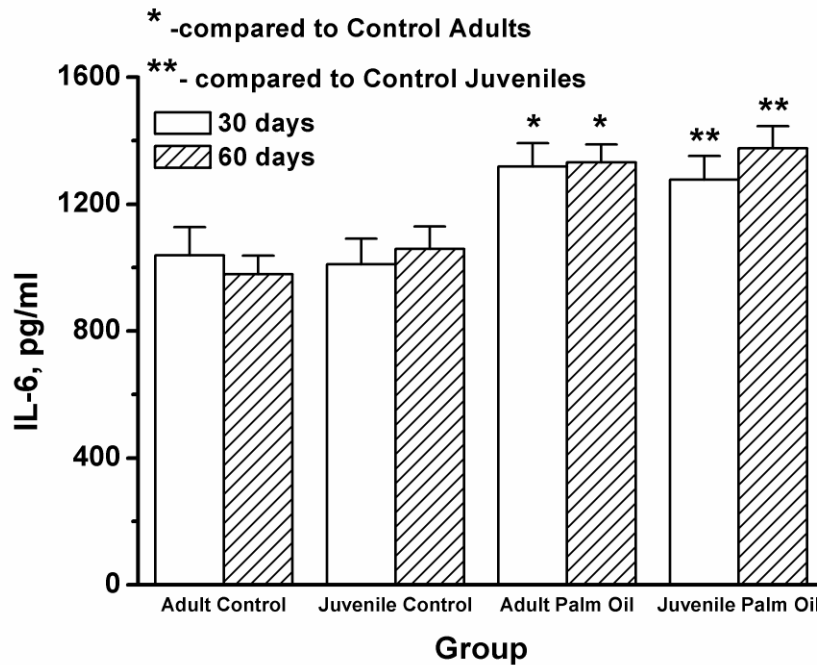


Figura 2. Efekti i vajit të palmës (30 dhe 60 ditë) ndaj niveleve të gjakut të IL-6 te minjët e vegjël (juvenile) dhe te të mëdhejt (adult) minjët mashkull.

Analizat statistikore të të dhënava

One way ANOVA: $P < 0.0004$. Variations among column means significantly differ than expected by chance; Bartlett: SD'd belong to the same population; Bonferony: critical $t = 3.418$.

Diskusioni

1. Apliki i vajit të palmës shkakton statistikë të rëndësishme ($P < 0.001$) të rritur në nivelet e IL-6 në të dy grupet
2. Në kushte të tjera të barabarta, nivelet e IL-6 nuk ndikohen nga vazhdimësia e hulumtimit, ashtu që rritja llogaritet , si në ditën e 30, ashtu edhe në ditën e 60 nga aplikimi i përditshëm i vajit të palmës.

Gjatë kohës së studimit u realizua edhe GTT (testi i tolerant glukozës) përkatsisht në ditën e 30 dhe 60.

Në figurën 3 (të radhës), prezentohen rezultatet nga GTT. Ata tregojn ndryshimet, të cilat janë më të rëndësishme në minutën e 90 dhe janë me të dhëna për paradiabet.

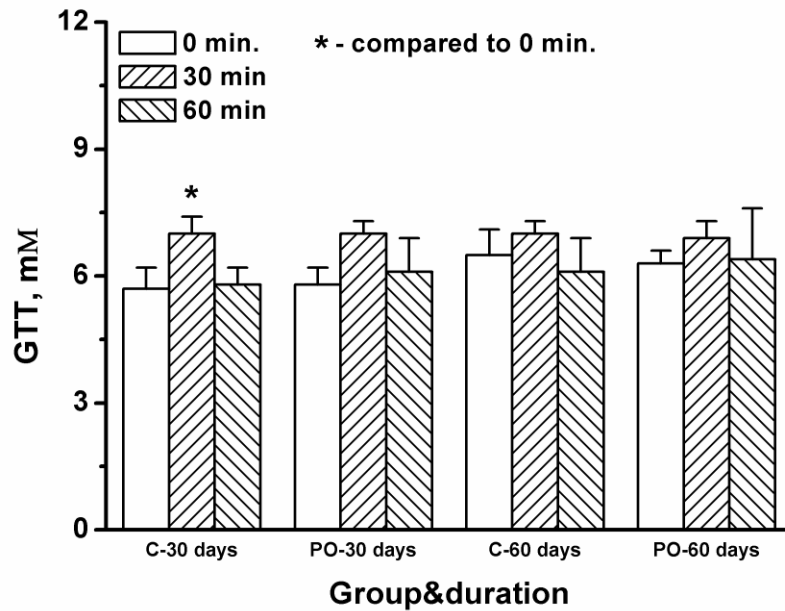


Figura 3. Ndikimi i vajit të palmës, e aplikuar 30 dhe 60 ditë ndaj GTT të minjëve të mëdhenj të gjinis mashkullore.

Rezultatet nga hulumtimet ndaj minjëve të vogë (jo të pjekur) nëpërmes GTT janë prezentuar në figurën e 4. Ata tregojn ndryshimet në GTT, të cilat janë me statistikë të rëndësishme në ditën e 60, ndaj 30 ditë nga trajtimi me vaj palme

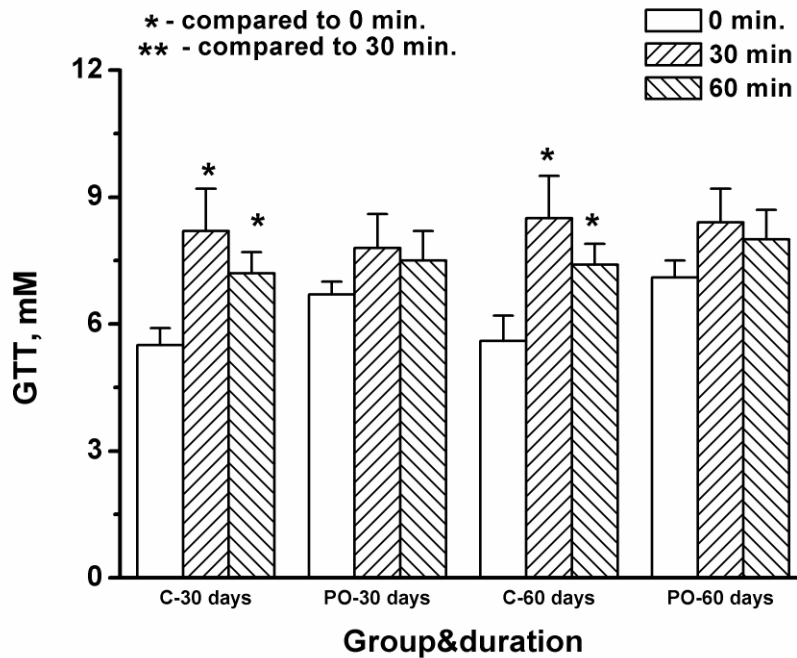


Figura 4. Ndikimi i vajit të palmës, e aplikuar 30 dhe 60 dië ndaj GTT të minjëve të vogël të gjinis mashkullore.

Diskusioni

Marur në përgjithsi, rezultatet demonstrojnë zhvillimin e paradiabetit me rezik më të rëndësishëm te minjët e vogël nën ndikimin e trajtimit kronik me vaj palme. Janë themeluar nivele të rritura të dy citokineve kundërinflamatore. Gjendja e paradiabetit korelon me rritjen e TNF- α dhe IL-6, për të parë ndryshimet në këtë studim demonstron për TNF- α dhe IL-6 në gjakun e minjëve eksperimental të gjinis mashkullore, nën ndikimin e aplikimit kronik të vajit të palmës. Është e njohur, se ki citokin mer pjesë në mekanizmin e ndezjeve (Michaud et al., 2013; Zhang et al., 2013). Në literaturë janë publikuar të dhënat, se inflamacioni ka rol në patogjenezën e sëmundjes së sheqerit. (Everard et al., 2013; Di marco et al., 2013; Pedicino et al., 2013; Game et al., 2013; Donathy, 2013; Cildir et al., 2013). Përveç kësaj të dhënat bindëse tregojn, se trashësia te disa

pacient është e ndërlidhur me rrezikun nga ndezja (Ramsay et al., 2013; Sobieska et al., 2013; Carillon et al., 2013; Grant et al., 2013; Borgesson et al., 2013; Wright et al., 2013).

Prezentimi i eksperimenteve hulumtuese dhe rezultatet e tyre demostrojnë, se vaji i palmës në gjakë i rrit nivelet e citokineve pro-inflamtoare. Kjo jep bazë që të supozojm se vërtetohet zhvillimi i paradiabetit te kafshët eksperimental ndodh për shkak të TNF- α dhe IL-6. Dallimet, të cilat janë dokumentuar në mes minjëve të mëdhenj dhe të vogël, japin bazë për të lejuar rrezik më të madh për zhvillimin e sëmundjes së sheqerit pas përdorimit të përditshëm dhe në vazhdimësi të vajit të palmës nga të rrit dhe adoleshentët. Këta të dhëna janë të parat në literaturë, por janë bazë për studimet e së ardhmes ndaj mekanizmave, nëpërmes të cilës vaj i palmës ndryshon si në citokinet e hulumtuara ashtu edhe metabolizmin e karbohidrateve.

Përfundimi

Adminstrimi i zgjatur 60 – ditor i vajit të palmës, te minjët eksperimental të moshës të pjekur madhore dhe të moshës minore (jo të pjekur), rrisin nivelet e TNF- α dhe IL-6 në gjakë dhe ndryshojn metabolizmin e karbohidrateve, me të dhëna për zhvillimin e gjendjes paradiabetike. Më e rëndësishme është rreziku që parqitet te kafshët eksperimental të moshës madhore jo të pjekur. Nëse bëjm një korelacion me njerëzit arijmë të konkludojm se personat që përdorin në vazhdimësi produkte ushqimore që përmbajnë vaj palme, do të jenë më të predisponuar për tu prekur nga kjo gjendje e çregullimit të shëndetit e cila shkakton paradiabet dhe deërgon pacientin drejt sëmundjes së sheqerit.

Rekomandimi

Nga rezultatet e prezentuar në këtë hulumtin shkencor, rekomandojm : kujdes me përdorimin e produkteve ushqimore që përmbajnë vaj palme, sidomos kujdes nga përdorimi i vazhdueshëm (çdo ditë) i këtyre produkteve ushqimeve. Kjo paraqet një edukim shëndetësor të mirfilltë sidomos për gjeneratat e reja dhe për adoleshentët

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Studimi i ndikimit të Metforminit ndaj efektit të vajit të palmës në metabolizmin e karbohidrateve

Doc.Dr. Besim Memedi¹ Prof.Dr.Nadka Bojaxhieva¹ Prof.Dr. Arian Idrizaj²

Universiteti i Tetovës. Universiteti i Mjekësis-Sofje. Universiteti Rezonanca-Prishtinë

Hyrje: Janë publikuar hulumtime për shpeshësin e përdorimit të metforminit te pacientët me komplikime të ndryshme nga përdorimi i ushqimit të pasur me yndyrna. Analiza e jonë ndaj një numri të madhë të studimeve tregon se: paralelisht me përmisimin e funksionit të mëlqis së zezë përmisohet edhe rezistenca insulinore. Viteve të fundit studiohen mekanizmat e ndikimit të metforminit ndaj rezistencës insulinore.

Qëllimi i prezentimit: përpjekja për tu studiuar ndikimin e metforminit te minjët eksperimental, që i trajtuam për një kohë të gjatë, në vazhdimsi me vaj të palmës.

Materiali dhe metoda: përpjekjet u realizuan ndaj minjëve meshkuj të rritur, të ndarë në 4 grupe, secila grup me nga 5-6 kafshë eksperimental. Vaji e palmës dhe metforminën e aplikojmë për os, periudha në mes vajit të palmës dhe metforminës ishte 4-5 orë dallim. Eksperimentet u realizuan në Universitetin shtetëror të Mjekësis në Sofje, pranë laboratorit për kërkime shkencore në katedrën e Farmakologjisë dhe Toksikologjisë

Rezultatet: Në **figurën 1**, janë vërtetuar dallime të rëndësishme statistikore në mes grupit hulumtues me vaj palme dhe grupit hulumtues me vaj palme + metformin në ditën e 60 të hulumtimit. Vazhdimsia e pranimit të Metforminit bashk me vaj palme zvoglon rritjen e peshës trupore të kafshëve eksperimental. Në **figurën e 2**, janë treguar rezultatet nga realizimi i TTG (Test tolerant i Glukozës). Analiza e këtyre të dhënave tregon se, nën ndikimin e vajit të palmës zhvillohet paradiabeti. Mirpo, nën ndikimin e metforminit pengohet kjo gjendje paradiabetie, kjo shihet nga rezultatet me vaj palme + grupi i trajtur me metformin. Në **figurën 3**, janë prezentuar rezultate e përcaktimit të INF- γ në ditën e 30 dhe 60 nga eksperimentet ndaj kafshëve të rritur eksperimental. Rezultatet tona tregojn, se vaji i palmës e bën uljen e nivelit të interferon gamës. Metformina antagonizon efektin supresiv të vajit të palmës në interferon gamën.

Fjalë kyçe : rezistenca insulinore, vaj palme, metformin.

Study on the impact of Metformin on the effect of palm oil in the metabolism of carbohydrates

Doc.Dr. Besim Memedi¹Prof.Dr.Nadka Bojaxhieva¹Prof.Dr. Arian Idrizaj²

University of Tetovo. Medical University Sofia. University of Medical Science Rezonanca-Prishtina

Introduction: Research has been published on the frequency of Metformin use in patients with various complications who have been using fat-rich foods. Our analysis of a large number of studies shows that: in parallel with the improvement of the function of the liver, insulin resistance is also improved. In the recent years, studies have been conducted on the impact of Metformin mechanisms on the insulin resistance.

Purpose of the Presentation: the attempt to study the impact of Metformin on the laboratory mice, treated for a longer period of time, continuously with palm oil.

Materials and Methods: experiments were conducted on adult male mice, divided into 4 groups, each group with 5-6 experimental animals. Palm oil and Metformin was applied per os. The period between palm oil and metformin appliance was 4-5 hours of difference. The experiments were carried out at the Medical University of Sofia, at the Scientific Research Laboratory of the Department of Pharmacology and Toxicology

Results:**Figure 1** shows significant statistical differences between the research group with palm oil and the research group with palm oil + Metformin, on the 60th day of the study. The continuation of Metformin's admission along with the palm oil reduces body weight gain of the experimental animals. **Figure 2** shows the results of TTG (Glucose Tolerance Test). The analysis of these data shows that, under the influence of palm oil, prediabetes is developed. However, under the influence of Metformin, this condition of prediabetes is prevented. This can be seen from the results of the use of palm oil + the group treated with Metformin. **Figure 3** shows the results of the determination of INF- γ on the 30th and 60th day of the experiments on the adult animals. The results show that palm oil reduces the level of interferon gamma. Metformin antagonizes the suppressive effect of palm oil in the interferon gamma.

Key words: insulin resistance, palm oil, Metformin

Hyrje

Janë publikuar hulumtime për shpeshhtësin e përdorimit të metforminit te pacientët me komplikime të ndryshme nga përdorimi i ushqimit të pasur me yndyrna. Angelico me bashkëpunorët (2007) publikojn studimet, të cilët dokumentojn rezultatet nga dhjetë studimet klinike, gjatë së cilës metformini ka përmisuar funksionin e heparit te pacientët me cirozë, distrofi të yndyrore, hepatitet (të gjitha sëmundjet jo-alkoolike ynydyrore të mëlqisë). Analiza e jonë ndaj një numri të madhë të studimeve tregon se, paralelisht me përmisimin e funksionit të mëlqisë së zezë përmisohet edhe rezistenca insulinore. Viteve të fundit studiohen mekanizmat e ndikimit të metforminit ndaj rezistencës insulinore. Ska të dhëna të publikuara për studimin e efektit të metforminit ndaj ndikimit të vajit të palmës dhe tregues të metabolizmit të karbohidrateve.

2. Qëllimi i prezentimit

Përpjekja për të studiuar ndikimin e metforminit te minjët eksperimental, që i trajtuam për një kohë të gjatë, në vazhdimisht me vaj të palmës.

3. Matriali dhe Metoda

Përpjekjet u realizuan ndaj minjëve meshkuj të rritur, të ndarë në 4 grupe, secila grup me nga 5-6 kafshë eksperimental

Grupa e parë : kontrulluese

Grupa e dytë: Eksperimentale me vaj palme – kafshët u trajtuan me vaj palme në vazhdimsi deri 60 ditë, një herë në ditë me dozë 1ml/100 gram

Grupa e tretë: Eksperimentale me metformin. Kafshët u trajtuan 30 ditë me metformin, një herë në ditë me dozë 100mg/kg nëpërmes gojës.

Grupa e katërt: Eksperimentale: kafshët u trajtuan në kombinim me vaj palme (1ml/100 gr) + metformin (100 mg/kg në ditë).

Eksperimentet u realizuan në Universitetin shtetëror të Mjekësis në Sofje, pranë laboratorit për kërkime shkencore në katedrën e Farmakologjisë dhe Toksikologjisë.

Të dy substancat aplikoheshin per os, periudha ne mes vajit të palmës dhe metformini ishte 4-5 orë dallim. Metforminin e kyçëm pas 30 ditve të parë pasi kishim aplikuar vaj palme në bazë të rezultateve që i kishim prezentur paraprakisht edhe në punime tjera me të dhëna për paraqitje e ndryshimeve të yndyrnave dhe karbohidrateve, pas trajtimit 30 ditor me vaj palme. (Kafshët nga grupa e 4 u trajtuan 60 ditë me vaj palme dhe 30 ditët e fundit edhe me metformin.

Rezultatet

Në **figurën e 1** prezentohen rezultatet nga përcaktimi i peshës trupore të kafshëve eksperimental. Janë vërtetuar dallime të rëndësishme statistikore në mes grupit me vaj palme dhe grupi me vaj palme + metformin në ditën e 60 të hulumtimit.

Vazhdimësia e Metforminin gjatë trajtimit me vaj palme zvoglon rritjen e peshës së kafshëve eksperimental. Tendenca e zvoglimit të masës së përgjithshme nën ndikimin e Metforminit vërehet gjithahstu gjatë krahsimit me grupin kontrollues.

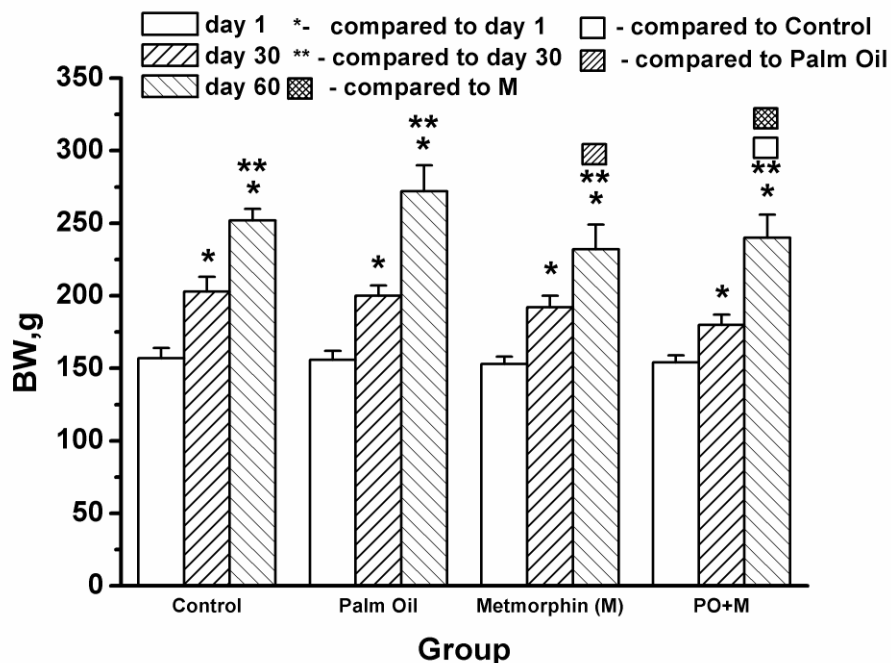


Figura 1. Ndikimi i bajit të palmës dhe metforminit ndaj peshës trupore të minjëve të mëdhenj eksperimental (hulumtimi kronik 60 – ditor)

Analizat statistikore

ONE-WAYANOVA: $P < 0.0001$; Bartlett: SD's belong to the same population; Bonferoni t critical=3.583.

Diskusioni

Statistikisht e rëndësishme është rritja e peshës trupore në tri grupet e kafshëve eksperimental. Rezultatite e 60-ditëve të studimit treguan dallime statistikisht të rëndësishme si më poshtë :

1. Kombinimi i vajit të palmës + metformin ndryshon peshën statistikisht të rëndësishme me grupin e kontrollit ($P < 0.001$) dhe në krahsim me grupin e trajtuar me metformin ($P < 0.001$).
2. Peshë e kafshëve eksperimental, të trajtuar me metformin dallon nga ajo peshë e kafshëve, të trajtuar me vaj palme

Në **figurën e 2** janë treguar rezultatet nga realizimi i TTG (Testi tolerant i Glukozës). Analiza e këtyre të dhënave tregon, se nën ndikimin e vajit të palmës zhvillohet paradiabeti. Statistikisht të rëndësishme janë ndryshimet në minutën e 90 nga testimi i ngarkimit të glukozës midis grupit të kontrollit dhe atyre që trajtohen me vaj palme për 60 ditë. Në ndikimin e metforminit pengohet paradiabeti, kjo shihet nga rezultatet me vaj palme + grupi i trajtuar me metformin. Rezultatet tregojn kthesa të ngushta të sheqerit në gjakë të kafshëve të grupit kontrollues. Vetë-administrimi i metforminës shoqërohet me rezultate të TTG, afër të atyre të grupit kontrollues.

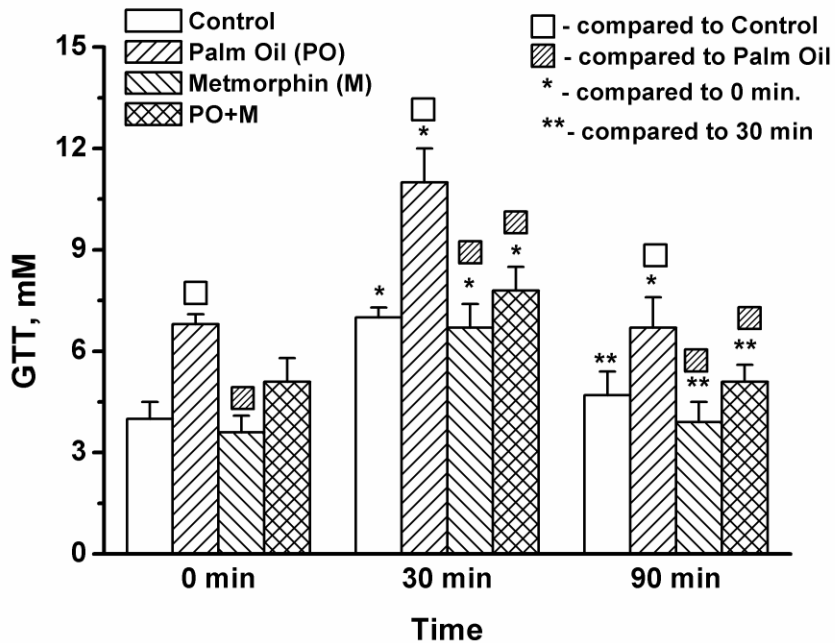


Figura e 2. Efekti i vajit të palmës dhe metformin ndaj rezistencës insulinore të kafshëve të rritur nga linja Wistar.

Analizat statistikore

One Way ANOVA: $P < 0.0001$; Bartlett: SD's belong to the same population; Bonferoni: critical $t = 3.598$

Diskusioni

Rezultatet e këtij studimi konfirmojn të dhënat e mësipërme se futja e vazhdueshme e vajit të palmës rezulton me një rritje të glukozës në gjak në të gjitha matjet në krahasim me grupin e kontrollit ($P < 0.001$ për tre vlerat e matura në minutën 0, 30 dhe 90). Aplikimi i Metforminit nuk ndryshon glukozën e gjakut të kafshët eksperimentale në krahasim me grupin kontrollues ($P > 0.05$ për tre vlerat e matura në minutën 0, 30 dhe 90). Dallimet janë vërejtur te kafshët që janë trajtuar vazhdimisht me vaj palme dhe metformina e cila është trajtuar gjatë 30 ditëve të fundit të studimit, te ata u vërtetua një ulje e glukozës në gjak dhe të dhëna për zvogëlimin e rezistencës insulinore gjatë analizës të rezultateve të TTG.

Përfundimi

Aplikimi i metforminit zvoglon rezistencën insulinore, e cila është zhvilluar gjatë përdorimit të vazhdueshëm të vajit të palmës. *Për herë të parë në literaturë raportohet ndikimi antagonistik i metforminit gjatë trajtimit me vaj palme.*

Hulumtimet i vazhduam me përcaktimin e insulinës në gjakun e kafshëve eksperimental. Në **tabelën 1** janë prezentuar rezultatet e insulinës në gjak. Efekti i vajit të palmës me ose pa metformin në përqëndrimet e gjakut të insulinë te minjët e mëdhenj të gjinis mashkullore * $P < 0.001$ krahsuar me grupin vetëm me vaj palme.

Grupa	Insulin në gjak нгр/мл
Kontrulluese	2.4 + 0.18
Metformin	2.1+ 0.2
Vaj Palme	2.9 + 0.05 a
Vaj palme + Metformi	2.0 + 0.09 *

Përpjekjet vazhduan me hulumtimin e efektit të vajit të palmës me ose pa metformin ndaj interferonit-gama, TNF- α dhe IL-6 të minjëve meshkuj.

Në **figurën 3** janë prezentuar rezultate e përcaktimit të INF- γ në ditën e 30 dhe 60 nga eksperimentet ndaj kafshëve të rritur eksperimental. Rezultatet tregojn, se vaji i palmës e bën uljen e nivelit të interferon gamës. Metformina antagonizon efektin supresiv të vajit të palmës në interferon gamën. Statistikisht e rëndësishme është diferenca midis vajit të palmës + metforminit dhe grupit vetem me vaj të palmës ($p < 0.001$)

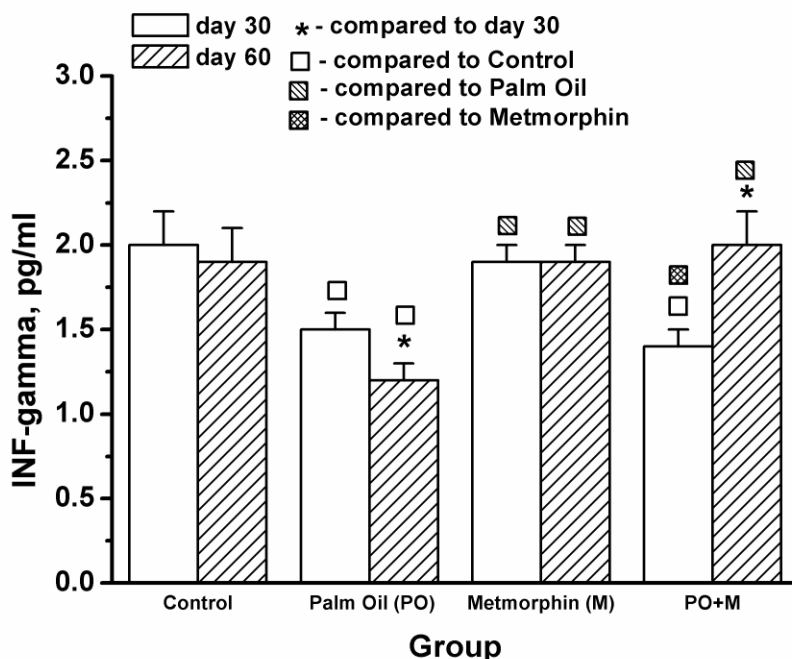


Figura e 3. Nivelet e INF- γ në gjakun e minjëve eksperimental të trajtuar vazhdimisht (30 dhe 60 ditë) me vaj palme, metformin ose kombinimi me vaj palme +metformin.

Analizat statistikore :

One-Way ANOVA: $P < 0.0001$, Bartlett: SD's belong to the same population, Bonferoni: critical $T = 3.308$.

Përfundimi : rezultatet te kafshët eksperimentaë nga hulumtimi i interferon gamës.

1. Trajtimi me vaj palme statistikisht rëndësishëm dërgon deri te zvoglimi i ($P < 0.001$ për të dy periudhat e hulumtimit) interferon gamës (INF- γ) për dallim me grupin kontrollues
2. Trajtimi vetëm me metformin nuk e ndryshon këtë tregues
3. Trajtimi i kombinuar me vaj palme dhe metformin ka efekte të ndryshme për periudhën nga 30 dhe 60 ditë : pas trajtimit të zgjatur 30 ditor, INF- γ kishte nivel tipik për grupën që kishte pranuar vaj të palmës ($P < 0.001$), kurse te grupi i trajtur në vazhdimsi prej 60 ditësh, ki nivel është afërsisht i ngjajshëm me grupin i cili ka pranuar metformin ($P < 0.001$).

Tabela 2. Nivelet e TNF $-\alpha$ (kg/ml) në gjakun e kafshëve të ritur eksperimental të trajtuar vazhdimisht (30 dhe 60 ditë) me vaj palme, metformin ose në kombinim me vaj palme +

metformin. * $P < 0.001$ në krahsim me grupin kontrollues, **a**- $P < 0.001$ krahsuar me grupin vetëm me vaj palme; **b**- $P < 0.001$ krahsuar me hulumtimin 30 ditor i grupit të njëjtë.

Grupi	TNF- α 30 ditë	TNF- α 60 ditë
Kontrollues	132.0 + 18.0	126.0 + 12.0
Metformin	116.0 + 9.0	111.0 + 14
Vaj Palme	184.0 + 11.0 *	198.0 + 8.0 *
Vaj palme +metformin (30 ditët e fundit)	179.0 + 7.2 *	121.0 + 11.8 a; b

Tabela 3. Nivelet IL-6 (kg/ml) në gjakun e kafshëve eksperimental të trajtuar në vazhdimsi (30 dhe 60 ditë) me vaj palme, metformin ose në kombinim me vaj palme + metformin.

Grupa	IL-6 30-ditë	IL-6 60-ditë
Kontrolluese	980.0 + 22.6	1060.0 + 16.4
Metformin	1020.0 + 31.4	840. + 28.6
Vaj Palme	1280.0 + 48.4	1320.0 + 36.1
Vaj Palme + Metformin	1140.0 + 25.9	1012.0 + 46.0

Përfundimi nga hulumtimi ndaj TNF- α dhe IL-6

1. Trajtimi me vaj palme e rrit nivelin e TNF- α dhe IL-6 edhe në ditën e 30 dhe në ditën e 60 të studimit.
2. Trajtimi vetëm me metformin nuk ndryshon të dy treguesit.
3. Metformini e zbrit si nivelin e TNF- α , ashtu edhe IL-6 në gjakun e minjëve, të trajtuar vazhdimisht me vaj palme.

Diskusioni

Hulumtimet me metformin demonstuan, se kjo substancë farmakologjike ka aftësi të antogonizon aktivitetin diabetogjen me vaj palme. Gjatë këtyre eksperimenteve prezantohet diçka e re në shkencën e mjeksisë, edhe atë : aplikimi i vazhdueshëm i vajit të palmës e zvoglon nivelin e interferon gamës në gjakë, e cila korrespondon me aktivitetin e tij diabetik. *RISI për efektin e metforminit është ndërveprimi me mekanizmat, nëpërmes të cilës vaji i palmës zvoglon intereferon gamën.* Rezultatet tregojn, se metformini antagonizon, si ndikimin diabetogjen të vajit të palmës ashtu edhe ndikimin ndaj interferonit. Rezultatet nga këta studime japin arsye për të supozojm, se vaji i palmës shkakton paradiabet nëpërmes zvoglimit edhe të interferon gamës, e cila është protektor i rëndësishëm i qelizave imune dhe e beta qelizat e pankreasit. (Durham et al 2008; Frank et al 2011; Tinworth et al., 2010; Tinworth et al., 2012; Wilcock et al., 1994).

Rezultatet të prezentuar më lartë për herë të parë demostrojn, se metformini antagonizon efektin e vajit të palmës ndaj 2 citokineve pro-inflamatore gjatë eksperimenteve shkencore. Është e njohur, se gjatë trashësis (obezitetit) këta citokine rriten. Aftësia e metforminit për të zvogluar përqëndrimin e saj është fakt i rëndësishëm për këtë substancë farmakologjike dhe barë, i aplikuar në praktikë. Marë së bashku rezultatet nga hulumtimet ndaj metabolizmit të karbohidrateve, intereferon gama dhe 2 citokinet pro-inflamatore japin arsye (bazë) për të pranuar efektin e metforminit në profilaktikën e beta-qelizave të pankreasit nëpërmes ndikimit të mekanizmave imunologjik. Studimet e ardhshme do të tregojnë rëndësinë e këtij supozimi.

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Study on the impact of Metformin on the effect of palm oil in the metabolism of carbohydrates

Doc.Dr. Besim Memedi¹ Prof.Dr.Nadka Bojaxhieva¹ Prof.Dr. Arian Idrizaj²

University of Tetovo. Medical University Sofia. University of Medical Science Rezonanca-Prishtina

Introduction: Research has been published on the frequency of Metformin use in patients with various complications who have been using fat-rich foods. Our analysis of a large number of studies shows that: in parallel with the improvement of the function of the liver, insulin resistance is also improved. In the recent years, studies have been conducted on the impact of Metformin mechanisms on the insulin resistance.

Purpose of the Presentation: the attempt to study the impact of Metformin on the laboratory mice, treated for a longer period of time, continuously with palm oil.

Materials and Methods: experiments were conducted on adult male mice, divided into 4 groups, each group with 5-6 experimental animals. Palm oil and Metformin was applied per os. The period between palm oil and metformin appliance was 4-5 hours of difference. The experiments were carried out at the Medical University of Sofia, at the Scientific Research Laboratory of the Department of Pharmacology and Toxicology

Results: **Figure 1** shows significant statistical differences between the research group with palm oil and the research group with palm oil + Metformin, on the 60th day of the study. The continuation of Metformin's admission along with the palm oil reduces body weight gain of the experimental animals. **Figure 2** shows the results of TTG (Glucose Tolerance Test). The analysis of these data shows that, under the influence of palm oil, prediabetes is developed. However, under the influence of Metformin, this condition of prediabetes is prevented. This can be seen from the results of the use of palm oil + the group treated with Metformin. **Figure 3** shows the results of the determination of INF- γ on the 30th and 60th day of the experiments on the adult animals. The results show that palm oil reduces the level of interferon gamma. Metformin antagonizes the suppressive effect of palm oil in the interferon gamma.

Key words: insulin resistance, palm oil, Metformin

Introduction

Research has been published on the frequency of metformin use in patients with various complications from the use of fat-rich foods. Angelico and colleagues (2007) published studies that document the results of ten clinical studies, during which metformin has improved hepatocytesfunction in patients with cirrhosis, lipodystrophy, hepatitis (all non-alcoholic fatty liver diseases). Our analysis of a large number of studies shows that, in parallel with the correction of black liver function, insulin resistance is also improved. In recent years, metformin's mechanisms of action against insulin resistance have been studied. There is no published data on the study of the metformin effect on palm oil influence and carbohydrate metabolism indicators.

2. Purpose of presentation

Attempting to study the impact of metformin on experimental mice, which we treated for a long time, consistently with palm oil.

3. Materials and Methods

Efforts were made on adult male mice, divided into 4 groups, each group with 5-6 experimental animals.

First group:controlling

Second group:Experimental with palm oil – the animals were treated with palm oil for up to 60 days, once a day with a dose of 1ml/100 grams

Third group:Experimental with metformin. The animals were treated for 30 days with metformin, once daily with a dose of 100mg/kg orally.

Fourth group: Experimental: animals were treated in combination with palm oil (1ml/100g) + metformin (100mg/kg per day).

The experiments were carried out at the State Medical University in Sofia, at the Department of Pharmacology and Toxicology Research Laboratory.

Both substances were applied per os, the period between palm oil and metformin application was 4-5 hours difference. Metforminwas introduced 30 days after we applied palm oil based on the results we had previously presented in other works with data on the presentation of changes in fats

and carbohydrates, after 30 days of palm oil treatment. (Animals from Group 4 were treated with 60 days of palm oil and the last 30 days with metformin).

Results

Figure 1 shows the results from the determination of body weight of experimental animals. Significant statistical differences were found between the palm oil and palm oil + metformin group on the 60th day of the study.

The continuation of Metformin during palm oil treatment reduces the weight gain of the experimental animals. The tendency of the reduction of the total mass under the influence of Metformin is also observed during the comparison with the control group.

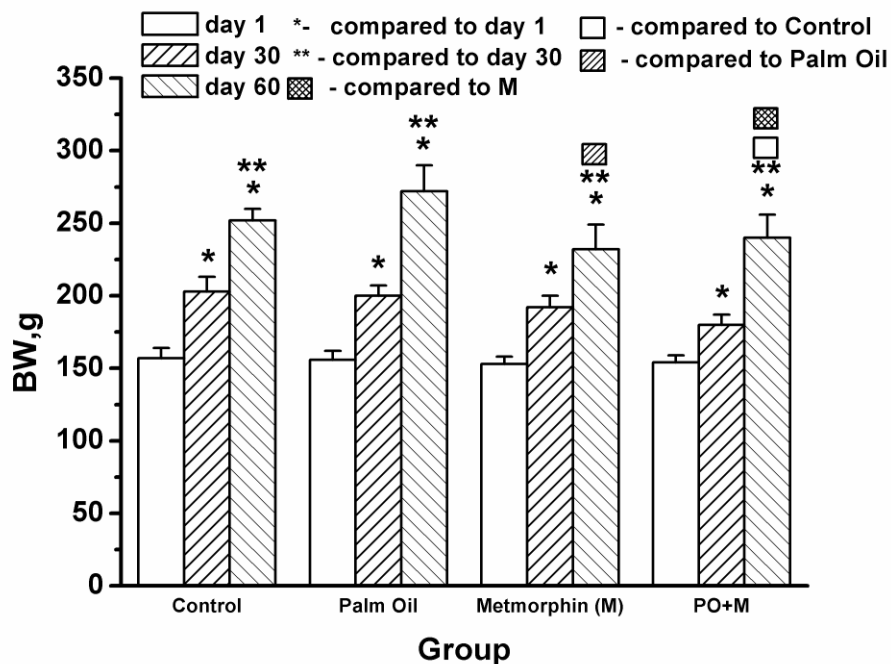


Figure 1. Influence of palm oil and metformin on the body weight of large experimental mice (60-day chronological research)

Statistical analysis

ONE-WAYANOVA: $P < 0.0001$; Bartlett: SD's belong to the same population; Bonferoni t critical=3.583.

Discussion

Statistically important is the increase in body weight in three experimental animal groups. The 60-day study results show statistically significant differences as follows:

1. The combination of palm oil + metformin changes the statistically significant weight with the control group ($P < 0.001$) and in comparison to the metformin-treated group ($P < 0.001$).
2. The weight of experimental animals treated with metformin differs from that of the animal weight, treated with palm oil

Figure 2 shows the results of the conducted GTT (Glucose Tolerance Test). The analysis of these data shows that under the influence of palm oil, prediabetes is developed. Statistically significant are changes in the 90th minute by the glucose loading test between the control group and those treated with palm oil for 60 days. Under the influence of metformin, prediabetes is prevented, which can be seen from the results of palm oil + metformin-treated group. The results show narrow blood sugar curves of the control group's animals. Metformin self-administration is associated with GTT results, close to those of the control group.

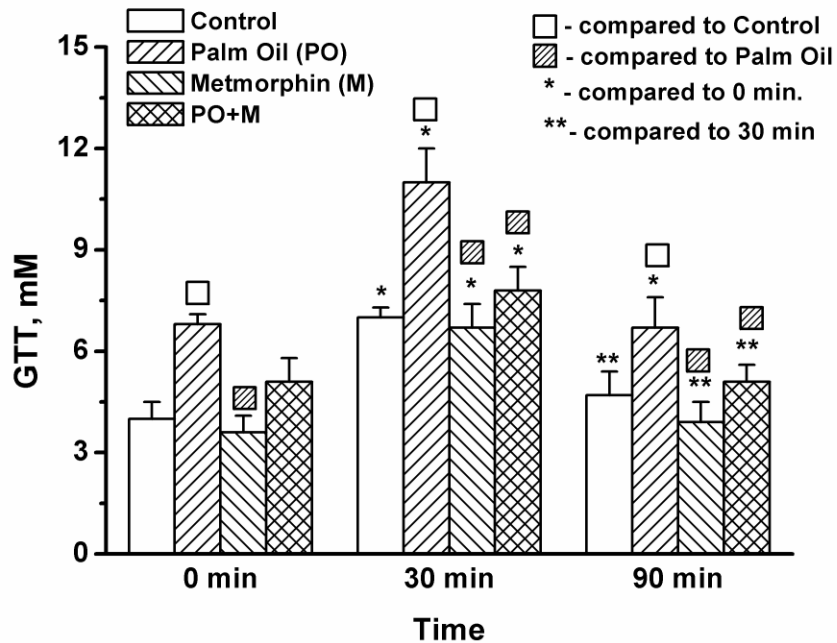


Figure 2. The effect of palm oil and metformin on insulin resistance of adult animals from the Wistar line.

Statistical analysis

One Way ANOVA: $P < 0.0001$; Bartlett: SD's belong to the same population; Bonferoni: critical $t = 3.598$

Discussion

The results of this study confirm the above data that the continuous intake of palm oil results in an increase in blood glucose in all measurements parallel to the control group ($P < 0.001$ for the three values measured at 0, 30 and 90). The application of Metformin does not alter blood glucose to the experimental animals alongside the control group ($P > 0.05$ for the three measured values at 0, 30 and 90 minutes). Differences have been observed in animals that have been treated continuously with palm oil and Metformin which was treated during the last 30 days of the study. They showed a decrease in blood glucose and data was obtained for the reduction of the insulin resistance during the GTT results.

Conclusion

The application of Metformin reduces the insulin resistance, which is developed during the continuous use of palm oil. *For the first time in the literature, the antagonistic metformin influence is reported in palm oil treatment.*

Research continued with the determination of insulin in the experimental animal blood. **Table 1** shows the results of insulin in the blood. The effect of palm oil with or without Metformin in the blood concentrations of insulin at the large male mice * $<<0.001$ compared with group with palm oil alone.

Group	Insulin in the bloodngr/ml
Control	2.4 + 0.18
Metformin	2.1+ 0.2
Palme oil	2.9 + 0.05 a
Palm oil + Metformin	2.0 + 0.09 *

Efforts continued with the research of the effect of palm oil with or without metformin to interferon-gamma, TNF- α and IL-6 of male mice.

Figure 3 shows the results of the determination of INF- γ on day 30 and 60 from experiments on experimental adult animals. The results show that palm oil reduces interferon gamma levels. Metformin antagonizes the suppressive effect of palm oil in the interferon gamma. Statistically significant is the difference between palm oil + metformin and palm oil only ($p < 0.001$)

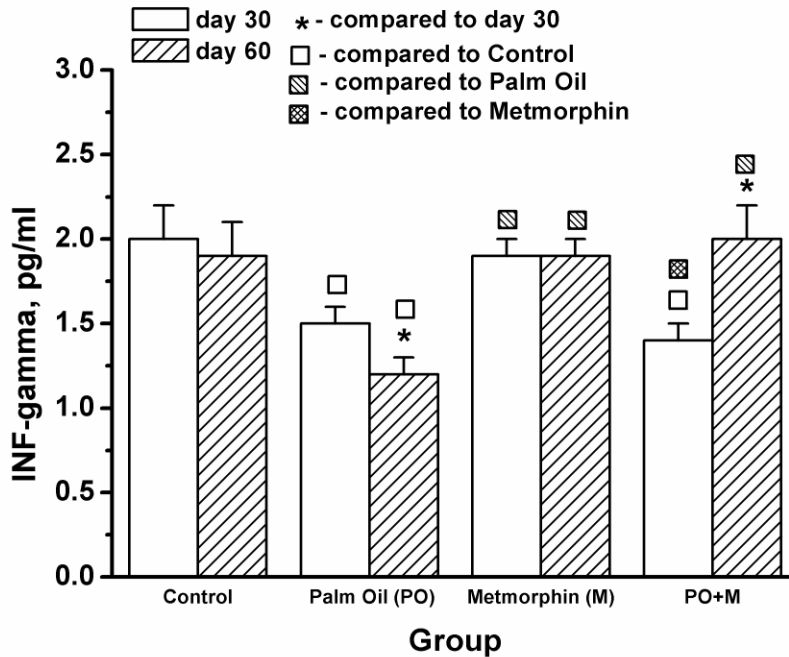


Figure 3. The levels of INF- γ in the blood of experimental mice continuously treated (30 and 60 days) with palm oil, metformin or combination with palm oil + metformin.

Statistical analysis:

One-Way ANOVA: $P < 0.0001$, Bartlett: SD's belong to the same population, Bonferoni: critical $T = 3.308$.

Conclusion: results of experimental animals from the interferon gamma research.

1. Treatment with palm oil is statistically significant leads to the decrease of ($P < 0.001$ for both study periods) interferon gamma (INF- γ) unlike the control group.
2. Treatment with metformin alone does not change this indicator.
3. The combined treatment with palm oil and metformin has different effects for the period of 30 and 60 days: after 30 days of prolonged treatment, INF- γ had a typical level for the group receiving palm oil ($P < 0.001$), whereas in the group treated continuously for 60 days, this level is approximately similar to the group that has received metformin ($R < 0.001$).

Table 2. TNF- α (kg/ml) levels in the blood of the experimental adult animals treated continuously (30 and 60 days) with palm oil, metformin or in combination with palm oil + metformin. * P <0.001 in comparison with the control group, **a**-P <0.001, compared with the palm oil group only; **b**- <<0.001 compared with the 30-day research of the same group.

Group	TNF- α 30 days	TNF- α 60 days
Control	132.0 + 18.0	126.0 + 12.0
Metformin	116.0 + 9.0	111.0 + 14
Palme oil	184.0 + 11.0 *	198.0 + 8.0 *
Palme oil+metformin (30 last days)	179.0 + 7.2 *	121.0 + 11.8 a; b

Table 3. Levels IL-6 (kg / ml) in experimental animal blood treated continuously (30 and 60 days) with palm oil, metformin or in combination with palm oil + metformin.

Group	IL-6 30-days	IL-6 60-days
Control	980.0 + 22.6	1060.0 + 16.4
Metformin	1020.0 + 31.4	840. + 28.6
Palme oil	1280.0 + 48.4	1320.0 + 36.1
Palme oil + Metformin	1140.0 + 25.9	1012.0 + 46.0

Conclusions from TNF- α and IL-6 research:

1. Palm oil treatment increases the level of TNF- α and IL-6 on day 30 and on day 60 of the study.
2. Treatment with metformin alone does not change the two indicators.

3. Metformin decreases both TNF- α and IL-6 levels in mice blood, treated continuously with palm oil.

Discussion

Metformin research has demonstrated that this pharmacological substance has the ability to antagonize the palm oil diabetogenic activity. During these experiments, something new is presented in the medical science: the continuous Palm oil application reduces the level of interferon gamma in the bloodstream, which corresponds to its diabetogenic activity. *An novelty for the effect of metformin is the interaction with the mechanisms through which palm oil reduces interferon gamma.* The results show that metformin antagonizes both the Palm oil's diabetogenic influence and its impact on interferon. The results from these studies give reason to suppose that palm oil causes prediabetes by reducing the interferon gamma, which is the important protector of immune cells and beta cells of the pancreas. (Durham et al 2008; Frank et al 2011; Tinworth et al., 2010; Tinworth et al., 2012; Wilcock et al., 1994).

The results presented above, for the first time demonstrate that metformin antagonizes the effect of palm oil to 2 pro-inflammatory cytokines during scientific experiments. It is known that these cytokines grow in case of obesity. Metformin's ability to reduce its concentration is important for this pharmacological substance and drug applied in practice. Taken together, the results from carbohydrate metabolism research, interferon gamma and 2 pro-inflammatory cytokines give reason (base) to accept the effect of metformin on prophylaxis of pancreatic beta cells through the influence of immunological mechanisms. Future studies will show the importance of this assumption.

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Adiponectin and the role of interleukin-6 (IL-6) in insulin resistency and diabetes mechanism and the role of tumor necrosis factor (TNF- α) in pre-diabetes, insulin resistency and obesity mechanisms

Besim Memedi, Selim Çerkezi, Bekim Ismaili, Muhamed Tairi

Faculty of Medical Sciences, University of Tetova

Abstract

Introduction: Diabetes represents an important social disease, which affects almost 5-8% of world population. (Kodama et al.,2013). The most common forms of diabetes are: type I diabetes, insulin dependent, or youth type, and type II diabetes, or non-insulin dependant, also adulthood diabetes.

Aim of study: Fructose effect on carbohydrate, adiponectin, insulin and other metabolic parameters metabolism. The role of interleukin 6 (IL-6) in mechanism of insulin resistency and diabetes and the role of tumor necrosis factor (TNF- α) in mechanisms of obesity, insulin resistance and pre-diabetes.

Material and methods: 128 Adult rats (3 months of age), Wistar species of both genders, weighting 160-180g and 65 white young rats (10-25 days) weighting 45-60g were taken for purposes of this study. The number of experimental animals in individual groups is between 5-8 while the details are added in the results section. The application of **palm oil** was conducted through special probe using oral administration straight into gastro-intestinal system.

Results: Figure 1 shows the differences of TNF- α after palm oil administration. There are statistically significant raised levels of this cytokines in blood of both young and adult rats compared to control cases. In Figure 2 it is emphasized the effect of palm oil (30 and 60 days) in IL-6 blood levels of young and adult male rats. Figure 3 shows the impact of palm oil, applied 30 and 60 days respectively against GTT of adult rats of male gender. The results of GTT studies on young rats are shown in Figure 4. They show the differences in GTT, which are statistically significant in day 60 compared to 30 days of palm oil treatment.

Conclusion: Elongated administration 60 days of palm oil in experimental rats of adult and young rats, raise the blood levels of TNF- α and IL-6, thus changing metabolism of carbohydrates with data supporting pre-diabetic conditions.

Key words: adiponectin, palm oil, cytokines

Adiponectin and the role of interleukin-6 (IL-6) in insulin resistency and diabetes mechanism and the role of tumor necrosis factor (TNF- α) in pre-diabetes, insulin resistency and obesity mechanisms.

Diabetes is an important social disease, affecting 5-8% of world population. The most common forms of diabetes are: type I diabetes, insulin dependent, or youth type, and type II diabetes, or non-insulin dependant, also adulthood diabetes.

The pathogenesis of diabetes is wide and many key factors are included like genetics, nourishing, infection, stress, enviroment pollutants and others, while in most cases there is a combination of two or more of those factors. The main pathogenic event is the distrupcion of pancreatic beta-cells function, which produce and secrete the hormone - insulin, important for carbohydrate metabolism in both human and a lot of experimental naimals. Diabetes is characterized with disruption of carbohydrate, protein and lipid metabolism and it is related with many risk complications.

Many factors leading to diabetes are not clear enough, though. We can mention here obesity, free radicals and some cytokines which may be responsible for diabetes development. Literature analysis of adiponectin role are published by (Gateva P, Besim M, Boyadjieva N, 2012). It is documented that adiponectin is produced and secreted by adipocytes. It belongs to anti-infective adipocites and there is data that proves that during weight gaining, adiponectin production and secretion is reduced. The research results clearly show that reduced production of adiponectin is positively correlated with insulin resistency, atherosclerosis and dislipidemy (Kadowaki, 2005). Adiponectin appears from different cytokines which are active during inflammation. It is also proved that TNF- α and PPAR- γ reduce gene expressin for adiponectin. Studies on substances which raise the sensitivity of insulin receptors for insulin, show that adiponectin levels in both human and rats are raised. Over-weight patients, after losing weight show the tendency of raising adiponectin levels and also improve insulin resistency. (Despres et al., 2005).

Generally, all the published facts show that adiponectin produced by cytokines and adipocytes has a role on type II diabetes pathogenesis. The widely fructose use as a replacement for glucose raise the question for its effect on adiponectin. There are only few studies on farmacological substances and food combinations. Some of the studies are focused on the effect of fructose towards indicator changes of carbohydrates and adiponectin.

Adiponectin is raised during adipocyte differentiation (Carbone et al., 2012). Three isoforms of adiponectin are well known: low molecular weight, medium- and high molecular weight (Magkos et al., 2007). HMW adiponectin is the most biologically active form and it establishes changes of total adiponectin during obesity (Almeda-Valdes et al., 2010). Adiponectin can also rasiie the cell oxidation, thus improving insulin resistency (Carbone et al., 2012), and also can stimulate gluconeogenesis in the liver, which physiologically reflects on lowering blood glucose levels. Adiponectin also has inflammatory activity and it's role is in insulin regulation of T-Cells (Carbone et al., 2012). IL-6 is added onto mechanisms, through

which obesity activates insulin resistance. Many authors establish the important role of TNF- α and IL-6 in activating insulin resistance. One of the hypothesis is that, during adipose tissue growth there is increased adipocyte hypoxia, which is expressed against transcriptional hypoxia inducible factor 1, with changes in NF- κ B activation, ultimately leading to adipocyte function changes and TNF- α and IL-6 secretion (Bruning et al., 2012).

The role of tumor necrosis factor-alpha (TNF- α) in mechanism of weight gaining, insulin resistance and pre-diabetes

TNF- α is an anti-inflammatory cytokine, which is produced by various types of cells, but mainly from macrophages and lymphocytes. There is data that suggest that is also produced by fat cells during weight gaining and also that its secretion is also raised. The studies on TNF- α and weight gaining show that there is difference between persons with and without fat mass increase. Also there are studies with show the contrary, that there is no correlation between these two parameters (Codoñer-Franch P et al., 2012). This has led researches to stand behind the hypothesis that leptin, adiponectin and other important metabolic factors regulate the secretion of TNF- α . Most of the researches have come to the conclusion that more studies are required to present role of TNF- α in humans in obesity, as most of the facts so far are based on experimental studies.

As TNF- α is an important inflammatory cytokine, its role in obesity development is undervalued. Clinical and experimental studies suggest that TNF- α regulates insulin sensitivity through insulin receptors. The studies on different nourishing and farmacological substances on TNF- α and diabetes risk are new and provide more facts on discussion about the role of cytokines in insulin sensitivity mechanism.

The role of interleukin 6 (IL-6) in insulin resistance and diabetes mechanism

As we have already state, IL-6 is produced and secreted by fat tissue. Curat et al (2004) studies show that adipocytes secrete IL-6 and that it does take part on glucose and lipid homeostasis regulation. Chung et al (2006) studies document decrease of GLUT4 transporters. There are studies that suggest that para-adipocytes secrete considerably more IL-6 than adipocytes (Mack et al., 2009). Also, we can agree that pro inflammatory cytokines take part on diabetes pathogenesis (Calle MC et al., 2012). Studies (Bahceci M et al., 2007) on healthy persons with/without obesity and on diabetic patients with/without obesity has shown different raised levels of IL-6 mainly on obesse ones. It is important the correlation between IL-6 raised levels and raised C-reactive protein on obesse people with or without diabetes. The highest values are shown where both factors are present, obesity and diabetes. Also data suggests that IL-6 stimulates insulin secretion through increased GLP-1 expression on pancreatic cells (Ellingsgaard et al., 2011).

Raised levels of IL-6 are linked with damaged function of the liver, and carbohydrate disturbance. Analysis show that increased production and secretion of IL-6 in obesity leads to increased production of insulin and thus leading to decrease of pancreatic beta cells function decrease. Provided all the published materials, it can be concluded that IL-6 produced by WAT,

skeletal muscle and liver, has a role on mechanisms of insulin sensitivity and type 2 diabetes development.

Aim of study

Fructose effect on carbohydrate, adiponectin, insulin and other metabolic parameters metabolism. Interleukin 6 role on diabetes and insulin resistance mechanism. The role of TNF- α in obesity, insulin resistance and pre-diabetes mechanisms.

Materials and methods

128 Adult rats (3 months of age), Wistar species of both genders, weighting 160-180g and 65 white young rats (10-25 days) weighting 45-60g were taken for purposes of this study. The number of experimental animals in individual groups is between 5-8 while the details are added in the results section.

Standard conditions of specific rooms and temperature were met as was needed for experimental animals care. For the control group it was provided palm oil while for experimental group pig oil was used. All of the rats had water acces of Ad Libitum type. During fructose studies rats had access on fructose solution, which is described better on result section.

Results

Results are shown in Figures below. Figure 1. shows TNF- α changes under palm oil influences. Raised levels of these cytokines in adult rats blood compared to control group are statistically significant. (* $p < 0.05$; ** $p < 0.001$).

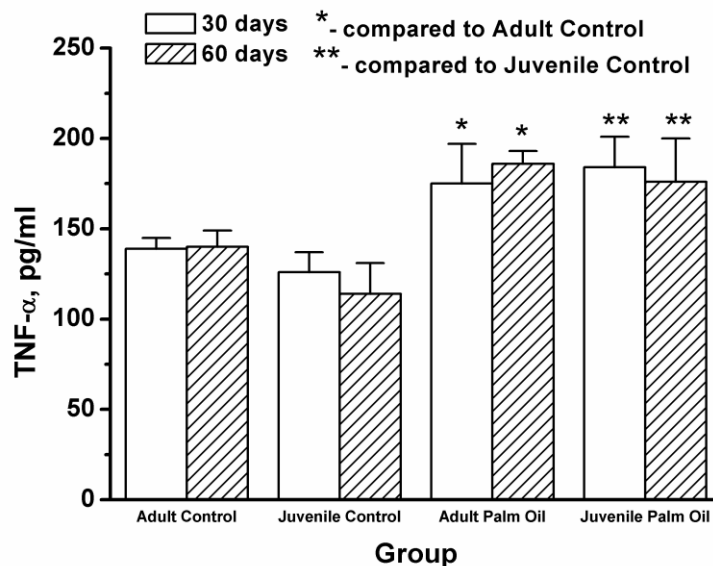


Figure 1. TNF- α levels in adult and young Wistar line rats serum with or without application of palm oil during 30 and 60 days.

Statistical analysis

One way ANOVA: $P < 0.0001$: the variances among column means are significantly greater than expected by chance.

Bartlett: The SD's belong to the same population.

Bonferoni: $t_{cr} = 2.697$; H_0 : if $t > t_{cr}$, then $P < 0.05$

Discussion

In each group of rats palm oil application bring to statistically significant raised levels of TNF- α in blood serum ($p < 0.001$ for young rats, $p < 0.01$ for adult ones). The power of this effect is not influenced neither from age nor from timing of palm oil application. Results of raised levels of TNF α under the influence of palm oil application show statistically significant results both on adult rats and young rats compared to control groups. It is well known that TNF α is a pro-inflammatory cytokine, and for the first time the results show that its levels raise after continued application of palm oil in experimental animals of different ages. Results show that there is an increased risk either on young or adults under palm oil influence.

We have continued studies on defining second cytokine IL-6. In figure 2 are shown changes of IL-6 levels under palm oil application. Reports show significant changes at young rats compared to adult rats, and also both groups show statistically significant between experimental group and control group.

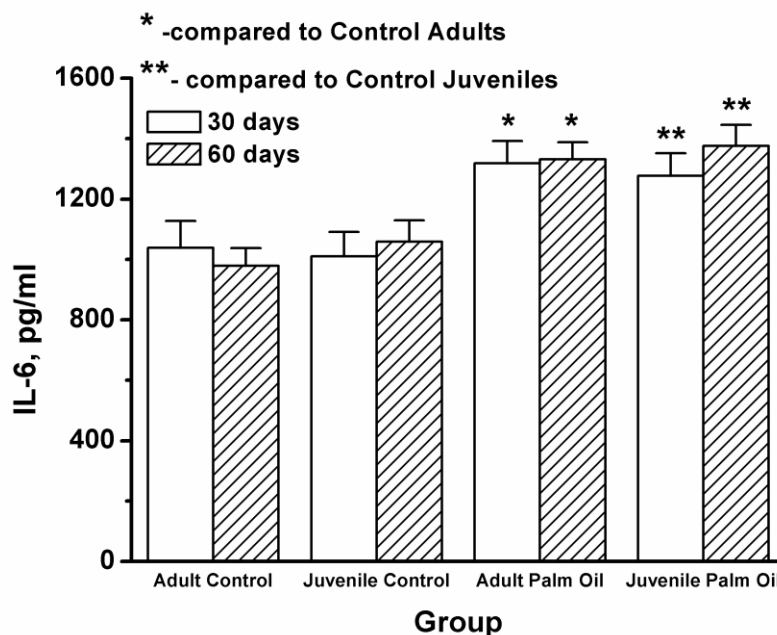


Figure 2. Palm oil effect (30 and 60 days) on blood IL-6 levels on juvenile and adult male rats.

Data statistical analysis

One way ANOVA: $P < 0.0004$. Variations among column means significantly differ than expected by chance; Bartlett: SD'd belong to the same population; Bonferony: critical $t = 3.418$.

Discussion

1. Palm oil application causes statistically significant raised levels of IL-6 in both groups.
2. In same conditions, IL-6 levels are not influenced by continuation of studies, so it is reckoned that increased levels will be the same in day 30 and 60 respectively

During studying time it was realised GTT in day 30 and 60 respectively. In Figure 3 are presented results from GTT. These results show the changed levels which are more important in minute 90 and speak same as data for pre-diabetes.

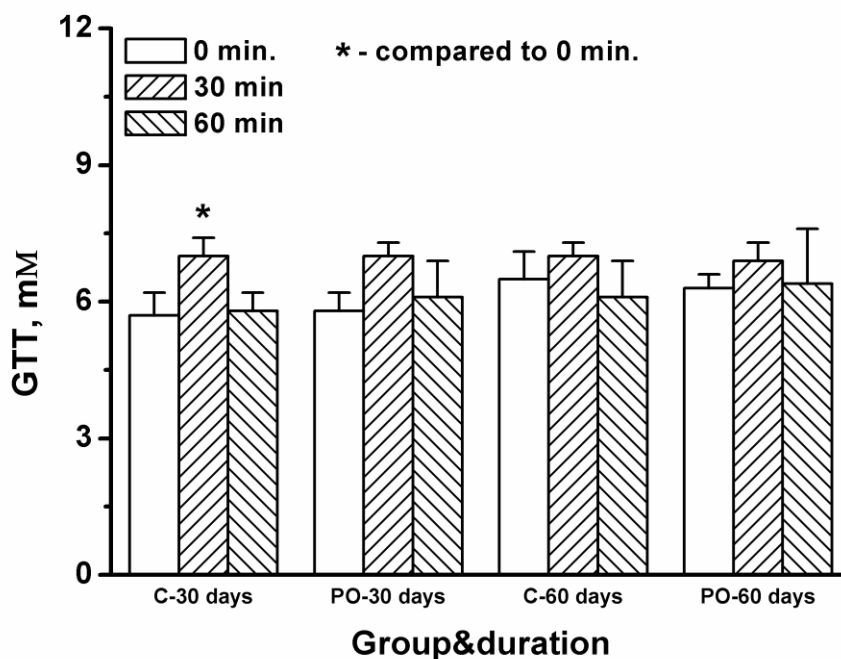


Figure 3. Palm oil effect, applied on day 30 and 60, on GTT levels of adult male rats

Results on studies of young rats GTT are presented on Figure 4. They show GTT changes which are statistically significant in day 60 compared to day 30, after palm oil treatment.

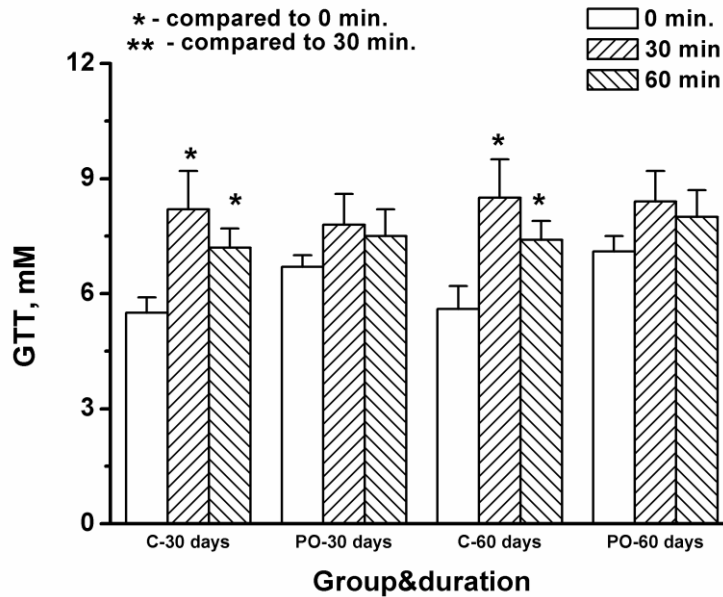


Figure 4. Palm oil effect on young male rats GTT applied on day 30 and day 60.

Discussion

Generally speaking, results show development of prediabetes with a significant risk at young rats chronically treated with palm oil. Raised levels of both cytokines are shown. Pre-diabetes condition correlates with raised levels of TNF- α and IL-6, and to see these changes in this study we have demonstrated those blood levels on male experimental rats, under palm oil effect. It is known that this cytokine takes part in inflammation mechanism (Michaud et al., 2013; Zhang et al., 2013). Studies have shown data in which inflammation has a role on diabetes pathogenesis (Everard et al., 2013; Di marco et al., 2013; Pedicino et al., 2013; Game et al., 2013; Donathy, 2013; Cildir et al., 2013). Furthermore, published data support that obesity is linked with risk of inflammation (Ramsay et al., 2013; Sobieska et al., 2013; Carillon et al., 2013; Grant et al., 2013; Borgesson et al., 2013; Wright et al., 2013).

Experimental studies and their result show that palm oil in blood raise levels of pro inflammatory cytokines. This can aid as a prove that prediabetes development in experimental animals happens because of TNF - α and IL-6. Differences, which has been documented between young and adult rats, prove to be a high risk for diabetes development after continuous usage of palm oil from youth and adolescents. This data are of the first published, but are a basis for future studies on mechanism through which palm oil changes both cytokines and carbohydrate metabolism.

Conclusion

60 days elongated administration of palm oil, in experimental rats of adult and young age, raise blood levels of TNF- α and IL-6 changes metabolism of carbohydrates with data also supporting the development of pre-diabetic condition. More important, is the risk which

appears in young, non mature experimental rats. If we make a correlation with humans we can conclude that individuals who use food products which contain palm oil, will be more predisposed to develop health disturbances which can cause pre-diabetic condition and later leading to diabetes.

Recommendation

From the results in our scientific research we can recommend: to take care in usage of food products which contain palm oil, specially from everyday usage of these foods. This represents a good health education for adolescents and youth individuals.

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Investigation of the Impact of Fructose on Tolerance of Glucose, Insulin, Adiponectin, Leptin and Triglycerides in Adult Experimental Rats

Besim Memedi¹ **Agron Zuferi**²

AM European University - Prishtina and UT, General Hospital - Gostivar

Introduction: Metabolic syndrome in recent years is being actively studied and is the focus of experimental research and clinical research. Insulin resistance is a major problem in metabolism and is related not only to obesity but also to the pathogenesis of type 2 diabetes.

Purpose: The effect of fructose on body weight in experimental adult rats and the effect of fructose on the content of glucose, triglycerides, uric acid, insulin, adiponectin and leptin in the blood of experimental animals.

Material and Method: The study was performed on 12 adult male rats divided into two groups: control group and study group (with fructose). Experimental animals were treated daily for a period of 8 weeks with 10% fructose solution, 1ml/100g by mouth (per os). The control group of animals was treated with a veiculum (physiological digestion).

Results: Table 1 presents changes in body weight of experimental animals under the influence of fructose. Statistically significant ($p < 0.05$) is the weight of fructose-treated experimental animals compared to the control group at the end of the experiment. Table 2 presents the results from the definition of other indicators. Animal blood glucose levels are elevated, treated with glucose compared to the control group. Statistically significant is an increase in blood triglyceride levels after 8 weeks of glucose treatment.

Conclusion: The analysis of insulin elevation and blood sugar rise gives us reasons for the development of “insulin resistance” under the influence of fructose. Experimental animals do not demonstrate the effect of insulin elevation, and fasting blood sugar is elevated after 8-week treatment with fructose.

Keywords: Fructose, obesity, insulin resistance.

Hulumtimi i ndikimit rë Fruktozës ndaj tolerancës së glukozës, insulinës, adiponektinës, leptinës dhe triglicerideve te minjët eksperimental të rritur

Besim Memedi ¹ Agron Zufferi ²

AM European Unversiteti Rezonanca –Prishtinë, Spitali i Përgjithshëm - Gostivarë

Hyrje : Sindromi metabolik viteve të fundit po mësohet në mënyrë aktive dhe është në fokusin e hulumtimeve eksperimentale dhe hulumtimeve klinike. Rezistenca insulinore është problem i madh në metabolizmin dhe ndërlihet jo vetëm më obezitetin, por edhe me patogjenezën e diabetit tip 2.

Qëllimi: Ndikimi i fruktozës ndaj peshës trupore te minjët e rritur eksperimental dhe ndikimi i fruktozës ndaj përmbajtjes të glukozës, triglicerideve, acidit urik, insulin, adiponektin dhe leptin, në gjakun e kafshëve eksperimental.

Material dhe Metoda: Hulumtimi u realizua ndaj 12 minjëve të rritur të gjinis mashkullore të ndarë në dy grupe: grupi kontrollues dhe grupi studiuës (me fruktozë). Kafshët eksperimental i trajtuam çdo ditë për periudhë kohore prej 8 javësh, me tretësirë 10% fruktozë, 1ml/100 gr nëpërmes gojës (per os). Grupin kontrullues të kafshëve u trajtua me vehikulum (tretje fiziologjike).

Rezultatet: Në tabelën e 1 prezentohen ndryshimet e peshës trupore të kafshëve eksperimental nën ndikimin e fruktozës. Statistikisht me rëndësi ($p < 0,05$) është pesha e kafshëve eksperimental të trajtuar me fruktozë në krahsim me grupin kontrollues në fund të eksperimentit. Në tabelën e 2 janë prezentuar rezultatet nga përcaktimi i treguesve të tjerë. Niveli i glukozës në gjakun e kafshëve është i rritur, të trajtuar me glukozë në krahsim me grupin kontrollues. Statistikisht e rëndësishme është rritja e nivelit të triglicerideve në gjakë pas 8 javë të trajtuar me glukozë.

Përfudnimi: Analiza e rritjes së insulinës dhe rritja e sheqerit në gjakë na jep arsye për zhvillimi e „rezistencës insulinore,“ nën ndikimin e fruktozës. Tek kafshët eksperimental nuk demonstrohet efekti i rritjes së insulinës, kurse sheqeri në gjakë esull është i lartë pas trajtimit 8-javor me fruktozë.

Fjalët kyçe: Fruktozë, obezitet, rezistencë insulinore.

I Hyrje

Obeziteti dhe sindromi metabolitik

Sindromi metabolitik mësohet aktiv viteve të fundit dhe është në fokusin e hulumtimeve eksperimentale dhe klinike. Gjatë vitit 1998, Reaven populullarizon konceptin për "sindroma X" kyçën shumë nga reziqet e sëmundjeve kardiovaskulare – hipertensioni arterial, trashësia, nivelet e rritura të triglicerideve dhe nivelet e ulta të HDL – holesterolit (Reaven, 1998). Ky sindrom është i njohur dhe diskutohet me dekada para këtij koncepti të parqitur. Për shembull: përshkrimi i sindromit është bërë nga Kylin qysh gjatë vitit 1923, i cili i bashkoi në një manifestim me hipertensionin arterial, trashësisin dhe podagrën.

Emra të ndryshëm janë sygjeruar për këtë sindrom, në këtë numër sindromin insulinorezistent, sindromi metabolitik kardiovaskular, kuartali vdekjeprues. (Groop, 2000)

Gjatë viteve të fundit shpesh përdoret edhe emërtimi sindromi insulinorezistent. Organizata botërore shëndetësore (OBSH-ja) propozoi definicionin dhe vërtetoi emërtimin sindromi metabolitik (Alberti & Zimmet, 1998).

Ndjejshmëria insulinore dhe faktorët për rezistencën insulinore

Rezistenca insulinore është problem i madh në metabolizmin dhe ndërlidhet jo vetëm me trashësinë, por edhe me patogjenezën e sëmundjes së diabetit tip 2. Elementi i rëndësishëm në zhvillimin janë prezentuar më lartë acidet yndrore, citokinet gjatë trashësisë. Duke pas parasysh, se trashësia në vendet perëndimore veç më pranohet si epidemi dhe është e ndërlidhur me shumë efekte të padëshiruar, lidhja në mes rezistencës insulinore – diabeti i sheqerit tip 2 – trashësisë është problem aktual në hulumtimet shkencore.

Rezistenca insulinore përcaktohet si përgjigje jo-adekuate e receptorëve insulinor në indet targete për ndikimin e insulinës si në hepar, muskujt skeletor dhe indin yndyror. Glukoza nuk përthithet siç duhet dhe përfitohet niveli i ritur i glukozës në gjakë, e cila lexohet klinikisht dhe mund të definohet si rezik për zhvillimin e sëundjes së diabetit.

II Qëllimi

III Material dhe Metoda

Hulumtimi u realizua ndaj 12 minjëve të rritur të gjinis mashkullore të ndarë në dy grupe: grupi kontrollues dhe grupi studiues (me fruktozë). Kafshët eksperimental i trajtuam çdo ditë për periudhë kohore prej 8 javësh, me tretësirë 10% fruktozë, 1ml/100 gr nëpërmes gojës (per os). Grupin kontrullues të kafshëve u trajtua me vehikulum (tretje fiziologjike).

Të dy grupet e kafshëve eksperimental ishin të lirë me qasje ndaj ujit dhe ushqimit, sipas standardit CHOW-paleti. Pas 8 javësh të trajtimit me fruktozë, kafshët ishin dekapituar nën narkozën me Netambutol, pastaj u mereshite gjak për përcaktimin e parametrave të ndryshëm, si më poshtë: glukozja mmol/l, trigliceridet mmol/l, acidi urik mg/ml, insulin ng/ml, adiponektin ng/ml, leptin g/ml. Para dekapitimit u bë përcaktimi i peshës së përgjithshme të kafshëve eksperimental.

You can read this study on page of mz dissertation.

IV Rezultatet

Rezultatet do ti paraqes në 2 tabela. Në tabelën e 1 prezentohen ndryshimet e peshës trupore të kafshëve eksperimental nën ndikimin e fruktozës. Statistikisht me rëndësi ($p < 0,05$) është pesha e kafshëve eksperimental të trajtuar me fruktozë në krahsim me grupin kontrollues në fund të eksperimentit.

Tabela.1 Ndikimi i Fruktozës ndaj peshës trupore të minjëve meshkuj të rritur

Grupa	Pesha në fillim të eksperimentit (g)	Pesha në fund të eksperimentit (g)
K (n=6)	130.0 ± 30.0	180.0 ± 33.0
H (n=6)	132.0 ± 25.0	250.0 ± 40.0*

Tabela 2. Ndikimi i Fruktozës ndaj përmbajtjes të glukozës, triglicerideve, acidit urik, insulin, adiponektin dhe leptin në gjakun e kafshëve eksperimental.

Grupa	Glukoza (mmol/L)	Trigliceridet (mmol/L)	Acidi urik (mg/dL)	Insulin (ng/ml)	Adiponektin (ng/ml)	Leptin (ng/ml)
K (n=6)	6.0 ± 0.4	1.2 ± 0.03	3.7 ± 0.2	2.8 ± 0.3	6.0 ± 2.1	28.7 ± 3.1
H (n=6)	18.2 ± 1.2*	4.6 ± 0.3*	6.5 ± 1.1*	3.5 ± 1.8*	2.4 ± 0.05*	35.9 ± 4.0*

Në tabelën e 2 janë prezentuar rezultatet nga përcaktimi i treguesve të tjerë. Niveli i glukozës në gjakun e kafshëve është i rritur, të trajtuar me glukozë në krahasim me grupin kontrollues. Statistikisht e rëndësishme është rritja e nivelit të triglicerideve në gjakë pas 8 javë të trajtuar me glukozë.

Për caktimin e insulinës, adiponektinës dhe leptinës në serum të gjakut, ishin të vërtetuar dallime në mes grupit kontrollues dhe hulumtues, sikur më poshtë:

- a) Rritja e nivelit të Insulinës në plazmën e kafshëve eksperimentale, trajtuar me glukozë ($p < 0,05$)
- b) Rritja e Leptinit, nën ndikimin e Fruktozës
- c) Statistikisht e rëndësishme **ishte ulja e nivelit të adiponektinës nën ndikim të fruktozës** (tabela 2).
- d) Gjatë bërjes së eksperimentit statistikisht u vërtetua rritje e rëndësishme e acidit urik në gjak ($p < 0,05$).

V Përfundimi dhe Diskusioni

Fruktoza shfrytëzohet gjerësisht në industrinë ushqimore. Efektet ndaj shëndetit njerëzor mësohen në mjekësi, por të dhënat në literaturë janë relativisht të pakta. Rezultatet nga hulumtimi tregojnë ndryshime ndaj metabolizmit të karbohidrateve dhe peshën e kafshëve pas trajtimit të zgjatur me fruktozë. Rritja e sheqerit në gjak përcakton rrezik nga sëmundja e sheqerit. Rezultatet gjithashtu dokumentuan ndryshime në nivelin e insulinës plazmatike.

Analiza e rritjes së insulinës dhe rritja e sheqerit në gjakë na jep arsye për zhvillimin e „rezistencës insulinore“, nën ndikimin e fruktozës. Tek kafshët eksperimentale nuk demonstron efektin e rritjes së insulinës, kurse sheqeri në gjakë esull është i lartë pas trajtimit 8-javor me fruktozë.

Në mbështetje të zhvillimit të rezistencës insulinore nën ndikimin e fruktozës, janë rezultatet e studimit të 2 hormoneve tjera të rëndësishme për karbohidratet dhe shkëmbimit yndyror – leptin dhe adiponektin. Rritja e leptinit nën ndikimin e fruktozës tregon, se ka ndryshime në metabolizmin dhe tendencë ndaj trashësis (obezitetit). Hiperleptinemija sqaron edhe rritjen e peshës së kafshëve eksperimental të trajtuar me fruktozë. Roli i leptinës në mekanizmin, apetit i kontrolluar dhe metabolizmi është dokumentuar në literaturën e (Ханджиева-Дърленска, Бояджиева Н., 2010 и др). Në përkrahje të të dhënave, se leptini luan rol në rezistencën insulinore ka shumë fakte të publikuara (Meersen et al., 2013; Gulcelik, 2013; Yanni, 2013; Trovati et al., 2013; Dong et al., 2013; Lakhadar et al., 2013; Wandell et al., 2013; Al-Suhaimi et al., 2013; Bardini et al., 2013; Bereiter-Hahn et al., 2013; Duque-Guimaser et al., 2013; Higashida K et al., 2013).

Rezultatet e prezentuara më lartë, mbështesin studimet e paraqitura. Mund të thuhet, se edhe zvoglimi i nivelit të adiponektinës luan rol si në ndryshimin e triglicerideve dhe në glukozën e gjakut, ashtu edhe në rezistencën insulinore. Ne theksojm se Adiponektina – e cila është citokin i rëndësishëm, i sekretuar nga adipocitet dhe ka rol në mekanizmin e rezistencës insulinore.

Për shembull : te pacientët me obezitet vërehen ndryshime në adiponektinën dhe rezistencën insulinore. Vërtetime të reja janë efektet fruktozës ndaj insulinës, adiponektinës dhe leptinës. Për here të parë dokumentohen reziqet për çregullime në metabolizmin e karbohidrateve dhe metabolizmin me të dhënat për zhvillimin e rezistncës insulinore.

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Investigation of the Impact of Fructose on Tolerance of Glucose, Insulin, Adiponectin, Leptin and Triglycerides in Adult Experimental Rats

Besim Memedi¹ Agron Zuferi²

AM European University - Prishtina and UT, General Hospital - Gostivar

Introduction: Metabolic syndrome in recent years is being actively studied and is the focus of experimental research and clinical research. Insulin resistance is a major problem in metabolism and is related not only to obesity but also to the pathogenesis of type 2 diabetes.

Purpose: The effect of fructose on body weight in experimental adult rats and the effect of fructose on the content of glucose, triglycerides, uric acid, insulin, adiponectin and leptin in the blood of experimental animals.

Material and Method: The study was performed on 12 adult male rats divided into two groups: control group and study group (with fructose). Experimental animals were treated daily for a period of 8 weeks with 10% fructose solution, 1ml/100g by mouth (per os). The control group of animals was treated with a veiculum (physiological digestion).

Results: Table 1 presents changes in body weight of experimental animals under the influence of fructose. Statistically significant ($p < 0.05$) is the weight of fructose-treated experimental animals compared to the control group at the end of the experiment. Table 2 presents the results from the definition of other indicators. Animal blood glucose levels are elevated, treated with glucose compared to the control group. Statistically significant is an increase in blood triglyceride levels after 8 weeks of glucose treatment.

Conclusion: The analysis of insulin elevation and blood sugar rise gives us reasons for the development of “insulin resistance” under the influence of fructose. Experimental animals do not demonstrate the effect of insulin elevation, and fasting blood sugar is elevated after 8-week treatment with fructose.

Keywords: Fructose, obesity, insulin resistance.

I Introduction

Obesity and Metabolic Syndrome

The metabolic syndrome is actively taught in the recent years and is the focus of experimental and clinical research. During 1998, Reaven popularized the concept of "X syndromes", the sum of the risks of cardiovascular disease - arterial hypertension, obesity, elevated triglyceride levels, and low HDL-cholesterol levels (Reaven, 1998). The syndrome is well known and has been discussed for decades before this concept was introduced. For example: Syndrome has been described by Kylin since 1923, who associated in one manifestation with arterial hypertension, obesity, and podagra.

Various names have been suggested for this syndrome, in this issue insulin-resistant syndrome, cardiovascular metabolic syndrome, lethal quartile. (Groop, 2000)

In recent years, the term insulin-resistant syndrome has often been used. The World Health Organization (WHO) defines and validates the designation metabolic syndrome (Alberti & Zimmet, 1998).

Insulin sensitivity and factors for insulin resistance

Insulin resistance is a major problem in metabolism and is related not only to obesity but also to the pathogenesis and type 2 diabetes mellitus. Important elements in the development are fatty acids, cytokines during obesity. Given that obesity in the western world is already widely accepted as an epidemic and is associated with many adverse effects, the link between insulin resistance - type 2 diabetes - obesity is a current problem in scientific research.

Insulin resistance is defined as an inadequate response of insulin receptors to target tissues for the influence of insulin such as hepatic, skeletal muscle, and fatty tissue. Glucose is not absorbed properly and results in increased blood glucose levels, which is clinically readable and can be defined as a risk for developing diabetes mellitus.

II Purpose

The effect of fructose on body weight in experimental adult mice and the effect of fructose on glucose, triglyceride, uric acid, insulin, adiponectin and leptin content in experimental animal blood.

III Material dhe MetodaMaterial and Method

I did this study at the state faculty of medicine – Sofia, near the laboratory of pharmacology (2009-2013).

The research was conducted on 12 adult male mice divided into two groups: the control group and the study group (with fructose). Experimental animals were treated daily for a period of 8 weeks with 10% fructose solution, 1 ml / 100g by mouth (per os). The control group of animals was treated with a veiculum (physiological digestion).

Both groups of experimental animals were free to access water and food, according to the CHOW-palette standard. After 8 weeks of fructose treatment, the animals were decapitated under Netambutol narcosis, then blood was taken to determine different parameters, as follows: mmol/l glucose, mmol/l triglycerides, mg/ml uric acid, insulin ng/ml. ml, adiponectin ng/ml, leptin g/ml. Before decapitation, the total weight of the experimental animals was determined.

You can read this study at my dissertation (page 56-59).

IV Results

I will present the results in 2 tables. Table 1 presents the changes in body weight of experimental animals under the influence of fructose. Statistically significant ($p < 0.05$) is the weight of fructose-treated experimental animals compared to the control group at the end of the experiment.

Table.1 Influence of Fructose on body weight of adult male mice

Group	Weight at the beginning of the experiment (g)	Weight at the end of the experiment (g)
K (n=6)	130.0 ± 30.0	180.0 ± 33.0

H (n=6)	132.0 ± 25.0	250.0 ± 40.0*
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Table 2. Impact of Fructose on glucose, triglyceride, uric acid, insulin, adiponectin and leptin contents in experimental animal blood.

Group	Glucose (mmol/L)	Triglycerides (mmol/L)	Uric acid (mg/d L)	Insulin (ng/ml)	Adiponektin (ng/ml)	Leptin (ng/ml)
K (n=6)	6.0 ± 0.4	1.2 ± 0.03	3.7 ± 0.2	2.8 ± 0.3	6.0 ± 2.1	28.7 ± 3.1
H (n=6)	18.2 ± 1.2*	4.6 ± 0.3*	6.5 ± 1.1*	3.5 ± 1.8*	2.4 ± 0.05*	35.9 ± 4.0*

Table 2 presents the results from the definition of other indicators. Animal blood glucose levels are elevated, treated with glucose compared to the control group. Statistically significant is an increase in blood triglyceride levels after 8 weeks of glucose treatment.

For the determination of insulin, adiponectin and leptin in blood serum, differences were established between the control and research groups, as follows:

- a) Increased insulin levels in plasma of experimental animals treated with glucose ($p < 0.05$)
- b) Leptin increase, under the influence of Fructose
- c) The statistically significant was the decrease in adiponectin levels under the influence of fructose (Table 2).
- d) Significant statistical increases in blood uric acid were observed during the experiment ($p < 0.05$).

V Conclusion and Discussion

Fructose is widely used in the food industry. The effects on human health are taught in medicine, but data in the literature are relatively scarce. Research results show changes in carbohydrate

metabolism and weight of animals after prolonged treatment with fructose. Increased blood sugar determines risk of diabetes. The results also documented changes in plasma insulin levels.

Analysis of the rise in insulin and the rise in blood sugar gives us reasons for the development of "insulin resistance" under the influence of fructose. Experimental animals do not demonstrate the effect of insulin elevation, and high blood sugar is elevated after 8-week treatment with fructose. In support of the development of insulin resistance under the influence of fructose, are the results of the study of 2 other important hormones for carbohydrates and fatty exchange - leptin and adiponectin. An increase in leptin under the influence of fructose indicates that there are changes in metabolism and a tendency to obesity. Hyperleptinemia also explains the increase in the weight of experimental animals treated with fructose. The role of leptin in mechanism, controlled appetite, and metabolism has been documented in the literature of (Ханджиева-Дърленска, Бояджиева H., 2010 et al). In support of the data, that leptin plays a role in insulin resistance has many published facts (Meersen et al., 2013; Gulcelik, 2013; Yanni, 2013; Trovati et al., 2013; Dong et al., 2013; Lakhadar et al. al., 2013; Wandell et al., 2013; Al-Suhaimi et al., 2013; Bardini et al., 2013; Bereiter-Hahn et al., 2013; Duque-Guimaser et al., 2013; Higashida K et al. ., 2013).

The results presented above support the presented studies. It can be argued that lowering adiponectin levels also play a role in altering triglycerides and blood glucose as well as insulin resistance. We note that Adiponectin - which is an important cytokine, secreted by adipocytes and has a role in the mechanism of insulin resistance.

For example: in obese patients, changes in adiponectin and insulin resistance are observed. New evidence is the effects of fructose on insulin, adiponectin and leptin. For the first time, risks for disorders of carbohydrate metabolism and metabolism with data on the development of insulin resistance are documented.

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FREQUENCY OF COMPLETE AND REMOVABLE PARTIAL DENTURE TREATMENT IN THE PRIMARY HEALTH CENTRES IN THREE DIFFERENT REGIONS OF KOSOVO FROM 2002 TO 2013

POGOSTOST OSKRBE S TOTALNO IN DELNO PROTEZO V PRIMARNIH ZDRAVSTVENIH CENTRIH V TREH RAZLIČNIH REGIJAH NA KOSOVU V OBDOBJU 2002-2013

Manushaqe SELMANI BUKLETA^{1,2}, Dashnor BUKLETA³, Mimoza SELMANI⁴, Milan KUCHAR^{5*}

¹University of Ljubljana, Medical Faculty, Doctoral School, Vrazov trg 2, 1000 Ljubljana, Slovenia

²Dental Clinic, Mdent Family Dentistry, Eqrem Qabej 74, 10000 Pristina, Kosovo

³Dental Polyclinic, Department of Oral Surgery, Nene Tereza NN, 30000 Peje, Kosovo

⁴AAB College, Zona Industriale, Fushe Kosove, 10000 Pristina, Kosovo

⁵University of Ljubljana, Medical Faculty, Department for Prosthodontics, Hrvatski trg 6, 1000 Ljubljana, Slovenia

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ABSTRACT

Keywords:

edentulism, prosthodontic treatment, complete dentures, removable partial dentures

Introduction: Edentulism and prosthodontic care are very common, especially in the elderly. The study investigated the treatment with complete dentures (CDs) and acrylic removable partial dentures (ARPDs) among people receiving new prosthodontic treatment in the Primary Health Centres of the three regions in Kosovo from 2002 to 2013.

Methods: The data on ARPDs delivery and CDs delivery was obtained from the archives of primary health centres from three Kosovo regions (Prizren, Peje, Ferizaj) from 2002 to 2013. The data was analysed concerning year of treatment, type of dentures, jaw, age, gender and urban or rural origin of the patients. The trend of treatment was determined, and the binomial logistic regression model was used for predicting odds of ARPD versus CD treatment by year of treatment and patient characteristics.

Results: From 2002 to 2013, 9,478 patients received 11,655 CDs and 4,401 ARPDs. Delivery of CDs significantly increased by 57.45 dentures per year ($R^2=0.609$) and delivery of ARPDs by 30.39 dentures per year ($R^2=0.569$). Each year the odds for ARPD versus CD increased by 4.2% (95% CI: 3.0%-5.4%). Younger patients have higher odds for ARPD rather than CD and odds for ARPD are decreasing as the age of patients rises. The gender, residence, and jaw all had a significant impact on prosthodontic treatment too.

Conclusions: In Primary Health Centres of Kosovo, there is a trend for higher frequencies of both dentures (more obvious for ARPD), and the frequency is highly dependent on the age of patients.

IZVLEČEK

Ključne besede:

brezzobost, protetična oskrba, totalne proteze, delne proteze

Uvod: Brezzobost in protetična oskrba sta zelo pogosti, še zlasti pri starejših osebah. V študiji so preučevali zdravljenje s totalnimi in akrilatnimi delnimi protezami pri osebah, ki so v obdobju od 2002 do 2013 prejele novo protetično oskrbo v primarnih zdravstvenih centrih v treh regijah na Kosovu.

Metode: Iz arhivov primarnih zdravstvenih centrov na Kosovu (Prizren, Peje in Ferizaj) so bili iz obdobja od 2002 do 2013 zbrani podatki o vstavljenih novih totalnih in akrilatnih delnih protezah. Analizirani so bili glede na leto vstavitve, vrste protez, prisotnosti protez v zgornji in/ali spodnji čeljusti, starost, spol in prebivališče (mesto, podeželje) oskrbovancev. Od leta 2002 do 2013 so določili trend pogostosti oskrbe s protezama. Za napovedovanje verjetnosti oskrbe z delno protezo v primerjavi s totalno protezo glede na leto, ko je bila proteza vstavljena, in značilnosti bolnikov so uporabili binomsko logistično regresijo.

Rezultati: V študiji je bilo zajetih 9.478 bolnikov, ki so jim vstavili 11.655 totalnih protez in 4.401 akrilatnih delnih protez. Število vstavitvev totalnih protez se je v letih 2002-2013 pomembno povečevalo za 57,45 na leto ($R^2 = 0,609$), vstavitvev akrilatnih delnih protez pa za 30,39 na leto ($R^2 = 0,569$). Vsako leto se je obet za vstavitvev akrilatne delne proteze glede na totalno protezo povečal za 4,2 % (95 %, CI: 3, 05, 4 %). Mlajši pacienti imajo večje obete za oskrbo z akrilatno delno protezo kot za oskrbo s totalno protezo. Verjetnost zdravljenja z akrilatno delno protezo se s staranjem zmanjšuje. Statistično značilen vpliv na protetično zdravljenje so imeli tudi spol in prebivališče pacientov ter lokacija proteze v ustih.

Sklepi: V primarnih zdravstvenih centrih na Kosovu se povečuje pogostost oskrbe z obema zobnima protezama (intenzivneje pri akrilatni delni protezi) in pogostost je močno odvisna od starosti pacientov.

*Corresponding author: Tel. + 386 41 778 621; E-mail: milan.kuhar@mf.uni-lj.si

1 INTRODUCTION

Thanks to preventive dentistry, which was introduced in the mid-20th century, most people are now aware that natural teeth can be maintained throughout an entire lifetime (1-3). In developed countries, the prevalence and extent of tooth loss has significantly decreased in recent decades (1, 2, 4, 5). The prevalence of edentulism decreased by approximately 4-10% over ten years (1, 5, 6). However, the growing population of elderly, which will continue to grow dramatically in the next few decades, is the main reason for the persistence of high levels of edentulism (1, 7). Consequently, prosthodontic care is prevalent, especially in the elderly, in most European countries (8, 9). Correlations between the prevalence of edentulism and prosthodontic care as well as national prosperity, irregular-regular visits to the dentist, urban-rural residence, age, gender, and education were also demonstrated (1, 10).

The primary purpose of prosthodontic care is to replace missing teeth and to restore function and aesthetics, as well as to preserve the health of the remaining oral tissues of partially or completely edentulous patients. The prosthodontic treatment combines several clinical and laboratory procedures that lead to the insertion of the fixed (crowns and bridges) or removable (partial and complete dentures) prosthodontic device (11). In principle, because treatment with various types of removable dentures is generally less successful than with fixed prosthodontic devices, removable prosthodontic treatment is indicated when clinical or financial factors exclude fixed prosthodontic treatment on teeth or dental implants (12-14). In the last three decades, the removable dentures supported by dental implants have proven to be a very successful form of prosthodontic treatment for edentulous people and treatment with them is continuously increasing. However, due to increased complexity (needs surgery) and higher costs are significantly less common than conventional denture treatment without dental implants (15, 16).

Currently, conventional mucosa-born complete dentures (CDs) are still the most common removable form of prosthodontic treatment for complete edentulous jaws, while conventional mucosa and teeth-born removable partial dentures (RPDs) of all types continue to be a standard treatment modality in the maxillary and mandibular partial edentulous arches (17). The RPDs with a metal framework (MRPDs) and acrylic based RPDs without a metal framework (ARPDs) are the most commonly used removable partial dentures (8). More expensive and technically more demanding MRPDs have some crucial advantages over ARPDS. The main advantages are better dental support, oral tissue release from the extended resin plate coverage, and facilitated maintenance of hygiene, which impacts the higher success rate of MRPDs.

Mobility of abutment teeth and fracture of dentures are observed more often in ARPDS than in MRPDS (18, 19). A study among general dentists in Ireland showed that the average survival rate of an ARPD is 5.7 years, whereas an MRPD has an average survival rate of 10.6 years (20).

Data on prosthodontic care in different countries, environments, and patient groups can be relevant, in particular to all those who are involved in planning and organizing healthcare, as well as in health education (15, 21). Epidemiological studies published from 2004 to 2013 show that, of all RPDs constructed, the acrylic without metal framework ones were used in 3.2-75% of cases in different countries (17, 20, 22-24). A review study of 43 articles, which related to 13 European countries, concluded that there is a trend toward a higher prevalence of fixed prosthodontic treatments, more various types of RPDs and a reduction in CDs in most European countries. The majority of these articles presented the prevalence of various prosthodontic devices at a given time, and only five articles presented the incidence of newly used prosthodontic treatments over a specific period (8). A study performed in Croatia shows that Croatian Health Insurance has been covering the cost of CDs, ARPDS, and MRPDS for the past twenty years. At the same time, an upward trend particular to MRPDS has been detected in the majority of Croatian regions (25). Since 2013, the costs of MRPD treatment have been covered by health insurance in Slovenia as well (26). Subjective clinical estimations show an upward trend in the use of MRPDS since that date but, unfortunately, this has not yet been scientifically confirmed. Ever since the war in 1999, primary health centres in Kosovo only provide low-cost prosthodontic treatment, which means that edentulous persons are treated with either ARPDS or CDs. A considerable number of patients seek medical and dental services in these centres, and most of them are socially deprived, war veterans, relatives of war victims and older adults. Treatment with MRPD as a more advanced form of the removable partial dentures is only available in the state-funded University Clinical Centre in Pristina, Kosovo. In other institutions, the patients need to pay MRPD, like fixed restorations, by themselves.

No epidemiological studies have been made on the use of any prosthodontic treatment in the Republic of Kosovo. The purpose of the study was to investigate the treatment with CDs and ARPDS among people receiving prosthodontic treatment in the Primary Health Centres of the three regions in Kosovo. Therefore, the aims of this study were: 1) to find out what was the trend in the frequency of new treatments with CDs and ARPDS from 2002 to 2013; 2) to analyse the relationship between two prosthodontic treatments (ARPDs versus CDs) and according to different factors (age, sex, jaw, living environment); 3) to determine the proportion of the population receiving new CD and ARPD treatment at the annual level by age.

2 METHODS

This study included all patients who received new prosthodontic treatment with CDs and ARPDs at Primary Health Centres of three Kosovo regions: Prizren, Peje (Peč) and Ferizaj (Uroševac) from 2002 to 2013 (Table 1). 35.2% of the total data comes from Prizren, 37.2% from Peja, and 27.6% from Ferizaj. The data collected from the archives of primary health centres included: the year of delivery of the denture, the type of the denture delivered (CD or ARPD), the location of the denture in the patient's mouth (maxilla, mandible), the patient gender, the urban or rural origin of the patient, and the age of the patient upon denture delivery.

This study includes 9,478 patients who received treatment with CDs and ARPDs. 72.1% of patients live in urban areas and only 27.9% in rural areas. The participants were divided into 6 age groups as follows: younger than 35

years of age (1.9%), 35 to 44 years of age (8.9%), 45 to 54 years of age (21.8%), 55 to 64 years of age (26.0%), 65 to 74 years of age (30.8%) and more than 75 years of age (10.5%).

Table 1. The number of patients according to the number and type of dentures they received in prosthodontic treatment in the Primary Health Centres of the three regions of Kosovo from 2002 to 2013.

Patients	Dentures		
	CD	ARPD	Total
4,483	8,966		8,966
1,647	1,647		1,647
1,042	1,042	1,042	2,084
1,053		2,086	2,106
1,253		1,253	1,253
Σ9,478	Σ11,655	Σ4,401	Σ16,056

The obtained data was statistically analysed using SPSS 22.0 statistical package. The separate linear trends for CD and ARPD treatment were calculated. To draw inference from our data and to lose the outliers, we have normalized the data into a range of between zero and one. The absolute number, as well as the normalized values of the delivered CDs and ARPDs for each year of delivery, are presented in the graphs.

A prediction of ARPD treatment versus CD treatment in people receiving prosthodontic care from 2002 to 2013 was statistically analysed using binomial logistic regression. Sex, age, residence and denture location in the jaw were proposed as categorical covariates and the year of

odds ratios (AORs) and 95% confidence interval (95% CI) are presented. For statistical significance, p-values <0.05 were considered.

To calculate the proportion of the population that is on an annual basis receiving new dentures in Public Health Centres, a four-year (2010-2013) average of patients who received CDs or ARPDs and population data from the last census were used. At the same time, patients and the population in all three investigated regions were arranged into six age groups (<35, 35-44, 45-54, 55-64, 65-74, 75+), in the age group 35+ and to all ages (total). The population for each age group was based on the data from the 2011 population census in the Republic of Kosovo (27). Of the 1,739,825 residents of Kosovo registered in the year 2011, the number living in Prizren, Peje, and Ferizaj was 382,841 (22% of the total number of residents in Kosovo) (Table 2).

Table 2. Population size in the three regions of Kosovo registered in 2011.

Age group	Prizren	Peje/Peč	Ferizaj/Uroševac	Total
<35	111,468	57,811	69,212	238,491
35-44	24,767	13,150	14,750	52,667
45-54	18,452	10,658	10,822	39,932
55-64	11,690	7,337	7,269	26,296
65-74	7,443	4,917	4,570	16,930
75+	3,961	2,577	1,987	8,525
Total	177,781	96,450	108,610	382,841

3 RESULTS

In the Primary Health Centres of all three regions of Kosovo, 9,478 patients received 16,056 removable dentures, of which 6,130 (64.7%) patients received only CDs, 2,306 (24.3%) patients received only ARPDs, and 1,042 (11.0%) patients received both CD and ARPD from denture delivery as a continuous covariate. The adjusted

The absolute number of the new treatments with CDs and ARPDs for each year of denture delivery, as well as the trends of the normalized frequencies of the treatment with CDs and ARPDs from the year 2002 to 2013, are presented (Figures 1 and 2). There is a significant upward trend line in the treatment with CDs: $p=0.003$, $R^2=0.609$. On average, the absolute number of CD deliveries increase by 57.45 CDs per year. Results regarding ARPDs show that there is a significant rising trend in their delivery as well: $p=0.005$, $R^2=0.569$. On average, the absolute number of ARPD deliveries increase by 30.39 per year. Although the average increase of the absolute number of CD deliveries is higher than the average increase of the

absolute number of the ARPD deliveries, the normalized data shows that the average number of ARPD deliveries increases more rapidly than the average number of CD deliveries. To further verify these findings, the odds for receiving ARPDs versus CDs - dependent on the years of delivery - were calculated.



Figure 1. The number of new treatments with CDs and ARPDs for each year of denture delivery in the Primary Health Centres of Kosovo.



Figure 2. The linear trend of CD and ARPD delivery in three regions of Kosovo from 2002 to 2013.

To determine the effects of age, gender, residence and denture location on the likelihood of the type of the denture treatment among the patients, a binomial logistic regression model was conducted (Table 3). The model explained 14.7% (Nagelkerke R²) of the variance in the type of denture predicted and correctly classified 73.9% cases.

The results indicate that the year of delivery was statistically significant: $p < 0.05$. Each year the odds for treatment with ARPD versus CD increased by 4.2% (95% CI: 3.0%-5.4%). Males have 29.1% higher odds for treatment with ARPD rather than CD. In comparison with the reference group (older than 75) patients younger than 34 years have the highest odds for treatment with ARPD (AOR=13.935; 95% CI: 10.296-18.861) rather than CD and odds for ARPD are decreasing as the age of patients rises. Moreover, in comparison with the same reference group (older than 75) all compared age groups have higher odds for treatment regarding ARPD versus CD. Significant results were also found regarding the residence of the patients. Compared with patients from urban residence, patients from rural residence have 10.2% higher odds for treatment with ARPDs over CDs. Results indicate that it is more likely for patients to receive ARPD than CD treatment on the mandibula than maxilla (AOR=2.996; 95% CI: 2.778-3.231).

Table 3. Binominal logistic regression model predicting odds of ARPD versus CD treatment by year of receiving the treatment (2002-2013) and patient characteristics.

Observed category	Reference category	AOR (95% CI) ARPD to CD	p
Year of delivery			
2013	2002	1.042 (1.030-1.054)	0.001*
Sex			
[Male]	[Female]	1.291 (1.197-1.392)	0.001*
Age			
[<34 years]	[75+ years]	13.935 (10.296-18.861)	0.001*
[35-44 years]	[75+ years]	6.201 (5.181-7.422)	0.001*
[45-54 years]	[75+ years]	3.541 (3.031-4.137)	0.001*
[55-64 years]	[75+ years]	2.519 (2.163-2.934)	0.001*
[65-74 years]	[75+ years]	1.512 (1.299-1.759)	0.001*
Residence			
[Rural]	[Urban]	1.102 (1.008-1.205)	0.033*
Jaws			
[Mandibula]	[Maksila]	2.996 (2.778-3.231)	0.001*

*marks statistically significant differences ($p < 0.05$)

From 2010 to 2013, at an annual level, 0.18% of the total population in three Kosovo regions received a new CD and 0.09% a new ARPD in the Public Health Centres. In the 35+ year-old population, the proportion of the population treated with new CDs was 0.48% and 0.23% for new ARPDs. The proportion gradually increased with the increasing age of recipients of dentures up to the 75+ age group, and a decrease was detected in the 75+ age group (Figure 3).

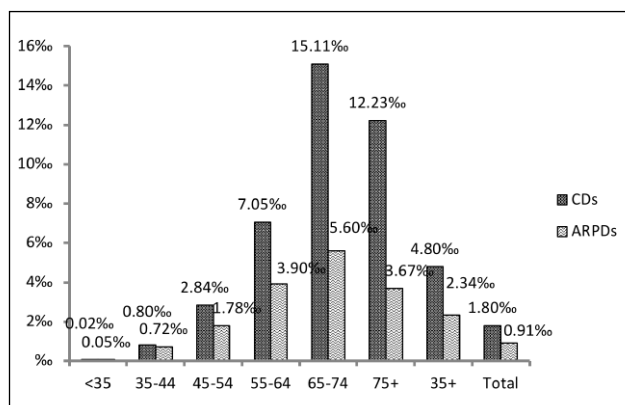


Figure 3. The distribution of the proportion of population treated with CDs and ARPDs from 2010 to 2013, at an annual level, by the age groups >35, 35-44, 45-54, 55-64, 75+, 35+ and in total.

4 DISCUSSION

In the primary health centres in the three Kosovo regions combined, where almost a quarter of Kosovo's inhabitants live, treatment with CDs was more than twice as frequent as treatment with ARPDs over the entire period of 2002-2013. Among those who were seeking prosthodontic care in these centres, most of them are completely edentulous, and the most commonly used prosthodontic devices are CDs. In the same centres, the absolute number of removable prosthodontic treatments with both dentures rose continuously from 2002 to 2013. The increase in treatment with CDs was significantly higher (more than 27 treatments per year on average) than in the case of treatment with ARPDs. In most European countries, however, there has been a clear trend toward reducing removable prosthodontic devices, especially CDs (7-9). In contrast to our study, the frequency of treatment with CDs in Croatia was significantly lower than the frequency of treatment with RPDs from 1996 to 2002 (23). Similarly, the trend toward increasing the frequency of fixed prosthodontics devices and RPDs and the reduction of CDs has been identified in thirteen other European countries (8, 9). Unfortunately, these studies differ significantly concerning age, socioeconomic status, and origin of subjects as well as providers of dental care. Therefore, we can only compare trends, while a direct comparison of data is difficult. The data of our study is obtained from Primary Health Centres, where only primary dentists perform prosthodontic care. While the study in Croatia also includes institutions where specialists perform prosthodontic care. It should also be noted that in these centres dental care is provided to the most vulnerable groups of people in Kosovo. Given that edentulism is the main factor dictating the need for treatment with the CD

(7, 8), we can conclude that there is a high probability that the number of edentulous patients in the Primary Health Centres in Kosovo has also increased from 2002 to 2013.

The normalized frequencies of the treatment with CDs and ARPDs and, in particular, logistic regression predictions of ARPD treatment probability compared to CD by the year of treatment (2002-2013) reveals more interesting data on trends that were previously covered with high absolute values of CD treatment. The probability of ARPD deliveries versus CD significantly increases over the years, and CD deliveries decrease. A more detailed analysis revealed that, in the background of unfavourable absolute values of prosthodontic treatment trends, there is a slight drop in CD delivery in comparison with ARPD delivery, which is also reflected by a slight drop in edentulism. It is also more likely that ARPDs are more commonly used for the prosthodontic treatment of younger patients and, vice versa, that CDs are more often used than ARPDs for the prosthodontic treatment of the elderly. The prevalence of edentulism is most affected by the aging of the population (1, 7, 10). According to the Kosovo Agency of Statistics, the number of people aged 35 years and older increased by 25.0% between 1991 and 2011 (27). Our results also show that the aging of the population is one of the important factors that affects prosthodontic treatment, as the probability of CD treatment increased with the age of the patient. The proportion of ARPDs compared to CDs decreased with the age of patients and at the same time increased over the years. As the number of lost teeth increases with age, it is quite reasonable that, with the increasing age of patients, the CD and ARPD treatment ratio gradually tilts to the side of the CD, which is evident in our and many other studies (8, 28). However, aging is not the only factor affecting the frequency of edentulism and its treatment, since treatment with CDs increased by more than 100% in this same period.

In addition to the year of treatment and age of patients, many additional factors are described that have an impact on the frequency of prosthodontic care and edentulism (8, 9). The additional factors that were available to us in Primary Health Centres - gender, urban versus rural residence and mandibula versus maxilla as denture sites - have had a significant impact on prosthodontic treatment. Treatment with ARPD is more likely to occur in male subjects and people in a rural environment, and CD treatment is more likely in women and people from an urban environment. An appropriate explanation for the more likely deliveries of CDs in an urban environment is currently difficult to justify. Further investigation will be necessary to clarify this.

The results of this study lead us to conclude that CD treatments were significantly more likely in the maxilla than in the mandible and ARPD treatments were more likely in the mandibula. Similarly, studies in Western European countries have found a higher frequency of treatment with a removable partial denture in the mandible (29, 30).

The present study shows that the proportion of the population treated with CDs and ARPDs per year steadily increased from the <35 up to the 75+ age group and decreased in the 75+ age group. Similarly, findings from a study in Croatia indicate that the delivery of prosthodontic appliances in the oldest age group dropped significantly. A less frequent delivery of CDs to persons who are 75 years of age or older can be explained by the fact that older adults rarely visit a dentist, wear prostheses for a more extended period than younger persons, and replace old dentures with new ones less frequently (15, 21, 25, 28).

An important fact is that every prosthodontic treatment also has unwanted side effects and, especially if it is inadequate, harms oral health and tooth loss (29-32). In the contemporary planning of prosthodontic treatment, RPD is indicated when indications for fixed prosthodontic treatment on teeth or implants are ruled out due to biological or socioeconomic factors. It is therefore not surprising that RPD treatment generally has a lower survival rate, more complications and is less comfortable for the patient than fixed prosthodontic treatment (12-14, 32). According to our study, the Primary Health Centres in Kosovo have witnessed a continually growing trend of ARPD delivery in the period from 2002 to 2013. Furthermore, both the absolute number of ARPDs delivered and their proportion in comparison to CDs has increased. In Croatia, Slovenia and many developed countries, the proportion of RPDs in comparison to CDs has also been increasing, but mainly due to an increase in the proportion of MRPDs and a decrease in the proportion of ARPDs (8, 22, 25, 28). MRPDs have significantly better survival and success rate and, above all, improve the survival of the supporting teeth compared to ARPDs (14, 18-20). In the Primary Health Centres of Kosovo, only ARPDs are used for the treatment of partial edentulism. Consequently, although many individuals in these centres met the clinical criteria for a fixed prosthesis or even an MRPD, they were treated with ARPDs, even though these were less appropriate. Except at the University Clinical Centre in Priština, the patients themselves cover the cost of MRPD treatment, which is beyond the reach of most patients looking for dental care in the Primary Health Centres in Kosovo. Treatment with ARPDs can lead to rapid loss of remaining teeth and can increase the overall level of complete edentulism, thereby increasing the need for treatment with a CD (31, 32).

This study undoubtedly has certain limitations, mainly because the prevalence of edentulism was deduced from new dentures treatments. We must be aware that the real prevalence of edentulism in Kosovo is significantly higher than the proportion of new denture treatment. Namely, the data from Primary Health Centres included no information on how long individual patients wore dentures for. According to the population census in Kosovo (25), 61.7% of the population was living in rural areas in 2011. On the other hand, almost three-quarters of patients who received dentures in Primary Health Centres came from the urban environment, which leads us to conclude that only a small proportion of the rural population received dentures in these centres. This may either mean that people in rural areas had better oral health and had less edentulism or, more likely, that they visited a dentist less frequently, were edentulous and without dentures or were using dentures for a long time and rarely changed them. When interpreting the results of this study, it should be borne in mind that our investigated population represents the most vulnerable groups of people in Kosovo.

The number of preserved natural teeth and, indirectly, the presence of prosthodontic restorations is a significant indicator as well as a factor of oral and general health (8, 33). Data on the trend in the frequency of prosthodontic treatment can be important for developing national dental health services and dental health policy, as well as for dental school and research programs in Kosovo and the wider region. Sufficient dental centres that offer adequate care should be made available. In the case of prosthodontic care, this means that partial edentulism should be treated based on the professional indication, including with fixed prostheses and MRPDs, not only ARPDs. Considering the model of the neighbouring countries, the possibility of financing dental treatment, at least with MRPDs, could also be considered in Kosovo.

5 CONCLUSIONS

The trend in the frequency of new CD and ARPD treatment increased linearly from 2002 to 2013 in the Primary Health Centres in three regions of Kosovo. The need for CD treatment in absolute numbers is more than twice as high as the need for treatment with ARPD.

However, the proportion of ARPDs compared to CDs significantly increased over the years. Younger people have higher odds for treatment with ARPD and odds for ARPD are decreasing as the age of patients rises. Males have higher odds for treatment with ARPD. Patients from the rural environment have higher odds for treatment with ARPDs. CD treatments are more likely in the maxilla, and ARPD treatments are more likely in the mandibula.

In the period of 2010-2013, the proportion of the population treated with new CDs and ARPDs per year steadily increased from the <34 to 65-74 age group and decreased in the 75+ age group. 0.48% of the population older than 34 years of age received new CDs, and 0.23% received new ARPDs in these centres.

Professional guidelines for treatment should be taken into consideration as much as possible, to improve oral health in people who need prosthodontic care, while efforts should also be made to reduce the impact of disadvantaged socio-economic factors on treatment decision, especially in older individuals and others seeking dental care in Primary Health Centres in Kosovo.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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There is no financial interest or risk.

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Impact of combined non-surgical and surgical periodontal treatment in patients with type 2 diabetes mellitus-a preliminary report randomized clinical study.

Dashnor Bukleta^{1,2}, Shaip Krasniqi³, Giangiacomo Beretta⁴, Armond Daci^{5*}, Arb Nila², Teuta Komoni⁶, Manushaqe Selmani⁶, Brikene Elshani⁷, Rok Schara¹

¹Department of Oral Medicine and Periodontology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

²Department of Oral Surgery, Dental Polyclinic of Peja, Peja, Kosovo

³Institute of Pharmacology Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Prishtina, Prishtina, Kosovo

⁴Department of Pharmaceutical Sciences, Università Degli Studi di Milano, Milan, Italy

⁵Department of Pharmacy, Faculty of the Medicine University of Prishtina, Prishtina, Kosovo

⁶School of Dentistry, Faculty of Medicine, University of Prishtina, Prishtina, Kosovo

⁷Faculty of Medicine, University of Prishtina, Prishtina, Kosovo

Abstract

Background/objectives: Scientific evidence regarding the effects of chronic periodontitis on Type 2 Diabetes Mellitus (T2M) is fragmentary and not definitive. This intervention study was designed to evaluate the effects of a Non-Surgical Procedure (NSP) in addition to a surgical procedure on systemic inflammation and glycaemic control in patients with T2M and periodontitis and Non-Diabetic (ND) patients with periodontitis.

Material and methods: A total of 100 patients with diabetes were randomly allocated to a treatment group and a control group. At least one tooth was extracted from each patient. After extraction, the control group (n=50) did not receive any other treatment until the 3-month follow-up. Patients in the treatment group (n=50) received Full-Mouth Scaling and Root Planing (FM-SRP). The Mean Probing Depth (MPD), Mean Attachment Level (MAL), Plaque Index (PI), Bleeding on Probing (BOP), fasting blood samples for the measurement of high-sensitivity C-Reactive Protein (hs-CRP), and glycated hemoglobin (HbA1c) were measured or taken at baseline and 3 months after treatment. To investigate the effect of diabetes on the therapeutic procedure's outcome, ND patients with periodontitis (n=60) were divided into two equal groups and subjected to the same procedures and analysis.

Results: HbA1c and hs-CRP decreased significantly in the diabetic groups and decreased more markedly when FM-SRP was added to tooth extraction (P<0.001). For ND patients, all of the examined periodontal parameters were in favor of the combination therapy (P<0.001).

Conclusion: Non-surgical periodontal treatment can help improve the outcome of surgical periodontal procedures by reducing systemic inflammatory status and improve glucose metabolism.

Keywords: Diabetes mellitus type 2, Inflammation, Periodontitis, Diabetes, Periodontal treatment, Diabetes.

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Introduction

Type 2 Diabetes Mellitus (T2DM) is a chronic disease that comprises a heterogeneous group of metabolic disorders expressed with altered glucose tolerance or impaired carbohydrate metabolism. In diabetic patients with poorly controlled metabolism, complications develop more rapidly and in more severe forms. Systemic complications

(retinopathy, nephropathy, neuropathy, macrovascular and microvascular disease) are associated with prolonged hyperglycemia [1,2].

The prevalence of diabetes has drastically increased over the past decade, with economic conditions playing an important role due to poor medication adherence [3-5].

Currently, dental professionals play an important role in the process of diagnosing and monitoring DM [6]. A major role was shown to play also dental hygienists in the screening of patients with periodontitis [7].

It has been shown that DM is linked to various oral complications that include periodontal disease, which is classified as the sixth complication of diabetes. Studies have indicated that individuals with type 2 DM are three times more vulnerable to developing periodontal oral complications than individuals without diabetes [8,9].

Moreover, periodontal disease is considered to be one of the major oral complications that are likely to occur in patients with poorly controlled type 2 DM [10]. The prevalence of periodontal oral complications has been shown to increase with age. The loss of teeth or dental formulae is considered to be 15 times higher for individuals with DM than for those without DM [11]. A higher prevalence of periodontal disease is reported in individuals who rarely seek medical attention [12,13].

Individuals with type 2 diabetes also exhibit poor oral health [14]. This implies, therefore, that glycaemic control is generally affected by oral complications such as periodontal infection and oral complications adversely affect periodontal health in individuals [15].

Similarly, periodontal disease increases the severity of DM and complicates glycaemic control. In general, improvements in glycaemic control improvements were shown to decrease the rate of acute and chronic systemic complications [16]. In addition, patients with well-controlled diabetes, as measured by the blood glycated hemoglobin levels, have the less severe periodontal disease than do individuals with poorly controlled diabetes [17]. The elimination of periodontal infection improves the glycaemic control of diabetes, as defined by a reduction in glycated hemoglobin. It was confirmed that even full-mouth tooth extraction raises the improvement magnitude [18].

It is known that inflammation plays a part in insulin resistance in patients with type 2 diabetes [19]. According to this finding, in periodontitis patients without other apparent diseases, CRP levels are higher than in patients without periodontitis [20]. Management of diabetic patients can maintain periodontal health with responding better to the periodontal treatment [21], and on the other side, the control of chronic periodontal infection is essential for achieving long-term control of T2DM [22].

Previous studies found that non-surgical periodontal therapy alone has an impact on the quality of life [23], and also such as full-mouth scaling and root planning resulted in an improvement of glycaemic control [24,25]. Moreover, another study demonstrated that non-surgical periodontal therapy was not proven to be effective in patients with T2DM with underlying obesity [26].

However, recent meta-analyses have suggested that this conclusion must be interpreted with care due to the limited

robustness and heterogeneity of the studies available in the literature [27,28].

Taking this into consideration together with the necessity for further clinical trial investigations for T2DM and periodontal disease, the aim of the present study was to evaluate the effects of surgical treatment (tooth extraction) alone or in combination with non-surgical periodontal treatment (tooth cleaning) on periodontal status, systemic inflammation and glycaemic control in diabetic and non-diabetic patients with periodontal disease.

Material and Methods

Study design

In this study conducted during 2015-2016, based on a literature review, the data for 200 patients who were admitted to the endocrinology department of "Peja's Regional Hospital" and Dental Polyclinic in the city of Peja were assessed and examined for eligibility by oral surgery specialists. All procedures performed in our study involving human participants were in accordance with the ethical standards. The study was approved by the Ethics Committee of the University Clinical Centre of Kosovo (Nr.4212/2) and informed consent was obtained from all individual participants included in the study. A priori sample size calculation was performed given: Effect size $d=0.5$, alpha error probability 0.08 and power 0.8 resulting in 26 patients for the group.

Personal interviews were used to collect baseline data from each participant using a pre-structured questionnaire. Patients were selected if they met the following inclusion criteria: had been diagnosed with type 2 DM; had a baseline HbA1c $\geq 6.5\%$, had at least 10 teeth in the functional dentition (excluding third molars); and had a clinical diagnosis of periodontal disease with at least one site with a Probing Depth (PD) ≥ 5 mm, two teeth with attachment loss ≥ 6 mm and no modification in the pharmacological treatment of diabetes during the study period. Exclusion criteria included pregnancy or lactation, major diabetic complications, and the use of antibiotic therapy or non-steroidal anti-inflammatory drug therapy within 4 months before the first visit. Patient Characteristics and Baseline characteristics of biochemical and clinical data's from the patients are shown in (Tables 1 and 2).

After clinical examination, 160 patients aged 30-70 y old were selected for this open-label, randomized clinical trial. These were further divided into four groups: type 2 DM groups (with or without initial periodontal therapy) and non-diabetic groups (with or without initial periodontal therapy) (Figure 1).

All the patients had periodontal disease, and at least one tooth extraction was performed for each patient. Prior to the surgical procedures, an adjunctive, non-surgical periodontal treatment to achieve a full-mouth tooth cleaning was performed for the patients in the treatment groups: Full-Mouth Scaling and Root Planing (FM-SRP) using an ultrasonic device (UDS-J Ultrasonic Scaler, Guilin Woodpecker Medical Instrument) and

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periodontal curettes for the mechanical debridement of supra- and subgingival plaque and calculus.

Post-operative rinsing was followed by the use of the antiseptic solution Listerine® (ethanol 21.6%, methyl salicylate 0.06%, menthol 0.042%, thymol 0.064% and eucalyptol 0.092%) as a mouthwash thrice a day for 3 w. The study was registered on Clinical.Trials.gov (NCT02874963).

Statistical analyses

All data were expressed as the means and Standard Deviations (SDs). Before the statistical analysis was performed, the normal distribution and homogeneity of the variances were tested. Associations between the experimental parameters were investigated using one-way ANOVAs, followed by t-tests of pairwise comparisons with the Least Square Difference (LSD) post hoc adjustment for multiple comparisons. Significant differences were also evaluated using the Mann-Whitney U-test, paired Student's t-test or the Wilcoxon rank-sum test. The results were considered significant when the P value was $P < 0.05$ (GraphPad Prism 5.0 software).

Results

Glycemic control and inflammation

The FM-SRP treatment after tooth extraction-induced significant improvements in the serum HbA1c values in T2DM patients with periodontal disease.

No significant difference was found in the HbA1c plasma serum levels of ND patients ($5.56 \pm 0.62\%$ vs. $5.65 \pm 0.65\%$; $P = 0.099$, $n = 30$), (Figure 2A) or in ND patients treated with the combined surgical/non-surgical therapy ($5.79 \pm 2.92\%$ vs. $5.58 \pm 0.74\%$, (-3.6%); $P = 0.036$, $n = 30$) (Figure 2B).

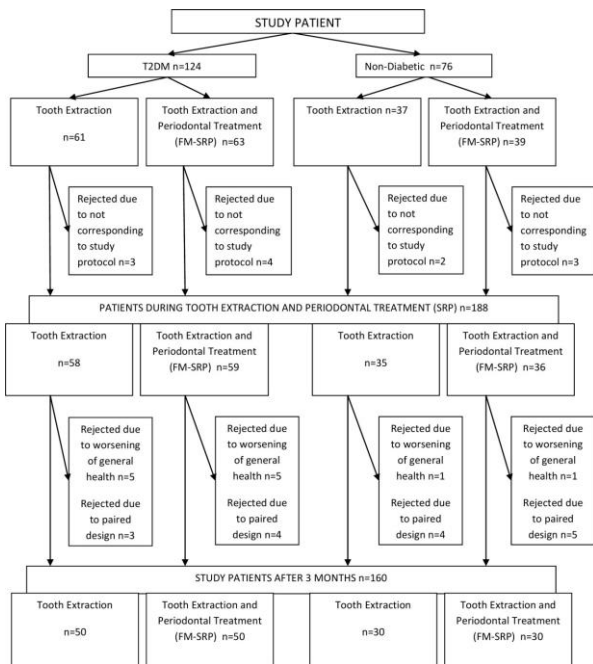


Figure 1. Study population flowchart.

Clinical data collection

The periodontal clinical examinations were performed according to the online periodontal chart (Department of Periodontology, School of Dental Medicine (ZMK), University of Bern) at baseline and 3 months after the periodontal treatment. All the parameters were recorded for six measurement points (mesial buccal, buccal, distal buccal, mesial lingual, lingual, distal lingual) on all teeth (excluding 3rd molars) during each of two visits.

Laboratory investigations

The levels of glycosylated hemoglobin (HbA1c) and of high-sensitivity C-reactive protein (hs-CRP) were measured using venous blood collected from patients at baseline and 3 months after the study procedures. All biochemical analyses were conducted in a biochemical laboratory in Peja (Laboratory Diagnostic Center, Peja, Kosovo) using a biochemistry analyzer (Select Pro XS, ELITech Clinical Systems, Paris, France) and enzyme-linked immunoassay kits (ELITech Clinical Systems, Paris, France).

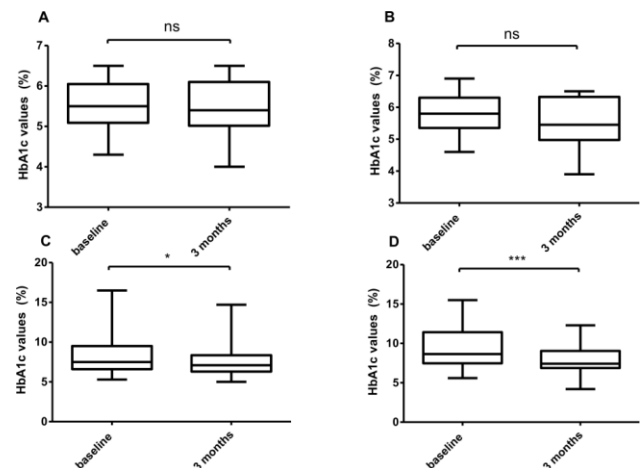


Figure 2. Graphical representation (box plot) of HbA1c values (%) at baseline and 3 months after tooth extraction in (A) non-diabetic patients with periodontal disease, (B) non-diabetic patients with periodontal disease treated with FM-SRP, (C) T2DM patients with periodontal disease and (D) T2DM patients with periodontal disease treated with FM-SRP. * indicates $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS: Not Significant.

With regard to periodontal disease, only the T2DM patients showed a significant HbA1c reduction after tooth extraction ($8.82 \pm 3.01\%$ vs. $7.69 \pm 2.09\%$, (-13%); $P = 0.018$, $n = 50$), (Figure 2C) and after tooth extraction followed by tooth cleaning with FM-SRP ($9.59 \pm 2.57\%$ vs. $8.03 \pm 1.67\%$, (-16.3%); $P = 0.0003$, $n = 50$) (Figure 2D). The variation in the serum levels of hs-CRP in ND patients was not statistically significant after tooth extraction (1.64 ± 1.06 vs. 1.64 ± 0.92 ;

P=0.913, n=30) (Figure 3A), but it was significant for patients with extracted teeth combined with FM-SRP ($3.63 \pm 2.19\%$ vs. $3.11 \pm 1.95\%$, (-14%); P=0.0006, n=30) (Figure 3B). In T2DM patients with periodontal disease, the reduction of hs-CRP after treatment with either tooth extraction only or with tooth extraction combined with FM-SRP was significant and had a higher magnitude than that of the ND patients (4.05 ± 2.20 vs. 3.24 ± 1.67 , (-20%); P=0.0003, n=50) and 4.44 ± 2.55 vs. 2.85 ± 1.96 , (-36.2%); P=0.0009, n=50) (Figures 3C and 3D, respectively).

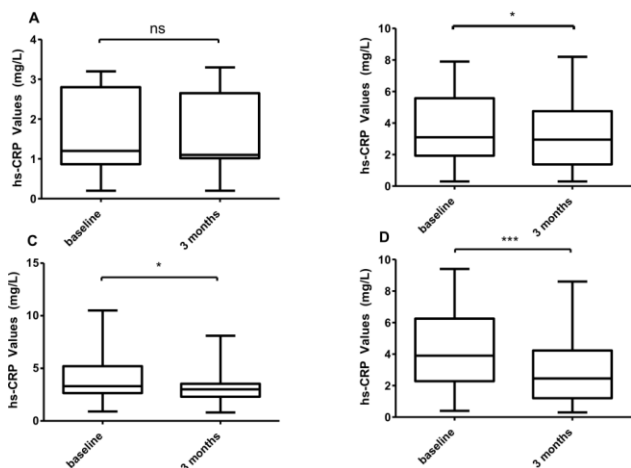


Figure 3. Graphical representation (box plot) of hs-CRP values (mg/L) at baseline and 3 months after tooth extraction in (A) non-diabetic patients with periodontal disease, (B) non-diabetic patients with periodontal disease treated with FM-SRP, (C) T2DM patients with periodontal disease and (D) T2DM patients with periodontal disease treated with FM-SRP. * indicates $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Periodontal conditions

The MPD value decreased, although not significantly, in ND patients after tooth extraction without FM-SRP treatment, while the decrease observed in non-diabetic patients reached the level of statistical significance in the FM-SRP treated group. This variation was significant and slightly higher in both T2DM groups: extraction only (6.60 ± 1.47 mm vs. 5.24 ± 2.17 mm; P=0.0004, n=50) and extraction followed by FM-SRP (6.52 ± 0.82 mm vs. 5.08 ± 1.10 mm; P=0.0001, n=50) (Figure 4A). The MAL value decreased significantly only in T2DM patients after tooth extraction followed by FM-SRP (-8.51 ± 1.55 vs. -7.32 ± 1.31 ; P=0.0001, n=50) (Figure 4B). Although the other groups showed a similar trend, those differences did not reach the level of statistical significance.

As expected, a significant reduction of PI was found in all groups with different magnitudes of reduction, depending on the FM-SRP application.

The lowest reductions were found in ND and T2DM patients after tooth extraction only ($95.07 \pm 7.14\%$ vs. $86.23 \pm 12.51\%$; P=0.0014, n=30) and ($93.02 \pm 15.78\%$ vs. $79.41 \pm 20.97\%$;

P=0.0001, n=50 respectively); the differences were enhanced by the FM-SRP treatment in both the ND and T2DM groups (96.57 ± 8.28 vs. 61.07 ± 25.27 (n=30); P=0.0028 and $98.06 \pm$

5.92 vs. 58.67 ± 22.76 ; P=0.0001, n=50 respectively) (Figure 5A).

BOP was reduced in all groups: ND patients after tooth extraction ($70.36 \pm 22.58\%$ vs. $55.70 \pm 20.57\%$; P=0.011, n=30) ND patients after tooth extraction followed by FM-SRP ($73.31 \pm 32.26\%$ vs. $48.57 \pm 29.72\%$; P=0.0031, n=30), T2DM patients after tooth extraction only ($71.55 \pm 34.65\%$ vs. $51.01 \pm 34.02\%$; P=0.0023, n=50), and T2DM patients after tooth extraction in combination with FM-SRP ($87.76 \pm 23.89\%$ vs. $28.94 \pm 21.65\%$; P=0.0001, n=50) (Figure 5B).

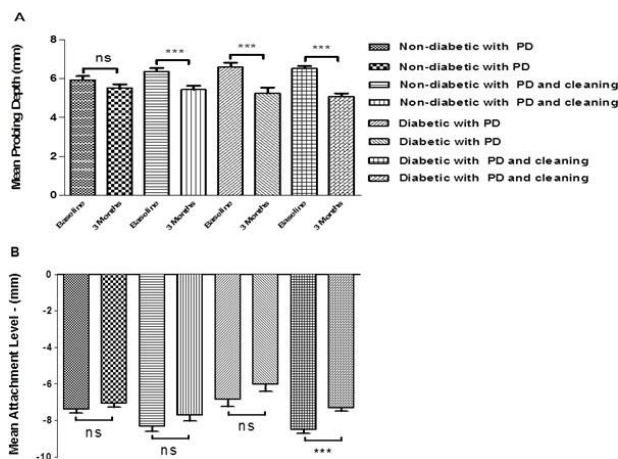


Figure 4. Comparison of mean probing depth (mm) (A) and mean attachment level (mm) (B) among groups with respect to time at baseline and 3 months after tooth extraction or extraction accompanied by periodontal treatment (FM-SRP). * indicates $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

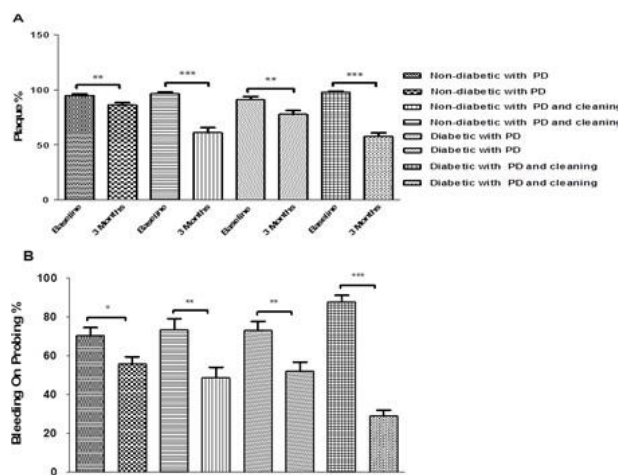


Figure 5. Comparison of plaque (%) (A) and bleeding on probing (%) (B) among groups with respect to time at baseline and 3 months after tooth extraction or extraction accompanied by periodontal treatment (FM-SRP). * indicates $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table I. Patient characteristics.

Characteristics	T2DM	Controls
Total patients (n)	100	60

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Sex (Male/female)	50/50	33/27	BMI	26.01 ± 3.64	26.49 ± 2.20
Age (y)	59.49 ± 10.82	56.66 ± 12.22	No. of teeth extracted	175 (1.75)	103 (1.72)
Weight (Kg)	76.03 ± 9.76	78.3 ± 9.76	Oral therapy	48	-
Height (cm)	174.64 ± 13.96	171.86 ± 6.81	Insulin	52	-

Table 2. Baseline characteristics of biochemical and clinical data's from the patients.

Parameters	Patients with diabetes		Patients without diabetes		Smokers
	Tooth extraction and FM-SRP	Tooth extraction	Tooth extraction and FM-SRP	Tooth extraction	Diabetes/without diabetes
No. of teeth extracted	87	88	54	49	88/37
PD (mm)	5.92 ± 1.06	6.37 ± 0.93	6.59 ± 1.47	6.53 ± 0.83	6.55 ± 0.75/6.08 ± 0.864
mAL (mm)	7.38 ± 1.31	6.8 ± 1.68	6.85 ± 2.74	8.52 ± 1.56	7.40 ± 2.79/7.75 ± 1.41
P (%)	95.07 ± 7.14	96.57 ± 8.28	93.02 ± 15.78	98.06 ± 5.92	96.41 ± 9.22/93.73 ± 10.05
BOP (%)	70.36 ± 22.58	73.31 ± 32.26	71.55 ± 34.65	87.76 ± 23.89	79.57 ± 30.99/72.74 ± 27.33
HbA1c (%)	8.82 ± 3.01	9.59 ± 2.57	5.56 ± 0.62	5.79 ± 2.92	8.98 ± 2.48/5.76 ± 0.63
hs-CRP (mg/L)	4.05 ± 2.20	4.44 ± 2.55	1.64 ± 1.06	3.63 ± 2.19	4.2 ± 2.07/2.37 ± 1.54

Discussion

The present study investigated the effects of surgical and non-surgical treatments on the periodontal status, glycaemic control (HbA1c) and systemic inflammation (hs-CRP) of T2M and ND subjects with periodontal diseases. This study was conducted to detect the beneficial properties of full-mouth tooth cleaning after tooth extraction in T2DM patients with moderate to severe periodontitis.

The critical analysis of different periodontal parameters in the T2DM group and the control group enabled us to find valuable evidence that indicates that the study interventions are effective in reducing oral complications, particularly in T2DM patients.

Our results show that the all measured periodontal clinical parameters (MPD, MAL, BOP and PI) improved significantly in T2DM patients. These results are consistent with those from previous studies in which the effect of FM-SRP, subgingival curettage, and tooth cleaning treatments on glycaemic control has been evaluated [26,29-32].

Our results demonstrated that concomitant cleaning and tooth extraction is more beneficial than tooth extraction alone in the course of periodontal disease treatment for both non-diabetic and T2DM patients.

It is known that the progression of the periodontal disease as a chronic infection is a risk factor for the development of diabetes, and this was also observed in our study groups [33].

In addition, we used the HbA1c and hs-CRP levels as indicators of diabetic control and inflammation in both groups. Other studies have evaluated periodontal treatments such as topical antibiotics and surgical procedures and have shown significant improvements in HbA1c through the reduction of

hs-CRP levels, and these results are consistent with our findings [31,34,35].

Similarly, following tooth extraction alone, patients tended to have improved HbA1c and hs-CRP values [17,36]. Oral hygiene plays an important factor in the control of periodontal status and in metabolic control [37].

It is well known that dental prophylaxis alone plays a role in periodontal health but does not affect the improvement of HbA1c levels [38].

Interestingly, in another study, full-mouth disinfection in type 1 diabetes patients has shown a positive effect on metabolic control [39]. In another meta-analysis, non-surgical periodontal treatment improved metabolic control in patients with periodontitis and diabetes [25]. Moreover, another recent study demonstrated that better oral hygiene improved periodontal health and metabolic control, which also confirms our findings [40].

These studies suggest a beneficial effect of surgical and non-surgical treatments on glycaemic control and periodontal status. Hence, taking this into consideration, we confirm that tooth cleaning through FM-SRP increases the positive effect of tooth extraction in patients with the periodontal disease with or without T2DM, and we shed light on their importance in dental oral practice.

Conclusion

By showing the critical effects of tooth cleaning accompanied by tooth extraction in the response to and efficacy of treatment for periodontal disease patients with T2DM and without DM, our work may set the stage for larger investigational studies aimed at evaluating the impact of non-surgical and surgical

approaches to the clinical management of periodontal disease and T2DM.

Compliance and Ethical Standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

"All procedures performed in our study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards". The study was approved by the Ethics Committee of the University Clinical Centre of Kosovo (Nr.4212/2).

Informed consent

"Informed consent was obtained from all individual participants included in the study".

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Authors' Contributions

The study design was planned by RC, DB, AD, and GB. Data collection was performed by DB, AN, TK, MS. Data analysis was conducted by RC, DB, AD, SK. All authors contributed to the interpretation of the data and manuscript preparation.

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***Correspondence to**

Armond Daci
Department of Pharmacy
Faculty of Medicine
University of Prishtina
Kosovo

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Approaches And The Management Of Patients In Oral Surgery During COVID- 19 Pandemic Kosovo Region.

Bukleta D¹, Cena Muhaxheri G², Krasniqi E³, and Selmani Bukleta M^{4*}.

¹Department of Oral Surgery. School of dental medicine. College of Rezonanca. Kosova.

²Department of Oral Medicine. School of dental medicine. College of Rezonanca. Kosova.

³Private dental practice. Pejë. Kosova.

⁴Department of Prosthodontics. School of dental medicine. College of Rezonanca. Kosova.

ABSTRACT

Treatment of Covid 19 positive patients in Oral surgery has a very high risk transmission of SARS Covid 2 because it is associated with the specific nature of dental interventions, which includes: face to face communication, usage of sharp instruments, saliva and other oral fluids that the patient might contain. Aspect discussions, data collection and the purpose of the review of managing in oral surgery during the COVID-19 pandemic is to provide protection for both, technical and human resources and to avoid unnecessary exposure to infected patient. The research methods are used in electronic bibliographic databases of relevant scientific literature such as, world health organization, Scisearch, etc. Practical Guidelines for Dentistry professional during the pandemic COVID-19 virus should be adjusted in accordance with the directions of the Center for Diseases Control and Prevention. Administration for Occupational Health and Safety, American Dental Association and Alberta Dental Association & Collage, Canada.

Keywords: Covid-19, Sars-Cov-2, Pandemic, Protective Equipment, Oral Surgery, Practical instructions for Dentistry Professionals.

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**Corresponding author*

INTRODUCTION

Treatment of Covid Positive Patients in Oral Surgery is associated with a high risk of transmission of COVID – 19, as it is associated with the specific nature of Dental Interventions which include face-to-face communication, use of sharp instruments and extensive exposure to saliva, and other oral fluids of the patient. It was obvious already at a very early stage of the pandemic that healthcare workers are affected in 29% of the cases, which is disproportionately high. During the SARS-CoV-2 pandemic, the specialty must organize patient treatment in such a way that infection transmission is reduced to a minimum, while all relevant treatment options are at hand to provide adequate patient care. Concepts have to be developed that take into account the possible need for triaging patients according to the degree of urgency of treatment in the field of Oral Surgery. Currently, specific guidelines and recommendations are just evolving (1).

MATERIALS AND METHODS

The research method is the electronic bibliographic databases of the relevant scientific literature such as: MEDLINE, EMBASE, CINAHL, ScienceDirect and Google search engine.

Literature searches were conducted for English language articles using index terms (e.g., Medical Entities Titles [MoH], Emtree) and free text keywords to identify acceptable reports.

The search terms used were "coronavirus 19 disease, COVID-19, severe coronavirus severe respiratory syndrome 2, SARS-CoV-2, transmission, pandemic, oral surgical procedures, oral surgery, dental equipment, personal protective equipment, prevention and infection control "The last search was conducted on 23.03 2021."

RESULTS

It is important to have a clear and well-communicated concept of the benefits of procedures in Oral Surgery.

Although it may be easy to distinguish between elector and emergency procedures, the boundaries between intermediate priority and urgent and emergency interventions can sometimes be blurred.

Therefore, relevant guidelines may vary between institutions or may depend on the individual characteristics of an institution.

If a conservative therapy is equivalent to surgery, it should be preferred at times with limited capacity for surgery.

Therefore, following the protective protocols in the COVID-19 crisis is of great importance for a dental environment where it can be done as much as possible to prevent the spread of the virus as well as to perform the necessary Dental services.

WHO through "Open data Kosova" (update) daily presents statistical data (collected by the National Institute of Public Health of Kosovo) on the number of infected persons and their gender within 24 hours (2).

The data presented in figure 1 are official data from the National Institute of Public Health of Kosovo.

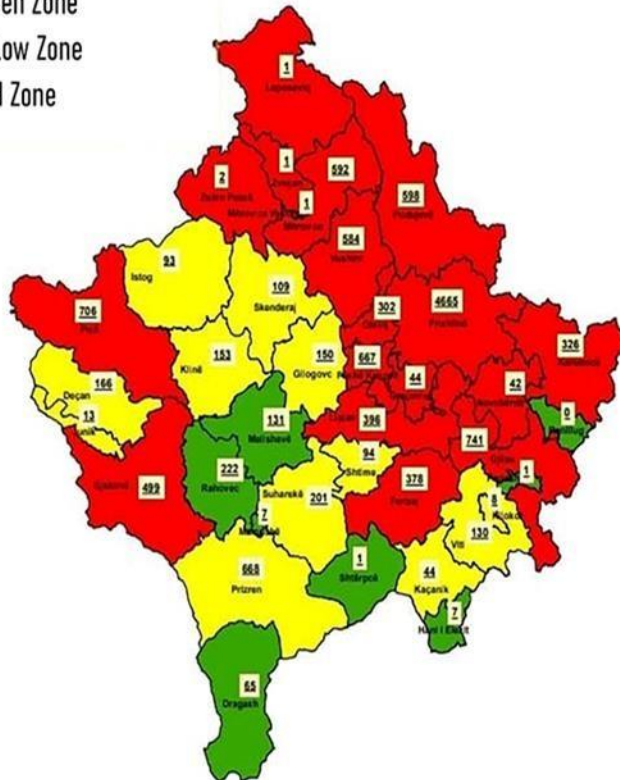
The following data, such as preventive measures and acquaintance with the patient's status, can be obtained through telephone or other electronic equipment's.

Epidemiological overview of active cases with COVID 19 in the Republic of Kosovo from 13 March - 22 March 2021

Day: 375	Infected during the last 24 hours	Total infected	Recovered during the last 24 hours	Total recovered	Dead during the last 24 hours	Total dead	Active cases	Tested during the last 24 hours	Total tested	Infected in 100k habitants	Revered in 100k habitants	Dead in 100k habitants	Population
Cases	705	83717	522	69123	10	1786	12808	3922	393713	4698	3879	100	1782115
%	17,98	21,26	0,62	82,57	0,01	2,13	15,30	0,22	22,09	0,26	0,22	0,01	

Municipality	Infected	Recovered	Dead	Active cases	Ratio cases to 100k habitants
Prishtina	32755	27823	255	4665	2151
Gjilan	4634	3754	109	741	972
Prizren	4027	3153	206	668	346
Peje	3897	3092	99	706	719
Fushë Kosovë	3870	3154	69	667	1712
Mitrovicë e Jugut	3164	3054	60	592	660
Podujevë	3055	3002	56	538	734
Ferizaj	3634	3145	105	378	340
Gjakovë	3588	2969	120	499	537
Vushtrri	2789	2132	73	584	942
Lopjan	2436	2006	34	396	690
Gjogjoc	1489	1291	48	150	248
Obiliq	1402	1074	26	302	187
Viti	1396	1211	55	130	274
Surrahkë	1263	1071	71	201	351
Kamenicë	1166	703	47	326	172
Skenderaj	1048	609	30	109	208
Deçan	968	777	25	166	393
Rahovec	959	675	64	222	294
Malishevë	932	754	47	131	234
Kisine	900	711	36	153	387
Shitme	701	596	21	94	348
Istog	676	553	30	93	277
Kaçanik	481	421	16	44	128
Dragash	373	275	33	65	191
Gracanicë	384	39	1	44	361
Novobërdë	127	84	1	42	590
Hani i Elezit	95	86	2	7	70
Jurine	92	77	2	13	204
Mamuzhe	63	50	6	7	120
Kilicët	58	50	0	8	296
Zubin Potok	58	54	2	2	30
Mitrovicë e Veriut	49	45	3	1	8
Zveçan	34	32	1	1	14
Shitërroc	31	27	3	1	15
Leposaviq	26	21	4	1	8
Ranilug	8	8	0	0	0
Partesh	5	4	0	1	5
Total	83717	69123	1786	12808	

Green Zone
Yellow Zone
Red Zone



Total cases hospitalized with COVID 19 on 23.03.2021		
Confirmed with COVID 19	Cases	%
Hospitalized	706	9,29
O ₂	669	94,76
CPAP	25	3,54
Respirator	11	1,56

Source: MSH, IKSH, AKK and ASK

1:500000

Figure 1: Number of infected residents with COVID -19 by Cities of Kosovo. Graphs and Statistic updated on 03/23/2021 07:25:10.

Urgency and Emergency in Oral Surgery

Dental urgency refers to conditions that require necessary interventions to stop major, intense pain and infections (3).

These conditions include:

1. Severe pain caused by inflammation of the pulp & apical periodontium;
2. Pericoronitis;
3. Postoperative osteitis, alveolitis.

Guide for applying dental interventions to patients with COVID 19:

- Localized abscess, pain and swelling;
- Soft and hard tissue trauma;
- Tooth traumas such as: avulsion, luxation;
- Dental interventions that precede medical interventions;
- Tissue biopsy;
- Patient interventions should be minimally invasive.

From rapid measures it is important to distinguish non-emergency dental interventions such as:

- First or systematic visit;
- Application of regular preventive measures;
- Removal of soft and hard deposits;
- Orthodontic interventions, except when they do not cause pain, infection or trauma;
- Asymptomatic tooth extraction;
- Restorative procedures which include asymptomatic carious teeth;
- Aesthetic dental interventions.

Measures to prevent health professional infection and nosocomial transmission at dental clinics (4):

- Obligatory, only one patient and / or attendant can stay in the waiting room;
- At the entrance / reception, there should be disinfectant and instructions for patients on how to use;
- All magazines, toys, etc. should be removed as a precaution against contamination / pollution;
- Given that the coronavirus stays on the surface for 24+ hours, it is very important to keep all surfaces clean and disinfected. This applies to the dental office, toilet, waiting area, and it is especially important to disinfect the operating room as often as possible;
- Commonly used disinfectants, such as 0.1% sodium hypochlorite or 62% -71% ethanol, have been proven to be very effective;
- The ventilation of the environment should be as natural as possible and the ventilation with air conditioner should be avoided;
- Extra care for instruments by applying the principles of Aseps and Antiseptics (2).

Precautions in the operating room

Prior to taking a patient to the surgical room, a test for SARS-CoV-2 should be performed. An emergency patient that leaves no time for testing should be treated as being infective.

In the operating room, negative pressure must be established with the aim to reduce dissemination of the virus. Before entering the operating room, every staff member needs to put on personal protective equipment. Besides an FFP3 respirator, it is also important to wear a face shield. At any time, the number of staff members in the operating room should be minimal.

The personal protective equipment of the surgical team should be completed by a water-tight sterile gown. Whenever possible an experienced team should perform the surgery. During the procedure, leaving or entering the operating room should be limited to a minimum.

If an extra oral approach is a relevant alternative to an intraoral one, it should be preferred. Reducing aerosol formation to a minimum should be a priority. Excessive water cooling for dental drill, saws, ultrasonic devices, and piezoelectric devices should be avoided. Instead of drilling screw holes, self-drilling screws should be used. The use of osteotomies should be considered wherever possible. Electric cautery should be avoided or performed with the lowest power possible and a smoke evacuation system (1).

Some dental procedures favor the creation / spread of aerosols, which can be potentially hazardous to Covid-19 transmission. Therefore, it is recommended that dentists limit such procedures to protect patients, staff and themselves. Pandemic Protective Equipment (PPEs) that do not create aerosols.

Current standards for infection prevention and control are applied with appropriate personal protective equipment: gloves, surgical mask, and goggles.

PPEs that create aerosols for dental procedures that create aerosols, in addition to current standards for the prevention and control of infection, additional personal protective equipment is needed: protective clothing, gloves, mask N-95 / respirator, suitable goggles or face shield (4).

Additional measures before treatment:

1. Use 1% 5cc hydrogen peroxide for rinsing for 30 seconds before examining the oral cavity;
2. Use Cofferdam for isolation;
3. Use high volume aspirators during dental procedures (4).

DISCUSSION

The COVID-19 pandemic puts pressure on the healthcare system, because of this there is a need for continuous adaptation of recommendations and guidelines.

In oral surgery elective procedures such as urgent and emergent procedures are performed.

When treating patients during the SARS-CoV-2 pandemic, a major issue is disease transmission from the patient to the medical staff. Patients with symptomatic COVID-19 should be treated in the field of oral and maxillofacial surgery only when the indication is urgent or an emergency. Symptomatic patients are a major source of viral transmission and therefore must be treated in an adequate infrastructure with personal protective equipment.

Additionally, asymptomatic patients and patients undergoing the incubation period can be carriers of SARS-CoV-2 and can be responsible for infection transmission. It is even debated whether patients in the recovery phase are potential sources of virus transmission. As with every other infectious disease, the approach to the situation must be, that the patient must be considered infective as long as the opposite is not proved. Obviously, there is a need for sensitive, reliable, and rapid testing of patients who enter the private practice or the hospital for an urgent or emergency treatment. The potential patients should be advised to first opt for a consultation on the telephone before they come for a face-to-face consultation. A relevant number of issues can be clarified by telephone, helping to avoid face-to-face contact (1).

CONCLUSION

The major aim is to protect patients as well as the medical team from unnecessary infection and to keep the healthcare system running effectively.

Although it might be easy to distinguish between elective and emergency procedures, the boundaries between interventions of intermediate and urgent priority might be blurred sometimes. Therefore, respective guidelines might differ between institutions or might be dependent on individual characteristics of an institution.

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RESEARCH ARTICLE

Comparison Of The Effectiveness Of Antibiotic Prophylaxis In Oral Surgery Department.

Bukleta D¹, Rexhaj E², Nila A², Berisha M², and Selmani-Bukleta M^{3*}.

¹Department Of Oral Surgery. School Of Dental Medicine. College Of Rezonanca. Kosova.

²Dental Polyclinic Oral Surgery. Peje. Kosova.

³Department Of Prosthodontics. School Of Dental Medicine. College Of Rezonanca. Kosova.

ABSTRACT

Antibiotic prophylaxis means the administration of antibiotics before surgical procedures in those cases when there is a possibility of postoperative complications or the possibility of transient bacteraemia. The methods are based on electronic literature, scientific papers which are researched in MEDLINE, PUBMED, LIBGEN, SCI-HUB and Google search engine. These studies were selected based on 2009 to the present time, surgical intervention, type of therapy and antibiotic dose. Three surgical procedures such as the extraction of third molar, the placement of dental implant and infective endocarditis has been researched with similar studies where a comparison has been made between them and the prescribed antibiotic therapy to see which of the therapies is more effective. Almost all studies have recommended antibiotic prophylaxis in these surgical procedures. In all three surgical procedures antibiotic prophylaxis is recommended in order to reduce the chances of postoperative complications, provided that these patients have a higher risk of these complications.

Keywords: antibiotic prophylaxis, dental implant, third molar surgery, oral surgery, endocarditis.

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**Corresponding author*

INTRODUCTION

The oral cavity contains a microflora with about 700 different microbial species.

The purpose of Antibiotic Prophylaxis in dentistry is to protect patients with a certain specific risk from local microbial contamination or systemic spread of oral bacteria during dental procedures which induce bleeding and a transient bacteraemia.

Dental procedures can cause a transient bacteraemia, with higher values in oral surgery (third molar extraction, periodontal surgery) (1). But is very important that the prescription of antibiotics should always be done after we have seen the general condition of the patient and see if there is an allergy to antibiotics (2).

Antibiotic prophylaxis to have effective results, we must adhere to antibiotic administration protocols such as determining the correct antibiotic and at the time of surgery, the dose of antibiotic should be present in the systemic circulation in order to prevent postoperative complications. The extraction of third molar is one of the most common surgical interventions requiring the need for antibiotic prophylaxis (3). The most common complications that may occur after third molar surgery are swelling, pain, trismus, infection etc. (4).

Dental implant placement is another surgical procedure that requires antibiotic prophylaxis to prevent postoperative infections and increase the success of implant longevity (5). Infective endocarditis (IE) also is a disease with high mortality. Most guidelines recommend giving antibiotics as prophylaxis to prevent IE in patients with specific predisposing cardiac conditions (6).

MATERIALS AND METHODS

The research method is the electronic bibliographic databases of the relevant scientific literature such as: MEDLINE, PUBMED, LIBGEN, SCI-HUB and Google search engine.

The first surgical procedure that we have been studied is the prophylaxis before and after surgical intervention related to the third molar. The research from Martin-Ares et al (7) has divided patients into two groups. One group of patients was administered 750 mg amoxicillin every 8 hours for 5 days after surgery and the second group placebo which means the administration of anti-inflammatory 50 mg diclofenac sodium every 8 hours for 4 days after surgery and analgesic 575 mg magnesiummetamizol every 6-8 h in cases when pain occurs.

The research from Milani et al is related also with the third molar (8). Here the patients were divided into three groups, the first group used 1 g amoxicillin 1 h before surgery + 500 mg every 8 h for 7 days. The second group used 1 g amoxicillin 1 h before surgery plus placebo, every 8 h for 7 days, while the third group used placebo 1 h before surgery and 500 mg every 8 h for 7 days.

The second surgical procedure that we have been studied is for the prophylaxis before the surgical procedure of the placement of dental implant. In the both mentioned studies, patients were divided into two groups. In the research of Arduino et al (9) the comparison of preoperative and postoperative antibiotics is made to see which is more effective in dental implants. The first group of patients were administered 2g of amoxicillin orally 1h prior to implant placement and the second group were administered 2g of amoxicillin orally 1h prior to implant placement; 1g in the evening of the day of surgery and 1g twice a day for 2 days following surgery.

In the research from El-Kholey (10) antibiotics were administered before and after surgery and a comparison was made to see as to which group of therapy was most effective. The first group of patients were administered 1g of amoxicillin preoperatively and in the second group were administered 1g of amoxicillin preoperatively and the antibiotic continued after surgery for three days.

The third surgical procedure that we have studied is the prevention of infective endocarditis before surgical intervention by prescribing antibiotics as prophylaxis. Articles are from American Heart Association and European Society of Cardiology (11, 12).

Both researches that we have been studied related with infective endocarditis, point that only a small number of cases of infective endocarditis might be prevented by antibiotic prophylaxis. Recent studies suggest prescribing of antibiotics only for patients with the highest risk for infective endocarditis. In both studies the preferred dose was 2 g amoxicillin 30-60 min before the procedure.

RESULTS

In this study, three surgical cases were researched and compared with similar studies. These studies were selected based on 2009 to the present time, then based on the surgical procedure, type and dose of antibiotics.

In table 1 were presented two cases with surgical intervention in the third molars. Here is a comparison between similar studies to determine which type of therapy is most effective in preventing the occurrence of infection after surgery in the third molars.

In the first case the patients were divided into three groups in which different therapies are given in order to compare which type of therapy is most effective in the case of third molar surgery. In the second case the patients are divided into three groups with different therapies and the comparison is made to determine which of the therapies is more effective.

In table 2 were presented two cases for surgical interventions of dental implants placement. Here is a comparison of these two studies with relevant therapy in order to prevent postoperative complications.

In the first case patients are divided into two groups and the comparison of preoperative and postoperative antibiotics is made, which is more effective to prevent infections during the procedure of dental implants placement. In the second case patients were divided into two groups and antibiotics were administered before and after surgery and a comparison was made to see which group of therapy was most effective.

Table 3 presents two researches on infective endocarditis with doses of antibiotics for prophylaxis.

The first case is from the organization of the American Heart Association while the second case is from the organization of the European Society of Cardiology.

These researches show the recommendations for antibiotic prophylaxis.

Transient bacteremia appears while working with teeth and periodontal tissues, and there is a wide variation in reported frequencies of bacteremia in patients resulting from dental procedures: tooth extraction (10% to 100%), periodontal surgery (36% to 88%), teeth cleaning (up to 40%), rubber dam matrix/wedge placement (9% to 32%), endodontic procedures (up to 20%), scaling and root planning (8% to 80%) (11).

DISCUSSION

The purpose of this study is to show efficiency of antibiotic prophylaxis in dental procedures, more precisely in three dental procedures which are: the extraction of the third molar, the placement of dental implants and infective endocarditis. In each of these surgical procedures a comparison was made with similar researches but having different therapies for antibiotic prophylaxis.

After detailed study related with antibiotic prophylaxis before and after surgical intervention related to the third molar, it has been confirmed that the group of patients who have used antibiotic therapy have shown higher efficacy in avoiding complications such as pain, inflammation etc. In other way it recommends that antibiotics should not be used for a long time because they can cause serious problems (7). However, they recommend that the use of antibiotics should not be done in cases when patients are healthy and adhere to strict antiseptic rules with good oral hygiene. Because the patient's defense mechanisms are sufficient and effective in preventing the occurrence of postoperative complications.

The use of antibiotics is indicated in patients with general immunosuppressive diseases such as uncontrolled diabetes, in patients who do not adhere to the strike rules of good oral hygiene (8). Based on the research related for prophylaxis before the placement of dental implants, can be concluded that it is sufficient to prescribe antibiotic preoperatively than to prescribe postoperatively (9, 10).

In other way prevention of infective endocarditis during oral surgery procedure it is very important. The ability of antibiotic therapy to prevent bacteremia associated with a dental procedure is controversial. Some studies reported that antibiotics administered before a dental procedure reduced the frequency, nature, and duration of bacteremia where as others did not (11).

Amoxicillin is the preferred choice for oral therapy because it is well absorbed in the gastrointestinal tract and reaches high serum levels. For individuals who are allergic to penicillins or amoxicillin, the use of cephalexin, clindamycin, azithromycin, or clarithromycin is recommended (11).

Table 1. Third molar case intervention and compared with similar researches

Authors	Year	Intervention	Dosage of antibiotics	Conclusions
Martin-Ares et al ⁷	2017	Third molar	Group 1- 750 mg amoxicillin p.o every 8 h for 5 days postoperative Group 2- Placebo	The first group has shown higher efficiency in presenting pain and inflammation that reduces the need for analgesics
Milani et al ⁸	2015	Third molar	Group 1- 1 g amoxicillin 1 h before surgery + 500 mg every 8 h for 7 days Group 2- 1 g amoxicillin 1 h before surgery plus placebo, every 8 h for 7 days Group 3- Placebo 1 h before surgery and 500 mg every 8 h for 7 days	No difference which group of antibiotics is better than the other in treatment

Table 2. Dental implant case intervention and compared with similar researches

Authors	Year	Intervention	Dosage of antibiotics	Conclusions
Arduino et al ⁹	2015	Dental implant	Group 1- 2 g of amoxicillin administered orally 1h prior to implant placement Group 2- 2 g of amoxicillin administered orally 1h prior to implant placement, 1 g the evening of the day of surgery and 1 g twice a day for 2 days following surgery	There are no statistical differences between the two groups but based on this study it is sufficient to prescribe 2 g of antibiotic preoperatively than to prescribe postoperatively
El-Kholey ¹⁰	2014	Dental implant	Group1 – 1 g amoxicillin preoperatively Group 2- 1 g amoxicillin preoperatively and the antibiotic continued after surgery for 3 days	No statistical differences between the two groups but based on this study 1 g amoxicillin preoperatively is sufficient

CONCLUSION

Based on the elaborations on this study, we can conclude that all three surgical procedures antibiotic prophylaxis is recommended in order to reduce the chances of postoperative complications, provided that these patients have a higher risk of these complications.

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Life expectancy's relationship with behavioral factors and polypharmacy in Western Balkan countries

Arben Boshnjaku^{1,2 *}, Ermira Krasniqi²

¹Faculty of Medicine, University of Gjakova "Fehmi Agani", Ismail Qemali, n.n., 5000 Gjakova, Kosovo

²College of Medical Sciences Rezonanca, Blloku te Shelgjet – Veternik, 10000 Prishtina, Kosovo

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Abstract

Ageing is a multidisciplinary studied process characterized with a gradual increased time of homeostasis and decreased time of reaction and performance. Expected life expectancy is an important measure of a populations' health status and healthcare system's performance, which is characterized with a gradual increase in the modern world. This increasing trend changes between different countries and societies, while being affected by several internal, external and behavioral factors.

This narrative review analyses and compares the countries of Western Balkans, all of whom classified as middle income countries.

Increasing physical activity, avoiding smoking as well as decreasing overweight and obesity present reliable mechanisms to invest in terms of providing a better lifestyle and quality of life. Polypharmacy presents another integral player into this process, which affects and interacts with each and every other factor. Altogether should be taken in consideration in policy makings, healthcare approaches and intervention plans.

Keywords: life expectancy, polypharmacy, western Balkan, overweight, obesity

Abbreviations

BMI – Body Mass Index, EU – European Union, GHO – Global Health Observatory, OECD - Organization for Economic Co-operation and Development, WHO – World Health Organization

Introduction

Ageing is a multidisciplinary studied process and phenomenon that starts from conception and continues throughout the life span, until the final moments of life, culminating with death. In the eyes of natural sciences, the ageing process follows several stages which culminate with senescence. The social sciences see ageing as a

phenomenon (besides as a process) that undergoes certain psycho-social evolution and challenges.

Homeostasis is one of the core processes enabling and assuring a decent contingency into the ageing process. An increase in time for equilibration is observed with ageing, which is mainly due to a number of different factors, gradually resulting in the decrease of the quality of life through the increase of prevalence of functional impairment, chronic diseases and mortality (Taylor et al., 2008).

Ageing (considering within the senescence period) is characterized by a tendency to increase body weight, fat content (including the redistribution from periphery towards center), and decrease of height, fat free mass (including muscle and bone mass) and physical activity (Kenney et al., 2012). There are two types of ageing: eugeric (true ageing / age-related changes) and pathogenic

* arbenboshnjaku@gmail.com

(pathological ageing / ageing process affected by external factors such as: environmental, genetic mutations and accidents) (Taylor et al., 2008).

Life expectancy at birth represents the average age at death if everyone experienced the prevailing death rates throughout their lifetime (Aburto et al., 2018), while being one of the most commonly used measures of the health status of a population and the performance of the healthcare system (WHO, 2016). In fact, it has been shown that a nation's life expectancy reflects its social and economic conditions and the quality of its public health and healthcare infrastructure (health spending per capita), whereas monumental improvements in life expectancy have been the predominant trend for high income, developed countries over the course of the 20th and 21st centuries (Bongaarts, 2006; Wilmoth, 2000). At the moment, worldwide expected life expectancy at birth is

72.4 years, out of which 70.2 in males and 74.7 in females (Worldbank, 2017). This number changes in different countries or populations due to several factors, starting from the quality and approach to healthcare system, morbidity, polypharmacy, level of education, genetic background, counties level of development and socio-economic aspects. Countries level of development unarguably presents an important factor on this matter, especially having in mind that many of the other factors are either directly or indirectly related to it. Therefore, notwithstanding the lack of a clear definition for developing countries, the common understanding is that these are countries generally characterized by low per capita income, an unequal distribution of resources, inadequate health care, bad education opportunities and high unemployment rate. Western Balkans is an encompassing name for a sub-region in Europe, formed by Albania, Bosnia and Herzegovina, North Macedonia, Montenegro, Serbia and Kosovo. According to the Organization for Economic Co-operation and Development (OECD) list of European developing countries only Kosovo is classified as being part of the Lower Middle Income Countries, whereas all the others belong to the Upper Middle Income Countries. The classification threshold lies upon per capita gross national income (GNI) of \$1 006 - \$3 955, in comparison to GNI \$3 956 - \$12 235 in upper middle income countries and ≤ \$1 005 in 2016 in low income countries (OECD, 2020).

Based on the upon mentioned facts, this narrative review aims to: (1) compare the expected life expectancy between the countries in the Western Balkan region, (2) analyze the impact of different covariates in the current state of art, (3) analyze the polypharmacy and its relation to morbidity in these populations.

Expected life expectancy in Western Balkans vs. EU-countries

The expected life expectancy in European Union (EU) is much higher than the world average (EU: 80.9

years in total, 78.4 in males and 83.8 in females, world: 72.4 years, out of which 70.2 in males and 74.7 in females) (Worldbank, 2017). These differences are seen mainly due to the countries level of development, which encompasses the generally better education, healthcare and social systems, altogether providing a better quality of life, lower morbidity and mortality. To this day, differences between lower and middle income countries vary between 68.3 years (66.7 in males and 70.1 in females) and 75.5 years (72.9 in males and 78.3 in females), respectively (Worldbank, 2017). Therefore, it is obvious that Kosovo as the only officially classified country in the lower group (within the studied countries) should have a lower expected life expectancy. Yet, this is not the case since Serbia (75.9 years in total, 73.5 in males, 78.4 in females) (Eurostat, 2020) presents a relatively lower life expectancy than Kosovo (76.7 years in total, 74.1 in males, 79.4 in females) (2011, Census), whereas North Macedonia (76.7 years in total, 74.6 in males, 78.8 in females) presents a relatively similar case. Montenegro is to some extent better (76.9 years in total,

74.5 in males, 79.3 in females), whereas Albania has the highest expected life expectancy in the region (78.9 years in total, 77.4 in males, 80.5 in females) (Eurostat, 2020). For comparison, developed European countries such as: Germany (81.0 in total, 78.6 in males and 83.3 in females), Austria (81.8 in total, 79.4 in males and 84.1 in females), Italy (83.4 in total, 81.2 in males and in 85.6 females) or France (82.9 in total, 79.7 in males and 85.9 in females) present a much higher expected life expectancy, even though these data could be changed in the wake of the aftermath of Covid 19 pandemics which undoubtedly presents a potentially demographical changing factor. It must be stated that there is a positive aspect in the studies data, which is the fact that these data are undergoing a constant increase in all the cases. This undoubtedly serves as a good indicator on the increase of the quality of life, as well as opportunities towards better service and goods.

The other interesting findings from these data are that excluding Serbia (19.4%), the number of older adults in this region (Montenegro: 14.4%, North Macedonia: 13.3%, Albania 13.1% and Kosovo: 8.1%) is lower than the EU's (19.4%), with North Macedonia leading in the transitional group 15 – 64 years (70.2%), followed by Albania (68.7%), Montenegro (67.4 %), Kosovo (68.8%) and Serbia (66.3%) (Eurostat, 2020). This is an aspect to be taken in serious consideration since with the increase of life expectancy comes the increase of the number of older populations. Thereafter, this leads to extra economic, psycho-social and healthcare burdens.

Impact of behavioral factors on life expectancy

It has been described that the life expectancy increases with time within a wide variation between different populations, all based on health spending per

capita and behavioral factors such as smoking and caloric intake (Zare et al., 2015). All the countries of this region (Western Balkans) are characterized for either undergoing of having gone through a transition period from a centralized governing system in the past, towards a rather decentralized democracy with competitive capitalist approach. This transition period led to the foundation of many private based healthcare providing competitors. Due to a combination of many factors, beginning from the lower number of elderly population, lack of adequate policies and profit oriented offer, the older generation and health spending on them might have been neglected.

Behavioral factors such as physical activity, smoking, overweight and obesity could also play their part. Unfortunately, there seem to be a general lack of data with respect to the behavioral habits of the elderly in western Balkan countries, with an emerging need for future studies on these fields. With respect to the physical activity level, we can only assume that it should be lower to other European countries, mainly due to the lack of organized infrastructure. This should be important especially knowing the fact that with ageing, comorbidities and impairments increase. This often leads to the bounding of physical activities to restricted paths and fields with extra caution.

Smoking is another factor described to strongly amplify the risk for mortality (Mons et al., 2015; Qin et al., 2013), which is presented in quite high prevalence within the studied countries. Smoking prevalence varies from 51.2% in males and 7.6% in females in Albania, 47.2% in males and 30% in females in Bosnia and Herzegovina, 43.6% in males and 39.7% in females in Serbia (WHO, 2015), 46.7% in males and 27.8% in females in North Macedonia (Tobacco control fact sheet, 2016), 47.9% in males and 44.0% in females in Montenegro (Worldbank, 2016) to 37.4% in males and 19.7% in females in Kosovo (Gashi et al., 2017). The importance of smoking consumption and its effect on health is observed especially when comparing with the European countries with higher life expectancy such as: Germany (32.4% in males and 28.3% in females), Italy (28.3% in males and 19.7% in females), France (29.8% in males and 25.6% in females) (WHO, 2015).

Last (but certainly not least), there are overweight and obesity as an ever-growing concern for public health. 39% of adults aged 18 years and over (39% of men and 40% of women) were classified as overweight by 2016 (officially defined as "abnormal or excessive fat accumulation that may impair health"), whereas this prevalence nearly tripled between 1975 and 2016; additionally, higher body mass index (BMI) is acknowledged as a major risk factor for non-communicable diseases such as: cardiovascular diseases, diabetes, musculoskeletal disorders and some cancers (WHO, 2020). Overweight and obesity present an already established factor correlated to populations in developing economies, and significantly related to higher mortality (Colpani et al., 2018; Tucker et al., 2001). To

this date, the overall prevalence of overweight and obesity amongst adults (ages 18+) is already high in these countries, starting from Montenegro 59.4% and 23.3%, respectively, North Macedonia 58.1% and 22.4%, Albania 57.7% and 21.7%, Serbia 57.1% and 21.5%, Bosnia and Herzegovina 53.3% and 17.9% (GHO, 2016), Kosovo 25.0% - 42.5% and 30% - 63% (Rexhepi et al., 2015). One ageing study performed in several countries including Albania, shows a prevalence of overweight and obesity in older subjects as high as 46.7% and 36.3%, respectively. Notwithstanding, there is a need for more data with respect of the overweight and obesity in older populations within the studied countries, whereas future studies analyzing this aspect could help bring more light on the issue. A fact to be noticed is that often health-inappropriate behaviors tend to be associated together, such as: physical inactivity with smoking, overweight or obesity, providing a potentially fatal combination that halts the increase in life expectancy, or even lowers it.

Morbidity, polypharmacy and life expectancy

Morbidity, polypharmacy and life expectancy are strongly correlated concepts. Medicines are used to prevent, treat or cure diseases, contributing in higher life expectancy. Regardless, higher life expectancy is accompanied with an increase of co-morbidities prevalence, showing the importance of age-related drug prescription (Shi et al., 2008).

In the existing literature, polypharmacy is not yet defined, since this is dependent from the present comorbidities. A systematic review on this issue emphasized that (most commonly) polypharmacy is considered when the patients is taking five or more medicines, while proposing the term of "appropriate polypharmacy" (Masnoon et al., 2017). The benefit of medicines usage is high in terms of quality of life, but polypharmacy has its negative consequences in health as well. This is mainly because of the adverse drug events and drug-drug interactions, which can lead to hospitalization and medicines induced morbidities. The prevalence of polypharmacy is expected to rise currently, especially when vulnerable group are patients above 65 years of age, as long as there are worldwide changes in proportion of older population (WHO, 2019).

Studies related to the current status of polypharmacy, its everyday life implications, morbidity and mortality are focused in developed countries, while this cannot be said for Western Balkans region. As pointed out by Kim et al. (2017), about 30% of adults aged 65 years and older take 5 or more medications in developed countries. But how is this situation in the countries of interest for this study?! There is a general scarce of comparable scientific evidence with their developed counterparts. In addition, existing studies have different methodologies, focus, inclusion criteria and year of publication which results in

inconsistency of the data. Some of these data show that the prevalence of polypharmacy (defined: ≥ 5 medicines) was found to be 10.4% in seniors in Serbia (Gazibara et al., 2013). Studies in Bosnia and Herzegovina found that this prevalence in population aged ≥ 65 years was 3.6% (Marković-Peković and Škrbić, 2016) and 48.1% (Alic et al., 2011). Another study in this country, found polypharmacy (defined: ≥ 3 medicines) in 74% of the hospitalized patients aged from 45 - 50 years (Trumic et al., 2012). To the best of our knowledge, there are still missing comparable data that could shed light on the matter. In terms of achieving higher life expectancies, observational, longitudinal and multi-centered research are needed. These measures would help these countries to build proactive and trustworthy policies which will contribute to the aim itself, as long as aging brings new challenges and opportunities in the health care system and age related medicines prescription.

Conclusion

Ageing is an important life process. Successful ageing is an achievement and a life milestone for everyone. A society's success should be estimated on the percentage of older populations and the quality of life these age groups live. Yet, this process can be a challenge in many countries, especially in developing ones. Countries from the western Balkans, even though geographically part of Europe, are amongst the latest remaining outside of the EU. Notwithstanding the turmoil most of them underwent in the last decades, the current development rate and socio-economic progress is promising. The continuous increase of expected life expectancy is a promising sign for a better quality of life for the whole population. Nevertheless, this will undoubtedly come with certain difficulties and obstacles, especially in terms of the continuous increase of older population.

Behavioral factors can affect the expected life expectancy. Increasing physical activity, avoiding smoking as well as decreasing overweight and obesity present reliable mechanisms to invest in terms of providing a better lifestyle and quality of life.

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Резиме

Однос помеѓу очекуваниот животен век, бихејвиоралните фактори и полифармацијата во земјите од Западен Балкан

Арбен Бошњаку^{1,2}*, Ермира Красниќи²

¹Медицински факултет, Универзитет во Ѓаковица „Фехми Агани“, ул. Исмаил Кемали б.б., 5000 Ѓаковица, Косово

²Колеџ за медицински науки Резонанца, Блок Шелѓет-Ветерник, 10000 Приштина, Косово

Клучни зборови: животниот век, полифармација, Западен Балкан, прекумерна тежина, дебелина




Старењето претставува мултидисциплинарен процес кој се карактеризира со постепено зголемено време на хомеостаза и намалено време на реакција и перформанси. Очекуваниот животен век е важна мерка за проценка на здравствената состојба на населението и перформансите на здравствениот систем, и истата се карактеризира со постепено зголемување во современиот свет. Овој зголемен тренд се менува помеѓу различните земји и општества, додека се под влијание на неколку внатрешни, надворешни и бихејвиорални фактори.

Овој труд претставува наративен преглед што ги анализира и споредува земјите од Западен Балкан, сите класифицирани како земји со среден приход.

Зголемување на физичката активност, избегнување на пушењето, како и намалување на прекумерната телесна тежина и дебелина, претставуваат веродостојни механизми за обезбедување подобар животен стил и квалитет на живот. Полифармацијата претставува друг интегрален дел во овој процес, кој влијае и е во корелација со сите други фактори. Сите овие фактори треба да бидат земени предвид при креирањето на политики, здравствени пристапи и планови за интервенција.

Review

Association between Polymorphisms in Vitamin D Pathway-Related Genes, Vitamin D Status, Muscle Mass and Function: A Systematic Review

Ermira Krasniqi ^{1,2,3} , Arben Boshnjaku ^{2,4}, Karl-Heinz Wagner ^{1,3}  and Barbara Wessner ^{1,2,*} 

¹ Research Platform Active Ageing, University of Vienna, Althanstraße 14, 1090 Vienna, Austria; ph.ermirakrasniqi@gmail.com (E.K.); karl-heinz.wagner@univie.ac.at (K.-H.W.)

² Centre for Sport Science and University Sports, University of Vienna, Auf der Schmelz 6, 1150 Vienna, Austria; arbenboshnjaku@gmail.com

³ Department of Nutritional Sciences, University of Vienna, Althanstraße 14, 1090 Vienna, Austria

⁴ Faculty of Medicine, University “Fehmi Agani” in Gjakova, Ismail Qemali n.n., 50000 Gjakovë, Kosovo

* Correspondence: barbara.wessner@univie.ac.at; Tel.: +43-1-4277-48875

Abstract: An association between vitamin D level and muscle-related traits has been frequently reported. Vitamin D level is dependent on various factors such as sunlight exposure and nutrition. But also on genetic factors. We, therefore, hypothesize that single nucleotide polymorphisms (SNPs) within the vitamin D pathway-related genes could contribute to muscle mass and function via an impact on vitamin D level. However, the integration of studies investigating these issues is still missing. Therefore, this review aimed to systematically identify and summarize the available evidence on the association between SNPs within vitamin D pathway-related genes and vitamin D status as well as various muscle traits in healthy adults. The review has been registered on PROSPERO and was conducted following PRISMA guidelines. In total, 77 studies investigating 497 SNPs in 13 different genes were included, with significant associations being reported for 59 different SNPs. Variations in GC, CYP2R1, VDR, and CYP24A1 genes were reported most frequently, whereby especially SNPs in the GC (rs2282679, rs4588, rs1155563, rs7041) and CYP2R1 genes (rs10741657, rs10766197, rs2060793) were confirmed to be associated with vitamin D level in more than 50% of the respective studies. Various muscle traits have been investigated only in relation to four different vitamin D receptor (VDR) polymorphisms (rs7975232, rs2228570, rs1544410, and rs731236). Interestingly, all of them showed only very low confirmation rates (6–17% of the studies). In conclusion, this systematic review presents one of the most comprehensive updates of the association of SNPs in vitamin D pathway-related genes with vitamin D status and muscle traits in healthy adults. It might be used for selecting candidate SNPs for further studies, but also for personalized strategies in identifying individuals at risk for vitamin D deficiency and eventually for determining a potential response to vitamin D supplementation.

Keywords: vitamin D; genetic variations; SNPs; GC; CYP2R1; VDR; CYP24A1; muscle-related traits



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1. Introduction

In recent years, the association between “optimal” serum levels of vitamin D with different healthcare conditions has been given important attention in medical research. Studies have shown that various factors such as season, latitude (ultraviolet B (UVB) availability), air pollution, clothing style, sunshine exposure, skin pigmentation, sunscreen cream, age, diet, and nutritional supplementation directly or indirectly affect vitamin D status [1–3].

In the body, vitamin D occurs in different forms with several enzymes being involved in their metabolism. Briefly, vitamin D₂ (ergocalciferol) built from the provitamin ergosterol and vitamin D₃ (cholecalciferol) originating from 7-dehydrocholesterol

(7-DHC) are converted into the circulating 25-hydroxyvitamin D (including 25(OH)D2 and 25(OH)D3) and the biologically active 1,25-hydroxy-cholecalciferol (including 1,25(OH)2D2 and 1,25(OH)2D3) [4]. Subsequently, the active form exerts its action on various cell types through a specific vitamin D receptor (VDR) [5,6]. The processes including metabolism, transportation and signaling of vitamin D are regulated by a number of proteins encoded by specific genes (i.e., 7-dehydrocholesterol reductase (DHCR7/), cytochrome P450-2R1 (CYP2R1), cytochrome P450-27B1 (CYP27B1), vitamin binding protein (GC/DBP), VDR, and retinoid-X receptor A (RXRA) [7].

Low levels of vitamin D have been reported across various geographical regions [8–10]. Interestingly, vitamin D deficiency is commonly detected among older adults and is strongly associated with a decline in physical performance in this age group particularly [11]. Additionally, vitamin D deficiency has been described to be among the factors that lead to sarcopenia [5,12], a progressive, generalized, and age-related skeletal muscle disorder [13]. Furthermore, the supplementation of vitamin D seems to ameliorate mobility and muscle strength [14,15], especially when combined with resistance training [16], but also enhance muscle performance [17,18], suggesting a direct connection between vitamin D and muscle function. One compelling review from Garcia and colleagues described most appropriately the need to assess vitamin D level as one way to minimize physiological and functional changes in skeletal muscle [5].

Besides environmental factors influencing vitamin D levels, a genetic impact has also been questioned in various studies [19,20], and some of them investigated whether single nucleotide polymorphisms (SNPs) in vitamin D-associated genes such as the VDR contribute to muscular strength and mass [21]. However, investigation and integration of studies that explored the association of vitamin D pathway-related genes with muscle traits other than VDR are still missing to date.

In an attempt to broaden this hypothesis, we further assume that genetic variants in several vitamin D pathway-related genes would affect vitamin D levels and, as a consequence, various parameters of physical performance. Therefore, the aim of this systematic review was to comprehensively identify published SNPs in genes known to be associated with the vitamin D pathway and then to systematically analyze their influence on vitamin D levels and/or muscle function in adults, including older adults.

2. Materials and Methods

Details of the protocol for this systematic review were registered on PROSPERO [22]. The report followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [23].

2.1. Search Strategy

In order to identify potential candidate genes, PathCards (<https://pathcards.genecards.org/>, accessed on 27 November 2018) was used to identify genes that are related to the vitamin D pathway ($n = 10$). PathCards is a constituent network of metabolic pathways with mapping genes, which provides researchers with a rich, searchable systems analysis resource [24]. Additionally, we searched for similar genetic association studies to identify other candidate genes encoding key player proteins (LRP2, CUBN, CYP3A4, and CASR (calcium-sensing receptor)) [19,20]. Information on the finally identified 14 genes is summarized in Figure 1. In order to find publications related to genetic variations in each of the genes, the respective NCBI entry in the gene database was linked first to the SNP database of each gene and then to the PubMed entries. Consequently, these results were connected to a conservative search strategy on PubMed using the respective gene name or their aliases connected with the MeSH term “genetic variation”.

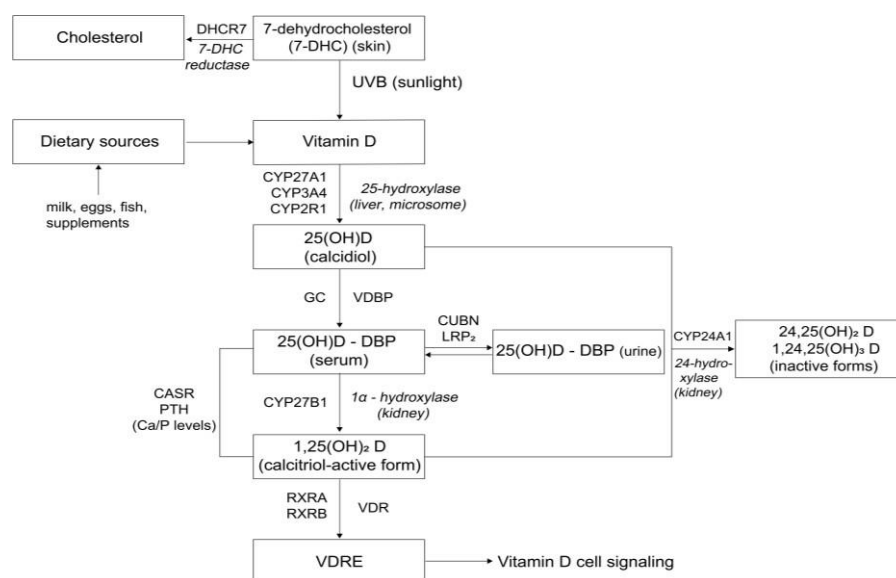


Figure 1. Vitamin D pathway, candidate genes (in bold), and associated enzymes. DHCR7 (7-dehydrocholesterol) gene encodes 7-DHC (7-dehydrocholesterol) reductase enzyme, which converts 7-DHC to cholesterol; CYP2R1 (cytochrome P450 family 2 subfamily R member 1), CYP3A4 (cytochrome P450 family 3 subfamily A member 4), and CYP27A1 (cytochrome P450 family 27 subfamily A member 1) genes encode 25-hydroxylation cytochrome P450 enzymes responsible for converting provitamin D that is absorbed from the diet or synthesized from the action of sunlight on the skin to the circulating form 25(OH)D (25-hydroxyvitamin D); vitamin D is transported bound to vitamin D binding protein (DBP) (encoded by GC gene); LRP2 and CUBN genes encode plasma membrane receptors megalin and cubilin, respectively (involved in re-absorption of 25(OH)D via receptor mediated endocytosis); CYP27B1 encodes the cytochrome p450 enzyme which converts 1- α -hydroxylates 25(OH)D to the active form 1,25(OH)₂D (1,25-Dihydroxycholecalciferol, Calcitriol); CASR (calcium sensing receptor) binds calcium in extracellular matrix, impacting calcium homeostasis; Ca homeostasis impacts the synthesis of parathyroid hormone (PTH gene—a protein coding gene) which stimulates the synthesis of 1,25(OH)₂D from 25(OH)D by upregulating renal 1- α -hydroxylase; CYP24A1 encodes a 24-hydroxylase enzyme which catalyzes the degradation of 25(OH)D and 1,25(OH)₂D in inactive metabolites; VDR encodes the vitamin D receptor, a nuclear receptor which binds 1,25(OH)₂D and forms a heterodimer with the gene product of RXR—the retinoid X receptor—to mediate the biological actions of vitamin D.

The final search for PubMed was conducted on 27 November 2018 and followed a protocol developed a priori. The search strategy aimed to identify all articles where the specific genetic variants (i) were aligned with search terms for either vitamin D status (ii), muscle traits (iii), or both (ii AND iii):

- (i) each of the 14 vitamin D-pathway-related genes (including their aliases variation [MeSH terms])
- (ii) vitamin D status (vitamin D[MeSH] OR 25(OH)D OR 25(OH)D₂ OR 25(OH) D₃ OR Vitamin D Deficiency[MeSH] OR “vitamin D status” OR “vitamin D level” OR “vitamin D inadequacy” OR “hypovitaminosis D” OR “avitaminosis D”) NOT Review[Publication Type], Filters: Humans; English; German
- (iii) muscle strength and function (Muscle, Skeletal[MeSH] OR Muscle Strength[MeSH] OR Physical Fitness[MeSH] OR Walking Speed[MeSH] OR “muscle mass” OR “lean body mass” OR “muscle quality” OR “physical performance” OR SPPB OR “short physical performance battery” OR “handgrip strength” OR “chair stand” OR “arm curl”) NOT Review[Publication Type], Filters: Humans; English; German

After conducting the initial structured search as outlined above, additional studies were added based on the reference lists of the finally selected studies (hand search).

2.2. Inclusion and Exclusion Criteria

Articles were included if studies:

- (i) were conducted among humans aged >18 years from both sexes;
- (ii) comprised candidate or genome-wide association studies being cross-sectional, cohort, case control, or intervention studies;
- (iii) investigated healthy subjects or contained at least a separate control group with normal health status;
- (iv) measured at least one genotype in a vitamin D pathway-related gene;
- (v) reported vitamin D status (circulating plasma/serum levels, 25(OH)D, 25(OH)D₂, or 25(OH)D₃ metabolites) and/or reported results for muscle mass or function, muscle strength parameters or scores for the Short Physical Performance Battery (SPPB);
- (vi) were published in English or German.

Articles were excluded if they:

- (i) reported only vitamin D intake or vitamin D metabolites from urine;
- (ii) investigated children (<18 years old);
- (iii) investigated participants with severe chronic or acute illnesses with a known impact on either vitamin D status and/or muscle mass and strength (myopathies, hypocalcemic hypercalcemia), or pregnant or lactating women;
- (iv) were published as case reports, systematic reviews, or meta-analyses.

2.3. Study Selection and Data Extraction

Study selection and data extraction was performed by two independent reviewers (EK, BW) in accordance with the above-mentioned inclusion and exclusion criteria. A third reviewer (KHW) was included in case of disagreements.

Data extraction from eligible studies included information as follows: (i) gene, (ii) SNP, (iii) bibliographic information, (iv) study design, (v) participants (age, gender, and ethnicity), (vi) sample size, (vii) main findings and outcomes, (viii) location, sampling season and analysis method of vitamin D level if available.

A narrative synthesis of the findings from the selected studies was performed using two frameworks in accordance with the following specific research questions: (i) association of vitamin D-related genetic polymorphisms and vitamin D status, and (ii) association of vitamin D-related genetic polymorphisms and muscle mass or strength. Results were described qualitatively rather than performing a quantitative meta-analysis as study designs and reported outcomes differed widely in order to quantitatively analyze them.

2.4. Risk of Bias (Quality) Assessment

In order to assess the quality of included studies, the STREGA recommendations (STrengthening the REporting of Genetic Association studies guidelines) were applied independently by two reviewers (three if there was any disagreement even after discussion) [25]. The quality of the studies was considered as “high” when the score was 18–22, “moderate-high” when the score was 13–17, and “low” with a score below 12. Results were reported, but no restrictions were made with respect to the inclusion or exclusion of the relevant studies.

3. Results

3.1. Study Selection and Characteristics

In total, 1292 studies were identified from the initial searches in PubMed ($n = 1282$) and through other sources ($n = 10$, Figure 2). As the searches were conducted separately for each of the 14 vitamin D pathway-related genes and then combined, 161 articles had to be excluded as duplicates. During title/abstract screening, a further 936 articles were excluded based on the predefined inclusion and exclusion criteria. The remaining 195 articles were assessed for eligibility based on the full texts. Among these, a further 106 studies were not suitable with respect to the research question. Consequently, 89 studies were included in

the systematic review, with 77 of them reporting the association of genetic polymorphisms of vitamin D-related genes and vitamin D status, and only 12 studies dealing with genetic variants of vitamin D-related genes and muscle mass and/or function. Study designs included 57 cross-sectional studies, 20 case-control studies, and 12 intervention studies.

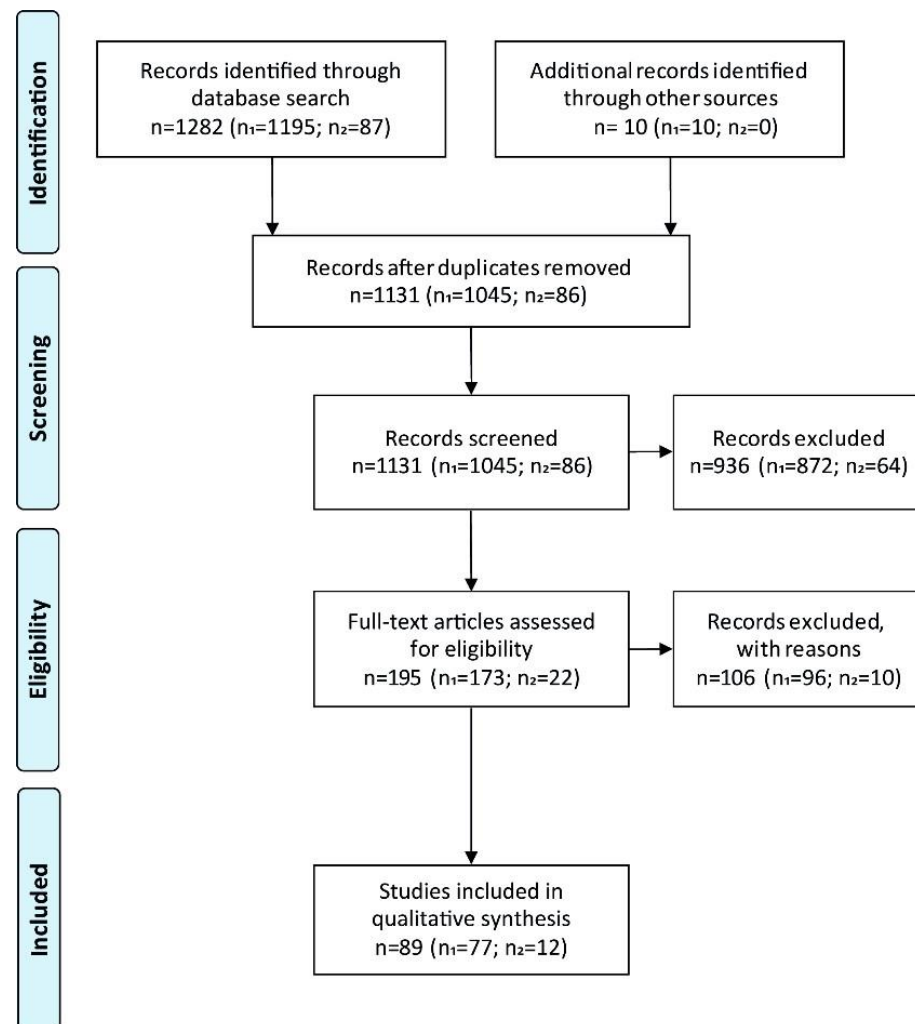


Figure 2. PRISMA-Flow diagram showing the selection of studies included in the systematic review. The number of studies reporting genetic variants and vitamin D status as well as the number of studies reporting genetic variants together with muscle mass and function, are given in parenthesis (n_1 and n_2).

3.2. Polymorphisms in Vitamin D Pathway-Related Genes and Vitamin D Status

As summarized in Table 1, 77 publications that have investigated a potential association between a certain genetic association and vitamin D levels were included in the report. Most of these publications were cross-sectional studies ($n = 46$); a further 19 studies were conducted as case-control studies and 12 studies as interventions. Altogether, these studies investigated 497 SNPs in 13 different genes (GC, CYP2R1, VDR, CYP24A1, DHCR7, CYP27B1, CYP27A1, CASR, PTH, CYP3A4, RXRA, CUBN, and RXRB). No suitable records were found for the gene lipoprotein receptor-related protein 2 (megalin, LRP2).

Table 1. SNPs in vitamin D pathway-related genes and vitamin D status.

Name Gene/ID	Description	Aliases	Studies	SNPs Investigated in Association with Circulating Vitamin D Levels	
				Significant Associations	Non-Significant
GC ID:2638	GC, vitamin D binding protein	DBP, DBP-maf, DBP/GC, GRD3, Gc-MAF, GcMAF, HEL-S-51, VDB, VDBG, VDBP	68 different SNPs reported by 56 studies [26–81]	rs115316390, rs1155563 *, rs11939173, rs12512631 *, rs16846876 *, rs16847015, rs17467825 *, rs222020 *, rs222040, rs222054, rs2282679 *, rs2298849 *, rs2298850 *, rs3755967 *, rs4588 *, rs7041 *, rs705119, rs705120, rs842999, rs9016	rs10011000, rs10488854, rs12640179, rs12644050, rs13117483, rs1352841, rs1352843, rs1352844, rs1352845, rs1491709, rs1491710, rs1491711, rs1491718, rs1491719, rs1565572, rs16846912, rs16846943, rs16847019, rs16847024, rs16847028, rs16847039, rs16847050, rs17383291, rs17830803, rs1873590, rs188812, rs2070741, rs222003, rs222010, rs222014, rs222016, rs222017, rs222023, rs222029, rs222035, rs222043, rs222049, rs2276461, rs3733359, rs3737549, rs3775152, rs4694105, rs4752, rs6817912, rs6835052, rs705117, rs705124, rs843006
CYP2R1 ID:120227	cytochrome P450 family 2 subfamily R member 1	-	29 different SNPs reported by 41 studies [27–36,38,39,42,44,46–57,60,48,50–52,55–,72–,62,63,65,67–69,74,76–83]	rs10500804 *, rs10741657 *, rs10766197 *, rs10832306, rs11023374 *, rs11023380 *, rs12794714 *, rs1562902 *, rs1993116 *, rs2060793 *, rs7116978, rs7935792	rs1037379, rs10832312, rs10832313, rs11023371, rs114050796, rs11819875, rs12418214, rs12419657, rs1496167, rs16930609, rs16930625, rs206793, rs7117967, rs7125348, rs7129781, rs7936142, rs952301
VDR ID:7421	vitamin D receptor	NR111, PPP1R163	111 different SNPs reported by 41 studies [27–30,33,37,41,42,46,47,50,52,55–57,60–63,65,67,71,74,75,77,78,84–98]	rs10783219 *, rs11568820, rs1544410 *, rs2228570 * (merged rs10735810), rs2239186 *, rs2408876, rs4516035, rs7139166, rs731236 *, rs7968585, rs7975232 *	rs10083198, rs10747524, rs10783215, rs10783218, rs10875693, rs10875694, rs10875695, rs10875702, rs10875712, rs11168264, rs11168266, rs11168268, rs11168275, rs11168277, rs11168287, rs11168288, rs11168292, rs11168302, rs11168314, rs11540149, rs11574024, rs11574026, rs11574027, rs11574038, rs11574042, rs11574044, rs11574065, rs11574077, rs11574110, rs11574113, rs11574138, rs11574141, rs11574143, rs11608702, rs11834903, rs12308082, rs12314197, rs12370156, rs12717991, rs12721364, rs12721365, rs12721370, rs1540339, rs17882106, rs1859281, rs1989969, rs2071358, rs2107301, rs2189480, rs2238135, rs2238136, rs2238138, rs2239179, rs2239180, rs2239181, rs2239182, rs2239184, rs2239185, rs2248098, rs2254210, rs2283342, rs2525044, rs2525045, rs2544027, rs2544038, rs2853559, rs2853560, rs2853564, rs3782905, rs3819545, rs3847987, rs4077869, rs4328262, rs4334089, rs4442605, rs4760648, rs4760655, rs4760658, rs4760674, rs6580642, rs7136534, rs7299460, rs7302038, rs7302235, rs7305032, rs7310552, rs7311030, rs739837, rs757343, rs7962898, rs7963776, rs7965281, rs7967152, rs7971418, rs7975128, rs7976091, rs881383, rs886441, rs9729, rs987849

Table 1. Cont.

Name Gene/ID	Description	Aliases	Studies	SNPs Investigated in Association with Circulating Vitamin D Levels	
				Significant Associations	Non-Significant
CYP24A1 ID:1591	cytochrome P450 family 24 subfamily A member 1	CP24, CYP24, HCAI, HCINF1, P450-CC24	65 different SNPs reported by 31 studies [26–33,35,38,42,46,52,55– 57,60–62,65,68,69,71– 75,78,80,81,97]	rs17216707, rs2209314 *, rs2762939 *, rs2762941, rs6013897 *, rs73913757	rs11907350, rs13038432, rs1555439, rs1570669, rs1570670, rs17219315, rs1870969, rs2021940, rs2181874, rs2244719, rs2245153, rs2248137, rs2248359, rs2248461, rs2274130, rs2296239, rs2296241, rs2426496, rs2426498, rs2585413, rs2585422, rs2585423, rs2585428, rs2585439, rs2762926, rs2762929, rs2762932, rs35051736, rs3787555, rs3787557, rs3886163, rs4809957, rs4809958, rs4809959, rs4809960, rs6013905, rs6022990, rs6022999, rs6023005, rs6023009, rs6023012, rs6068810, rs6068812, rs6068816, rs6068824, rs6097797, rs6097801, rs6097805, rs6097809, rs6127112, rs6127119, rs73913755, rs751090, rs765058, rs765059, rs8124792, rs912505, rs927650, rs927651
DHCR7 ID:1717	7- dehydrocholesterol reductase	SLOS	25 different SNPs reported by 28 studies [27–29,31– 33,35,36,38,39,42,44,46,51, 52,55–57,60,67,69,72– 74,77,78,81,99]	rs11603330, rs12785878 *, rs1790349 *	rs11233570, rs11234027, rs11606033, rs12419279, rs12800438, rs1540129, rs1540130, rs1790325, rs1790329, rs1790334, rs1790373, rs1792272, rs1792284, rs3794060, rs3829251, rs4316537, rs4944957, rs4945008, rs7122671, rs7944926, rs7950649, rs949178
CYP27B1 ID:1594	cytochrome P450 family 27 subfamily B member 1	CP2B, CYP1, CYP1alpha, CYP27B, P450c1, PDDR, VDD1, VDDR, VDDRI, VDR	15 different SNPs reported by 22 studies [26–28,30,33,37,41,42,46, 52,55,56,60,62,65,74,75,77, 80,81,97,100]	rs10877012 *	rs1021469, rs1048691, rs10877011, rs10877013, rs12368653, rs2269720, rs3782130, rs4646536, rs4646537, rs4760169, rs703842, rs8176344, rs8176345, –1077
CYP27A1 ID:1593	cytochrome P450 family 27 subfamily A member 1	CP27, CTX, CYP27	27 different SNPs reported by 7 studies [26,28,30,33,74,80,81]	–	rs116071925, rs11677711, rs12623740, rs12694443, rs12987009, rs12990447, rs13013510, rs13382651, rs17470271, rs4646535, rs4674338, rs4674344, rs4674345, rs6436084, rs6436094, rs645163, rs647952, rs6709815, rs6716642, rs6723334, rs6740004, rs6751527, rs7566656, rs7568196, rs7594289, rs7603709, rs933994

Table 1. Cont.

Name Gene/ID	Description	Aliases	Studies	SNPs Investigated in Association with Circulating Vitamin D Levels	
				Significant Associations	Non-Significant
CASR ID:846	calcium-sensing receptor	CAR, EIG8, FHH, FIH, GPRC2A, HHC, HHC1, HYPOC1, NSHPT, PCAR1, hCasR	71 different SNPs reported by 6 studies [27,60,71,74,80,101]	rs17251221, rs1801725,	rs10222633, rs1042636, rs10934578, rs11715859, rs11716910, rs12485716, rs13093602, rs13324814, rs13327652, rs1354162, rs1393198, rs1501892, rs1501898, rs1501900, rs16832787, rs17203502, rs17203516, rs17282008, rs1801726, rs1814740, rs1847029, rs1973490, rs1979869, rs2036399, rs2134223, rs2134224, rs2173961, rs2202127, rs2221266, rs2270916, rs2279802, rs3749203, rs3749207, rs3792288, rs3792291, rs3804592, rs3804593, rs3804595, rs3845918, rs4677900, rs4678013, rs4678029, rs4678031, rs4678035, rs4678173, rs4678174, rs6438705, rs6438706, rs6438712, rs6764205, rs6764544, rs6768109, rs6776158, rs6799828, rs7614486, rs7617603, rs7628990, rs7635128, rs7639847, rs7644981, rs7647405, rs7648041, rs937626, rs9740, rs9820206, rs9826770, rs9866419, rs9875101, rs9875636
PTH ID: 5741	parathyroid hormone [Homo sapiens (human)]	PTH1	12 different SNPs reported by 6 studies [30,74,80,81,96,102]	rs10500783, rs1459015,	rs2593570, rs6254, rs6256, rs6264, rs694, rs10500784, rs177706, rs192802, rs3099597, rs751610
CYP3A4 ID:1576	cytochrome P450 family 3 subfamily A member 4	CP33, CP34, CYP3A, CYP3A3, CYP11A3, CYP11A4, HLP, NF-25, P450C3, P450PCN1	9 different SNPs reported by 5 studies [28,30,62,80,81]	rs2242480	rs12333983, rs2246709, rs2687116, rs2740574, rs35599367, rs3735451, rs4646437, rs6956344
RXRA ID: 6256	retinoid X receptor alpha	NR2B1	48 different SNPs reported by 3 studies [52,71,80]	rs11185644 *	rs1045570, rs10785870, rs10881577, rs10881578, rs10881580, rs10881582, rs10881583, rs11102986, rs11103473, rs11103482, rs11185647, rs11185659, rs12004589, rs12004786, rs1536475, rs1805348, rs1805352, rs12339187, rs3118523, rs3118526, rs3118536, rs3118570, rs3118571, rs3132294, rs3132296, rs3132299, rs3132300, rs34677682, rs35603635, rs3818738, rs3818739, rs3818740, rs4240705, rs4917347, rs4917352, rs4917353, rs4917354, rs6537944, rs7039190, rs7861779, rs7864987, rs7871655, rs842196, rs872298, rs877954, rs881657, rs914853

Table 1. Cont.

Name Gene/ID	Description	Aliases	Studies	SNPs Investigated in Association with Circulating Vitamin D Levels	
				Significant Associations	Non-Significant
CUBN ID:8029	cubilin [Homo sapiens (human)]	IFCR, MGA1, gp280	14 different SNPs reported by 1 study [81]	-	rs10904881, rs11254370, rs1687705, rs1801222, rs1801223, rs1801224, rs1801225, rs1801231, rs1801232, rs1801234, rs1801241, rs2271462, rs3740165, rs703064
RXRB ID:6257	retinoid X receptor beta [Homo sapiens (human)]	DAUDI6, H-2RIIBP, NR2B2, RCoR-1	4 different SNPs reported by 1 study [81]	-	rs6531, rs2076310, rs3117040, rs9277935

SNPs-Single Nucleotide Polymorphisms, * significant associations with circulating vitamin D levels reported in more than one study.

The publication date of the included papers ranged from 2002 to 2018. In total, 81,896 healthy participants were investigated, whereby the number of study participants ranged from 31 (case-control) [89] to 8417 (cross-sectional studies) [57]. Participants with certain diseases such as type 1 [33] and type 2 [83] diabetes mellitus, osteomalacia [89], COPD [45], coronary artery disease [64], hemodialysis [87], Crohn's disease [86], pulmonary tuberculosis [100], melanoma [34], prostate [26,42], breast [32,61,73,97], colorectal [69] and non-small cell lung cancer [75] comprised the cases in the included case-control studies. However, the results of these participants were not included in the analyses of this systematic review.

The most frequently studied gene comprised the vitamin D binding protein (GC) which was investigated in 56 studies, followed by CYP2R1, coding for a vitamin D 25-hydroxylase, which was mentioned in 41 studies, and the vitamin D receptor (VDR) having been subject to 41 studies. In total, 59 SNPs located within 10 different genes showed a significant association with vitamin D levels in at least one study. Most importantly, 23 of these SNPs were confirmed to be related to vitamin D status in at least two other studies (Table 1). For genetic variants in the CYP27A1 gene (vitamin D 25-hydroxylase), CUBN gene (cubilin), and RXRB gene (retinoid-X receptor B), none of the studies reported a significant association with vitamin D level [26,27,30,33,71,101].

SNPs that were studied in at least 15 different studies showed significant associations in 8–77% of the respective studies. The highest confirmation rates were found for SNPs in the GC gene [rs2282679 (association to vitamin D status confirmed in 23 out of 30 studies (77%)); rs4588 (confirmed in 27 out of 37 studies (73%)); rs1155563 (confirmed in 12 out of 17 studies (71%)); rs7041 (confirmed in 27 out of 39 studies (69%))] and in the CYP2R1 gene [rs10741657 (confirmed in 21 out of 32 studies (66%)); rs10766197 (confirmed in 9 out of 15 studies (60%)); rs2060793 (confirmed in 8 out of 15 studies (53%))]. Further frequently studied SNPs located in the DHCR7 gene [rs12785878, confirmed in 6 out of 19 studies (32%)], the CYP24A1 gene [rs6013897, confirmed in 3 out of 18 studies (17%)], and the CYP2R1 gene [rs10877012, confirmed in 2 out of 15 studies (13%)]. Interestingly, SNPs in the VDR gene were frequently investigated, but their confirmation rate was very low [rs7975232 (alias ApaI, confirmed in 3 out of 18 studies (17%)); rs2228570 (confirmed in 4 out of 25 studies (16%)); rs1544410 (confirmed in 4 out of 28 studies (14%)); and rs731236 (confirmed in 2 out of 24 studies (8%)); rs11568820 (confirmed in 1 out of 16 studies (6%))]. A complete list can be found in Supplementary Table S1.

Most of the studies were performed in Europe (21 studies from Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Netherlands, Norway, Estonia, UK, France, Italy, Greece, Spain, Scotland, Sweden, United Kingdom), followed by the US (14 studies) and China (11 studies).

Measurement methods for vitamin D levels were very diverse and included radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), chemiluminescent immunoassays, high performance liquid chromatography (HPLC), and liquid chromatography–tandem mass spectrometry. Mostly, total 25(OH)D (summing up 25(OH)D2 and 25(OH)D3) was measured, although some studies [20,26,34,37,39,41,42,49,52,54,56,70,76,83,92,94,95,100] discriminated between different vitamin D metabolites (Supplementary Table S1).

3.3. Polymorphisms in Vitamin D Pathway-Related Genes and Muscle Mass and Function

As reported in Table 2, 12 publications were included in the qualitative synthesis, whereby 11 studies were conducted as cross-sectional studies and one study as a case-control study [103]. All the selected studies were focusing on potential associations between VDR gene polymorphisms and muscle traits, investigating only four different SNPs in this gene [rs7975232 (alias ApaI), rs1544410 (alias BsmI), rs2228570 (alias FokI, including the merged SNP rs10735810), and rs731236 (alias TaqI)].

Table 2. SNPs in the VDR genes and their association with various muscle traits.

SNP/Traditional Name	Region, Reference	Participants (Number, Gender, Age)	Outcomes for Muscle Mass and Function	Main Findings
rs7975232/ Apa1	Tianjin, China [104]	<i>n</i> = 109 f (AA: 19.57 ± 0.53 y, Aa: 20.00 ± 1.20 y, aa: 19.89 ± 1.05 y)	Concentric and eccentric peak torque of knee extensors and flexors at 30°/s, 60°/s, and 180°/s; concentric peak torque of elbow extensors and flexors at 30°/s and 120°/s	→ aa + aA genotypes → higher knee extension peak torque at 120°s, higher elbow flexion at 120°/s and 30°/s than AA
	Fuki Prefecture, Japan [105]	<i>n</i> = 180 f; 60.1 ± 6.6 y	Handgrip strength; isokinetic concentric peak torque of knee extensors and flexors; isometric and isokinetic concentric and eccentric peak torque of trunk flexors and extensors	No significant differences in any muscle strength parameter between Apa1 genotype groups (AA, Aa, aa) (data not shown in article)
	Northern Italy [106]	Centenarians (<i>n</i> = 102, 102.3 ± 0.3 y) versus septuagenarians (<i>n</i> = 163; 73.0 ± 0.6 y)	Handgrip strength	FF→significantly higher handgrip strength than Ff + ff
rs1544410/ Bsm1	Sirente area, Italy [107]	<i>n</i> = 259 (87 m + 172 f); 85.0 ± 4.5 y	Handgrip strength, short physical performance battery (SPPB)	No significant differences between genotypes (BB, Bb, bb) in handgrip strength and SPPB score
	Baltimore, USA [108]	<i>n</i> = 864 (489 m + 375 f); 22–90 y;	Total and appendicular fat-free mass (DXA); handgrip strength; concentric peak torque of knee extensors at 30°/s and 180°/s; isometric peak torque at 120° and 140° knee ankle	No significant differences in fat-free mass Only modest differences in strength measurements: knee extensor isokinetic peak torque at 30°/s in females (bb > bB > BB)
	Northern Italy [106]	Centenarians (<i>n</i> = 102, 102.3 ± 0.3 y) versus septuagenarians (<i>n</i> = 163; 73.0 ± 0.6 y)	Handgrip strength	No significant differences in handgrip strength between genotypes
	Istanbul, Turkey [109]	<i>n</i> = 120 m; 69.0 ± 6.9 y	Fat-free mass (BIA); isokinetic peak torque of knee extensors, flexors at 60	BB higher knee extensor strength as compared to Bb + bb; No differences for flexors, muscle mass, and vitamin D level
	UK, Germany, France [110]	APUSS cohort (<i>n</i> = 3234 f; 54.3 ± 2.3 y); OPUS cohort (<i>n</i> = 1970 f; 66.9 ± 7.0 y)	Handgrip strength; chair rise test (difficulty and power with force plate); data only from OPUS cohort	BB + bB genotypes→higher max power, fewer difficulties to stand up from a chair No differences in handgrip strength
London, UK [103]	CPD (<i>n</i> = 107; 75 m + 32 f; 63.5 ± 9.5 y); age matched controls (<i>n</i> = 104; 48 m + 56 f; 61.8 ± 8.5 y)	Handgrip strength; quadriceps strength, calculated as quadriceps maximum voluntary contraction force; fat-free mass (BIA)	No significant differences between genotypes for fat-free mass and strength measures	
Leuven, Belgium [111]	493(253 m (54.9 ± 10.2 y) + 240 f (41.5 ± 13.2 y))	Fat-free mass (estimated from skinfolds); handgrip strength; Isometric knee extension strength at 150°, 120°, and 90° knee ankle; Isometric knee flexion strength at 120°	No significant differences in any of the measured parameters [data not shown]	
Tianjin, China [104]	<i>n</i> = 109f (AA: 19.57 ± 0.53 y, Aa: 20.00 ± 1.20 y, aa: 19.89 ± 1.05 y)	Concentric and eccentric peak torque of knee extensors and flexors at 30°/s, 60°/s, and 180°/s; concentric peak torque of elbow extensors and flexors at 30°/s and 120°/s	BB + Bb genotypes→higher knee flexion peak torque at 180°/s than bb group No other differences	

Table 2. Cont.

SNP/Tra- ditional Name	Region, Reference	Participants (Number, Gender, Age)	Outcomes for Muscle Mass and Function	Main Findings
	Uppsala, Sweden [112]	$n = 175$ f; 29.6 ± 5.9	Handgrip strength; Isokinetic knee-flexion and extension strength at $90^\circ/s$; lean body mass (DXA)	BB \rightarrow higher hamstring strength as compared to bb No differences in lean mass
	Monongahela Valley, USA [113]	$n = 302$ m; 58–93 y	Peak and average isometric quadriceps strength at 125° knee ankle; appendicular and total fat-free mass (DXA)	No differences in any measured parameter
	n/A [114]	$n = 501$ f; >70 y (mean age: 75 y)	isometric muscle strength of the quadriceps; handgrip strength	bb + Bb \rightarrow higher quadriceps strength than BB in non-obese females, but not in obese females
rs2228570/ FokI	Sirente area, Italy [107]	$n = 259$ (87 m + 172f); 85.0 ± 4.5 y	Handgrip strength, short physical performance battery (SPPB)	No significant differences between genotypes (FF, Ff, ff) in handgrip strength and SPPB score
(rs10735810)	Baltimore, USA [108]	$n = 864$ (489 m+ 375 f); 22–90 y;	Total and appendicular fat-free mass (DXA); handgrip strength; concentric peak torque of knee extensors at $30^\circ/s$ and $180^\circ/s$; isometric peak torque at 120° and 140° knee ankle	No significant differences in fat-free mass; Males: no significant differences for any strength variable measured; Females: ff \rightarrow higher isometric quadriceps strength (120°) in comparison to Ff and FF
	Northern Italy [106]	Centenarians ($n = 102$, 102.3 ± 0.3 y) versus septuagenarians ($n = 163$; 73.0 ± 0.6 y)	Handgrip strength	FF \rightarrow significantly higher handgrip strength than Ff + ff
	Istanbul, Turkey [109]	$n = 120$ m; 69.0 ± 6.9 y	Fat-free mass (BIA); isokinetic peak torque of knee extensors, flexors at $60^\circ/s$	No significant differences between genotypes (FF, Ff, ff)
	UK, Germany, France [110]	APUSS cohort ($n = 3234$ f; 54.3 ± 2.3 y); OPUS cohort ($n = 1970$ f; 66.9 ± 7.0 y) cases with stable COPD ($n = 107$; \pm 63.5 9.5 y) and healthy age-matched controls ($n = 104$; 61.8 ± 8.5 y)	Handgrip strength; chair rise test (difficulty and power with force plate); data only from OPUS cohort	No significant differences between genotypes (FF, Ff, ff)
	London, UK [103]	63.5 9.5 y) and healthy age-matched controls ($n = 104$; 61.8 ± 8.5 y)	Handgrip strength; quadriceps strength, calculated as quadriceps maximum voluntary contraction force; fat-free mass (BIA)	FF \rightarrow significantly lower quadriceps strength than Ff + ff Females: Ff \rightarrow lower isometric knee extension strength \rightarrow at 120° and 90° than both FF and ff
	Leuven, Belgium [111]	493(253 m (54.9 ± 10.2 y) + 240 f (41.5 ± 13.2 y))	Fat-free mass (estimated from skinfolds); handgrip strength; Maximal isometric knee extension strength at 150° , 120° , and 90° knee ankle; Maximal isometric knee flexion strength at 120° knee ankle;	Males: no significant differences for any of the strength measurements \rightarrow
	Monongahela Valley, USA [113]	$n = 302$ m; 58–93 y	Peak and average isometric quadriceps strength at 125° knee ankle; appendicular and total fat-free mass (DXA)	FF \rightarrow significantly lower \rightarrow appendicular and total fat-free mass than Ff and ff; FF \rightarrow significantly lower peak and average isometric quadriceps strength than ff

Table 2. Cont.

SNP/Tra- ditional Name	Region, Reference	Participants (Number, Gender, Age)	Outcomes for Muscle Mass and Function	Main Findings
rs731236/TaqI	Tianjin, China [104]	n = 109f (AA: 19.57 ± 0.53 y, Aa: 20.00 ± 1.20 y, aa: 19.89 ± 1.05 y)	Concentric and eccentric peak torque of knee extensors and flexors at 30°/s, 60°/s, and 180°/s; concentric peak torque of elbow extensors and flexors at 30°/s and 120°/s	No significant differences between genotypes (TT, Tt, tt)
	Fuku Prefecture, Japan [105]	n = 180 f; 60.1 ± 6.6 y	Handgrip strength; isokinetic concentric peak torque of knee extensors and flexors; isometric and isokinetic concentric and eccentric peak torque of trunk flexors and extensors	No significant differences between genotypes (TT, Tt, tt)
	Baltimore, USA [108]	n = 864 (489 m + 375 f); 22–90 y;	Total and appendicular fat-free mass (DXA); handgrip strength; concentric peak torque of knee extensors at 30°/s and 180°/s; isometric peak torque at 120° and 140° knee ankle	No significant differences in fat-free mass; Males: no significant differences for any strength variable measured; Females: ff→higher isometric quadriceps strength (120°) in comparison to Ff and FF
	Northern Italy [106]	Centenarians (n = 102, 102.3 ± 0.3 y) versus septuagenarians (n = 163; 73.0 ± 0.6 y)	Handgrip strength	FF→significantly higher handgrip strength than Ff + ff
	Istanbul, Turkey [109]	n = 120 m; 69.0 ± 6.9 y	Fat-free mass (BIA); isokinetic peak torque of knee extensors, flexors at 60°/s	No significant differences between genotypes (TT, Tt, tt)

n-number, y-years, f-females, m-males.

In total, 5342 healthy subjects were included, with the number of participants ranging from 104 (case-control study) [103] to 1970 (cross-sectional study) [110]. The most frequently studied SNP was rs1544410 (BsmI), investigated by 11 studies, whereby five studies were reporting significant associations between its genotypes (BB, Bb, bb) and muscle traits such as knee flexion peak torque [104], knee extensor strength [109], maximal power [110], hamstring strength [112] and quadriceps strength [114]. Four studies included only female subjects [104,110,112,114].

The rs2228570 (FokI, rs10735810) SNP was mentioned in eight studies, five of which showing a significant association between its genotypes (FF, Ff, ff) and muscle traits: quadriceps strength [103,108,113], handgrip strength [106], and knee extension strength [111].

Rs7975232 (ApaI) was investigated by three studies [104–106], one of them showing significance between aa + aA genotypes and muscle strength [104]. For the genotypes of the SNP rs731236 (TaqI), none out of five studies reported any significant association [104–106,108,109].

3.4. Quality of Included Studies

The STREGA quality score for the studies relating the respective SNPs to vitamin D status was 18.8 ± 2.3 showing low to high quality with a range between 11 and 22. While for studies relating SNPs to muscle traits, the mean STREGA score was 16.8 ± 1.8 with a range between 13 and 19, indicating moderate to high quality.

4. Discussion

We have systematically evaluated the available scientific data showing the association of certain genotypes to vitamin D deficiency, and hence, poor muscle status. Variations in GC, CYP2R1, VDR, and CYP24A1 genes were reported most frequently, whereby especially SNPs in the GC (rs2282679, rs4588, rs1155563, rs7041) and CYP2R1 genes (rs10741657, rs10766197, rs2060793) were confirmed to be associated with vitamin D plasma level in more than 50% of the respective studies. Various muscle traits have been investigated only in relation to four different VDR polymorphisms (rs7975232, rs2228570, rs1544410, and rs731236). Interestingly, all of them showed only very low confirmation rates (6–17% of the studies).

Synthesized or consumed with the diet, in the liver, vitamin D is converted in its circulating form 25-hydroxyvitamin D (calcidiol), a process mediated by enzyme 25-hydroxylase, which is encoded by the CYP2R1 gene. Polymorphisms in this gene may impact vitamin D metabolism, while it shows catabolic effects toward Vitamin D2 and D3 by modulating 25-hydroxylase's activity and expression [115]. Accordingly, a significant impact of 9 polymorphisms on this gene (Table 1) with vitamin D status has been found to be replicated in more than two studies. Rs10741657, located in non-coding region 5'-UTR, was the most frequently studied SNP of this gene, with a significant association confirmed in 66% of the conducted studies with the beneficiary allele being the minor one. This data is in line with a recent meta-analysis on the effects of CYP2R1 gene variants on vitamin D levels [116]. Being located in 2-kb CYP2R1 mRNA transcript, it is believed that this SNP is able to change enzyme activities and affect vitamin D metabolism [117]. At the same time, two other SNPs, rs12794714 and rs10766197, located in the coding region of introns with the possibility of altering transcription rate [117] are confirmed in 79 and 60%, respectively, with the minor allele being the risk allele in the two aforementioned SNPs (Supplementary Table S1).

The transport of 25(OH)D toward target tissues for utilization and processes is enabled by vitamin D binding protein (DPB). This protein binds 85 to 90% of vitamin D circulating form, having the role of both carrier and reservoir. The remaining 10–15% of the circulating vitamin D is bound to albumin, or unbound, representing the available fraction based on the free hormone hypothesis [97]. This multifunctional and polymorphic protein is encoded by the GC gene (located on chromosome 4q12-q13), whose DNA sequence may impact the binding ability to vitamin D since its isoforms have different binding affinities,

possibly impacting the half-life of circulating 25(OH)D [28]. From at least 120 identified isoforms, the most common ones Gc1f and Gc1s (rs7041 locus), as well as Gc2 (rs4588 locus), refer to the two functional SNPs in exon 11 with rs7041 causing an Asp Glu amino acid change and rs4588 causing a Thr Lys exchange in the vitamin D binding protein [118]. Interestingly, those two SNPs were found to be significantly associated with the vitamin D level in 69 and 73% of the respective studies included in this review. In the aforementioned SNPs, the major allele was the beneficiary one in the majority of the studies (70 and 93%, respectively). In addition, this gene's polymorphisms are believed to influence the circulating concentration of DBP, which may alter the bioavailable circulating vitamin D [119]. This association might also be linked to the possible impact of these SNPs in the affinity of DPB to actin while modifying its actin-binding region and affecting 25(OH)D uptake and retention into skeletal muscle cells. Furthermore, it has been shown that C2 myotubes and primary rat muscle fibers express megalin and cubilin receptors, which enable endocytosis of DBP [120]. Studies on megalin and cubilin published after the search closing date of this systematic review did not reveal new findings [121,122]. In addition to the previously mentioned functional polymorphisms in the GC gene, there are also intron-located SNPs, which were found to be associated with vitamin D status, whereas the underlying mechanism remains unclear. One such, rs2282679, an intron variant (in linkage disequilibrium with rs4588) [123], was found to be significantly associated with vitamin D in 77% of the identified studies, all confirming the major allele as the beneficiary one. Finally, rs1155563 (an intron variant) was confirmed in 71% of the studies (of which 71% confirmed the major allele as the beneficiary one).

Furthermore, another important part of the vitamin D pathway chain is the vitamin D receptor, a high-affinity nuclear receptor encoded by the VDR gene. Vitamin D exerts its biological roles when its active metabolite 1 α ,25-dihydroxyvitamin D₃ [1 α ,25(OH)₂D₃] binds to VDR, causing a transactivation function of VDR [124]. The resulting complex 1,25D-VDR-RXR then binds to vitamin D response elements in the DNA [125]. Consequently, VDR is involved in the regulation of many cellular functions such as phospholipid metabolism, apoptosis, cell differentiation, and oxidative stress. It also affects the expression of the vitamin D metabolism-related genes CYP27B1 and CYP24A1 [126,127]. Several studies aimed to prove the association between VDR gene polymorphism and vitamin D status. Among one of the most studied SNPs, Fok1 is a polymorphism located at the start codon of the coding part, whose polymorphic form produces a protein shorter by amino acids [128], altering the length of the VDR [106]. However, an association with vitamin D level has been confirmed in only 16% of the studies included in this systematic review. The exact role of other frequently studied polymorphisms in VDR is not fully elucidated. Three SNPs located in the 3' end of the VDR gene rs1544410 (Bsm1), rs731236 (Taq1), and rs7975232 (Apa1), considered to be in high linkage disequilibrium with 3' UTR polymorphisms [129]. An effective role of these genetic variants on vitamin D level is very unlikely as they have been associated with the vitamin D level in only 14, 8, and 17% of the studies, although investigated in 28, 24, and 18 studies, respectively. Although the regions around these SNPs are not translated to the VDR protein, they might have a role in mRNA stability because of their neighborhood to the poly-A tail [130].

Taken together, there seems to be limited evidence that genetic variations in the VDR gene will exert a meaningful association with vitamin D level, given the small number of studies showing significant associations in relation to the high number of studies investigating a potential association. This is further supported by the notation that even in those studies showing a significant association, there was no clear direction with respect to the identification of a beneficiary allele (Supporting File S1).

In addition, two SNPs on the DHCR7 genes have been highly investigated. This gene encodes the enzyme 7-dehydrocholesterol reductase, a key metabolite enzyme that catalyzes the conversion of 7-dehydrocholesterol to cholesterol [28]. Rs12785878 and rs1790349 were found to be significantly associated with the vitamin D level in 32 and 50% of the included studies, while the major allele could be identified to be the beneficiary

one in 83 and 80% of the studies. Therefore, no clear conclusion can be drawn on the involvement of these SNPs in affecting vitamin D level, which is confirmed in recent studies on adolescents with rs12785878 genotype showing no association to hypovitaminosis D [131], but an interaction between 25(OH)D levels and rs12785878 genotype in DHCR7 on overall survival of patients with metastatic colorectal cancer [132].

While vitamin D deficiency is an important public health topic, at least some vitamin D-related gene polymorphisms seem to play an important role in vitamin D status. However, an in-depth analysis of the study characteristics revealed (see Supplementary Table S1) that the included studies were characterized by heterogeneous methodology, including varying sample sizes, age groups, and, most importantly, different vitamin D measurement techniques. After concerns were raised about the accuracy of different vitamin D assays and the possibilities of misleading assessment of vitamin D levels [133], EFSA recommended liquid chromatography-tandem mass spectroscopy (LC-MS/MS) as the reference method in regard to 25(OH)D concentrations [4]. Notwithstanding, LC-MS/MS was found to be used in only 17 out of 77 studies (22.1%) included in this qualitative analysis, while the radioimmunoassay method (RIA) was the most used one (in 22 of 77 studies, 28.6%). It also must be noted that the search for genotypes differed as well—as most of the studies used a candidate genotype approach, although some SNPs were identified via SNP arrays [26–28,30,37,38,52,60,62,65,74,80,81,97].

As vitamin D level has been shown to be low in a significant proportion of adults worldwide [134], vitamin D supplementation remains an important method to achieve optimal levels. Its ability to enhance muscle strength [135], physical performance [17,18], including the lowering of the risk of falling in older adults with low serum 25(OH)D [17,136], has already been demonstrated. Notwithstanding, genetics might also affect the metabolic response toward vitamin D supplementation [80]. Accordingly, existing data implicate that polymorphisms in CYP2R1 (rs10766197, rs10741657), GC (rs4588, rs7041, rs2282679,) and VDR gene (rs2228570,) are associated with vitamin D dose-response, in view of the fact that these were individually replicated in at least two different intervention studies [27,35,40,41,54,56,60,74,78,80]. Although this was not the primary aim of this systematic review, it might comprise important information towards the necessity of personalized vitamin D treatment due to a possible intra-individual variability. It should be noted that we have not encountered studies investigating the direct link between vitamin D pathway-related gene polymorphisms and the effect of vitamin D supplementation on muscle traits. Although, data supporting the impact of these genes' polymorphisms in vitamin D status implicates their potential effect of vitamin D status in health outcomes such as muscle performance, particularly in vitamin D supplementation improvement in older adults with 25(OH)D levels <37–45 nmol/L [17,18].

While some outcomes of vitamin D deficiency, such as osteomalacia and osteoporosis are well known, the implications of vitamin D in muscle strength and function are still being investigated. Studies on vitamin D pathway-related genotypes and muscle traits were exclusively focused on the VDR gene. Genomic and non-genomic pathways might explain the effects of vitamin D on muscles. While the genomic effect is mediated through the already mentioned 1,25D-VDR-RXR complex, the non-genomic effect involves intracellular calcium and phosphate homeostasis resulting from transcriptional regulation of specific proteins in organs such as intestines, bone, and parathyroid gland [125]. In this respect, it has been shown that muscle fibers of VDR-null mice were smaller, more variable in size, and accompanied by abnormal expression of myoregulatory transcription factors (myf5, myogenin, and E2A). Hence, it is believed that VDR may be involved in transcriptional down-regulation of these factors during muscle differentiation [137]. Whereas these implications and underlying biology are still being studied [138], this review shows rs2228570 and rs1544410 within the VDR gene to be the most frequently investigated polymorphisms also with respect to their impact on skeletal muscle traits. Interestingly, the identified studies showed non-conclusive results as, i.e., upper body strength (major allele—the beneficiary one) [106] was controversially affected by the FokI (rs2228570) genotype

than the lower body strength (minor allele-the beneficiary one) [103,108,111,113]. Nevertheless, this demands further mechanistic investigations. For BsmI (rs1544410) genotype, the positive impact of major alleles in lower body muscles was confirmed in four studies [104,109,110,112]. A recent study, not being included in the systematic review focusing on further SNPs in the VDR gene (rs9729, rs17882106, rs7136534, rs11568820, rs10735810, rs4516035, and 11574024) did not reveal new findings, as neither muscle strength nor physical performance were associated to these genotypes [139]. To date, studies investigating the direct impact of vitamin D pathway-related genes (other than VDR gene) and muscle traits are still lacking.

Finally, this systematic review highlighted that there are promising candidate SNPs in vitamin D pathway-related genes that might impact vitamin D level and eventually muscle traits. However, it should be noted that heterogeneity among the selected studies represents a potential limitation, which also caused the decision to refrain from conducting a meta-analysis. Despite this limitation, the strength lies in the extensive information on individual SNPs in most of the relevant vitamin D pathway-related genes. To extract this detailed information from all included studies caused the rather long duration from the underlying systematic search to reporting the results. However, no conflicting results were found when comparing the outcomes of our study to recently published data [121,122,140–149]. The focus of recently published data remains in the same gene's polymorphisms: GC, CYP2R1, VDR, CYP24A1, and CYP27B1. Except for these genes, Fediriko et al., 2019 and Jorde et al., 2019 identified potentially novel SNPs in vitamin D-related candidate genes (LRP2 and CUBN), but none of those were statistically significant [121,122].

5. Conclusions

To the best of our knowledge, this systematic review presents a very comprehensive update of the association of polymorphisms in vitamin D pathway-related genes with vitamin D status in healthy adults. While especially SNPs in the GC (rs2282679, rs4588, rs1155563, rs7041) and CYP2R1 genes (rs10741657, rs10766197, rs2060793) were confirmed to be associated with vitamin D levels in more than 50% of the respective studies, various muscle traits have been investigated only in relation to four different VDR polymorphisms (rs7975232, rs2228570, rs1544410, and rs731236) and outcomes remain inconclusive. Taken together, these data could be used in various ways: (1) to use the identified SNPs as candidate genes to be validated in further studies, (2) to identify individuals at potential risk, and (3) to optimize potential interventions with all these suggestions being important for precision nutrition.

Supplementary Materials: The following data are available online at <https://www.mdpi.com/article/10.3390/nu13093109/s1>, Table S1: SNPs overview.

Author Contributions: E.K. and B.W. designed the study, performed the literature research, and completed the selection of studies; K.-H.W. was consulted in case of disagreement; E.K. and A.B. extracted data from the selected articles; E.K. and B.W. drafted the manuscript; A.B. and K.-H.W. critically revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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A Preliminary Study of Natural Radioactivity Levels in Soils from Kosovo: a case of study in Pristina region

Merita Kaçeli Xhixha^{1, a)}, Fadil Hasani^{2, b)}, Manjola Shyti^{3, c)} and Gerti Xhixha^{4, d)}

¹University Aleksandër Moisiu Durrës, Faculty of Professional Studies, Str. Currila 14, Durrës, Albania.

²College of Medical Sciences ‘Rezonanca’, Str. Blloku te Shelgjet Veternik, Pristina, Kosovo

³University of Tirana, Institute of Applied Nuclear Physics, Str. Thoma Filipeu, Qesarakë, Tirana, Albania.

⁴University of Tirana, Faculty of Natural Sciences, Zogu I Blvd., AL-1001 Tirana, Albania.

^{a)}Corresponding author: merita.xhixha@gmail.com

^{b)}fadil.226@gmail.com

^{c)}manjolahyti@yahoo.com

^{d)}gerti.xhixha@fshn.edu.al

Abstract. This preliminary survey has been undertaken as part of a systematic study to provide the soil radiological map of the Republic of Kosovo. A systematic unaligned grid sampling strategy was adopted using the European Environmental Agency reference grid of 10 km x 10 km. Soil samples were collected from fifteen locations in the region of Pristina. The activity concentrations of the radionuclides of ⁴⁰K, ¹³⁷Cs and progenies in the ²³⁸U, ²³²Th decay chains were measured by using high-resolution gamma ray spectrometry. The average activity concentrations were 398 ± 85 , 24 ± 6 and 35 ± 7 Bq/kg for the ⁴⁰K, ²³⁸U and ²³²Th respectively. The corresponding abundances were calculated to be, respectively 1.3 ± 0.3 % wt. K, 2.0 ± 0.5 µg/g eU and 2.0 ± 0.5 µg/g eTh with a characteristic eTh/eU ratio of average value of 4.4 indicating a common geological source origin and similar mobility. The activity concentration of ¹³⁷Cs was found to vary from 0.2 to 48 Bq/kg, which was found to be comparable with similar studies in the region. The outdoor air absorbed dose rate and the corresponding annual effective dose rate vary from 34 to 64 nGy/h and 210 to 390 µSv/y, respectively. These results represent the starting point for studies on natural background radiation, geochemical processes and epidemiological investigations.

INTRODUCTION

Naturally occurring radionuclides are generally found in various concentrations in the earth's crust. These primordial radionuclides are ubiquitously distributed in the environment, including in soil, air, water, foods and building materials and are the major source of the background radiation and specifically of the external gamma radiation. The most significant component of the dose rate received by humans arises from ⁴⁰K and ²³²Th and ²³⁸U decay chain progenies, accounting to a worldwide average of 58 nGy h^{-1} [1]. In Kosovo, the use of depleted uranium has been an issue of concern [2]. However, other sources like the contamination due to radioactive fallout with ¹³⁷Cs [3, 4] and Naturally Occurring Radioactive Materials (NORMs) from particular industrial activities can also elevate the level of radiation dose received by the human population [5, 6]. To our knowledge, no systematic surveys were conducted to study the distribution of the levels of the environmental activity concentration across the Kosovo.

The current work aims to present and discuss the criteria adopted to choose the number of measurements and the action plan for the realization of the natural radioactivity map of soils in Kosovo. Some preliminary results, taken from a previous project focusing the radiological assessment around the lignite power plants in Kosovo [3], are re-analyzed by hyper-pure germanium (HPGe) gamma-ray spectrometry and used to discuss the strategy for the realization of the natural radioactivity map. Soil samples were analyzed for ²¹⁰Pb, ²²⁶Ra, ²²⁸Ra, ²²⁸Th, ⁴⁰K and ¹³⁷Cs. The equilibrium in the ²³⁸U and ²³²Th decay chains is checked by the ²¹⁰Pb/²²⁶Ra and ²²⁸Ra/²²⁸Th ratios, respectively. These data are used to evaluate the radiological hazard for the population.

MATERIALS AND METHODS

Sampling Strategy, Sample Collection and Preparation

The European Environmental Agency reference grid of $10 \times 10 \text{ km}^2$ was adopted at the first stage to cover the entire surface. As a result, around 150 samples are foreseen to be collected considering an unaligned grid sampling strategy. Additionally, a denser number of samples per cell (four sub-cells of $5 \times 5 \text{ km}^2$) were proposed for geochemical and environmental studies (Figure 1). The coordinates are recorded in geographical coordinate system and projected either in GIS (Geographic Information Systems) or in Google Earth (key mark language format). In Figure 1 are shown the spatial distribution of fifteen samples collected in the first stage.

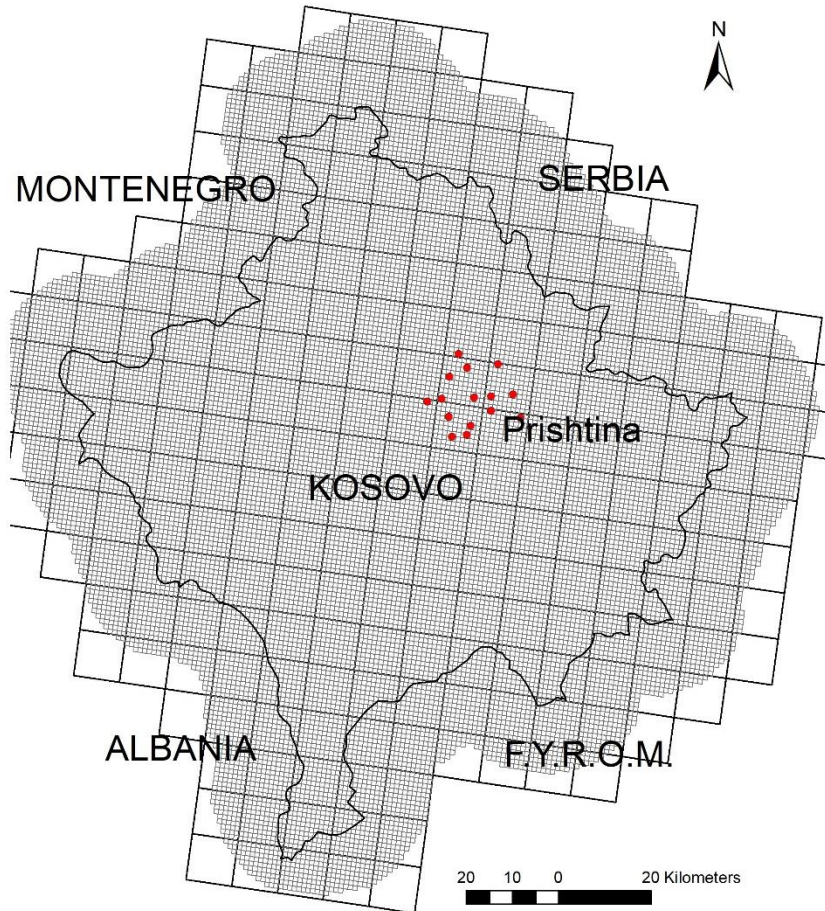


FIGURE 1. The division in $10 \times 10 \text{ km}^2$ of the Kosovo territory and the location of fifteen samples.

Soil samples were collected up to a depth of 5-10 cm, with each sample weighting around 1 kg. Impurities such as vegetation or stones were hand removed. Samples then were dried for 24 h at a temperature of 105°C or until a constant weight was achieved. The dried samples were homogenized to a grain size of less than 2 mm and then transferred into the Marinelli beakers (500 mL), sealed with PVC tape and carefully labelled. The sealed samples were left undisturbed for at least 4 weeks to establish a radioactive equilibrium in the ^{226}Ra decay chain due to ^{222}Rn escape during sample preparation. All samples were measured using high-resolution gamma-ray spectrometry system.

Gamma-Ray Spectrometry Measurements

The activity concentration in soil samples was determined by using a n-type coaxial HPGe (high-purity germanium) detector with 55% relative efficiency and an energy resolution of 2 keV at 1332.5 keV (^{60}Co). The HPGe detector was shielded with lead and copper-cadmium foils in order to reduce the environmental background. The absolute photopeak efficiency was determined using a gamma mix standard source solution of ^{241}Am , ^{109}Cd , ^{139}Ce , ^{57}Co , ^{60}Co , ^{137}Cs , ^{113}Sn , ^{85}Sr , ^{88}Y , ^{203}Hg , ^{54}Mn and ^{65}Zn (Amersham QCY48) and interpolated in the energy range 60 keV to 1850 keV. The combined uncertainty of measured absolute efficiencies was determined to be less than 7.5% [7].

Each sample was measured for 24 hours. After correcting for background and assuming secular equilibrium, the spectra were analyzed through the prominent gamma-ray lines associated with specific radionuclide decays. The natural activity concentration of ^{226}Ra was determined through ^{214}Pb and ^{214}Bi gamma-ray lines. The activity of ^{228}Ra was determined by measuring the gamma-ray lines of ^{228}Ac and ^{228}Th by measuring the gamma-ray lines of ^{212}Pb , ^{212}Bi and ^{208}Tl . The determination of the activity concentration through different gamma lines is given as an example in Figure 2 showing a good agreement between the results. The activity concentration of ^{40}K and ^{137}Cs was determined by measuring the 1460 keV and 662 keV gamma-ray, respectively. The minimum detectable activity (MDA) concentration was calculated for each energy of interest as described by Currie [8].

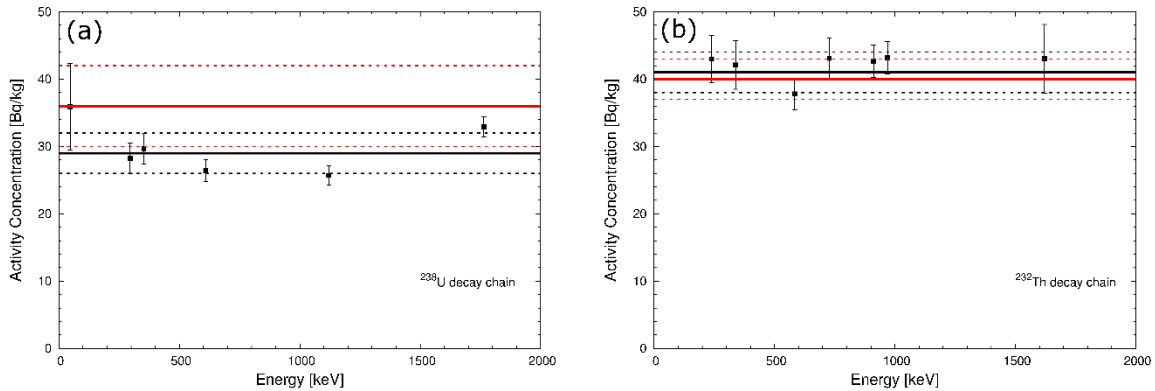


FIGURE 2. An example (sample 142/iii) of the determination from different gamma-ray lines of the activity concentration (a) for ^{238}U decay chain and (b) ^{232}Th decay chain.

RESULTS AND DISCUSSION

The activity concentrations of natural and artificial radionuclides in the soil samples are given in Table 1. The average activity concentrations of ^{40}K , ^{210}Pb , ^{226}Ra , ^{228}Ra , ^{228}Th and ^{137}Cs in the soil samples are found to be 398 ± 85 Bq kg^{-1} , 34 ± 14 Bq kg^{-1} , 24 ± 6 Bq kg^{-1} , 35 ± 9 Bq kg^{-1} , 35 ± 9 Bq kg^{-1} and 19.2 ± 15.1 Bq kg^{-1} , respectively. This first data show that exist a certain variability within the 10×10 km^2 cells and therefore support the decision of diving the cells in 5×5 km^2 . These activity concentrations in soil are found to be comparable with those of similar studies available in the literature (Table 1) and the world average value of 412 Bq kg^{-1} , 33 Bq kg^{-1} and 45 Bq kg^{-1} , respectively for ^{40}K , ^{238}U and ^{232}Th [1]. The range of the activity concentration of ^{137}Cs in soil samples is found to be comparable with the data in the literature regarding similar studies in the region.

Figure 3 shows the relationship between the activity concentration of ^{226}Ra and ^{210}Pb and that between the activity concentration of ^{228}Ra and ^{228}Th . The results seem to be in good agreement within the uncertainty, with exception of two samples in the case of ^{238}U decay chain. This fact needs to be investigated in more details, since the measurement of ^{210}Pb is affected by higher uncertainty. However, the correlations made among these radionuclides indicate the existence of secular equilibrium in the investigated soils and the assumption on secular equilibrium seems to be reasonable.

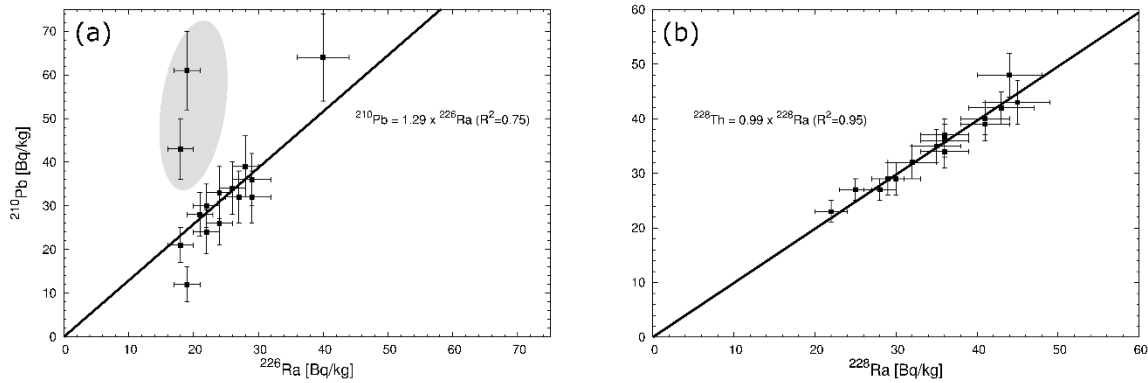


FIGURE 3. The correlation between (a) ^{210}Pb - ^{226}Ra in the ^{238}U decay chain and (b) ^{228}Ra - ^{228}Th in the ^{232}Th decay chain.

TABLE 1. The activity concentrations of natural and artificial radionuclides in the soil samples are reported together with available data from literature. The calculated absorbed and annual effective dose rates are given.

Sample ID	^{40}K (Bq kg ⁻¹)	^{238}U		^{232}Th		^{137}Cs (Bq kg ⁻¹)	D (nGy h ⁻¹)	E (μSv h ⁻¹)
		^{226}Ra (Bq kg ⁻¹)	^{210}Pb (Bq kg ⁻¹)	^{228}Ra (Bq kg ⁻¹)	^{228}Th (Bq kg ⁻¹)			
122/iii	359±27	22±2	30±5	30±3	29±3	12.4±0.8	45	270
141/iii	398±30	24±2	26±5	36±3	34±3	9.7±0.6	50	310
141/iv	463±35	19±2	12±4	25±2	27±2	0.2±0.1	44	270
141/i	224±17	19±2	61±9	22±2	23±2	15.8±1.0	34	210
141/ii	444±34	27±2	32±6	36±3	36±3	36.5±2.2	58	350
142/iii	333±25	29±3	36±6	41±3	40±3	47.7±2.9	58	360
142/i	513±39	18±2	21±4	32±3	32±3	32.4±2.0	54	330
142/ii	414±32	40±4	64±10	41±3	39±3	33.3±2.0	64	390
142/iv	360±27	24±2	33±6	35±3	35±3	16.1±1.0	49	300
143/i	436±33	28±2	39±7	45±4	43±4	40.1±2.4	63	390
156/iii	541±41	29±3	32±6	44±4	48±4	4.8±0.3	64	390
156/ii	430±33	26±2	34±6	43±4	42±3	4.2±0.3	56	340
157/iv	291±22	18±2	43±7	28±2	27±2	9.1±0.6	39	240
157/ii	450±34	21±2	28±7	29±2	29±3	21.0±1.3	49	300
157/i	310±24	22±2	24±5	36±3	37±3	5.1±0.3	46	280
range	224-541	18-40	12-64	22-45	23-48	0.2-47.7	34-64	210-390
media	398±85	24±6	34±14	35±9	35±9	19.2±15.1	52±9	320±60
Slovenia [9]	800±520	63±44	-	77±33	-	-	110±49	-
Greece [10]	355±220	29±19	-	21±16	-	-	40	-
Cyprus [11]	105±95	7±9	-	5±7	-	-	9±8	-
Turkey [12]	643	50	-	42	-	85	77	-
Turkey [13]	298	18	-	21	-	-	67	-
Serbia [14]	536	47	-	41	-	26	69	-
Serbia & Montenegro [15]	620±166	34±11	-	42±12	-	-	-	-
Bosnia and Herzegovina [16]	331	32	-	32	-	37	50	-
F.Y.R.O.M. [17]	546±118	39±15	-	44±18	-	42±40	67±21	-

The abundances of the corresponding elements are found to be 1.3 ± 0.3 % wt. K, 2.0 ± 0.5 $\mu\text{g g}^{-1}$ eU and 8.6 ± 1.7 $\mu\text{g g}^{-1}$ eTh (equivalent U and Th assume secular equilibrium). The ratio eTh/eU show an average value of 4.4 with low variability between samples, indicating a common geological source origin and similar mobility. Indeed, the geology of this area is mainly characterized by alluvium, deluvium and proluvium sediments with heterogeneous grain size.

These data can be used to assess the radiological hazard of the population due to external gamma radiation present in soil. As demonstrated in Xhixha et al. [3] the results give a good agreement with direct dose rate measurements. The absorbed dose rate (D) and the annual effective dose rate (E) in air from the external gamma radiation at one meter height above ground due to the presence of natural radionuclides in soil is calculated according to UNSCEAR [1]:

$$D (\text{nGy h}^{-1}) = 0.462 \cdot C_{226\text{Ra}} + 0.604 \cdot C_{232\text{Th}} + 0.0417 \cdot C_{40\text{K}} \quad (1)$$

$$E (\mu\text{Sv y}^{-1}) = 0.7 \cdot 8760 \cdot D \cdot 10^{-3} \quad (2)$$

where $C_{226\text{Ra}}$, $C_{232\text{Th}}$ and $C_{40\text{K}}$ are the activity concentrations of ^{226}Ra , ^{232}Th and ^{40}K (Bq kg^{-1}), the coefficient 0.462, 0.604 and 0.0417 are respectively the absorbed dose rate conversion coefficient (nGy h^{-1} per Bq kg^{-1}) and 0.7 (nSv nGy^{-1}) is the conversion factor to the effective dose received by an adult. Considering an outdoor occupancy factor of 20%, the results give an average annual effective dose rate of $63 \mu\text{Sv y}^{-1}$ which is comparable with the worldwide average values of $70 \mu\text{Sv y}^{-1}$ [1].

CONCLUSIONS

- The European Environmental Agency reference grid of $10 \times 10 \text{ km}^2$ grid is found to appropriate for the sampling action plan foreseeing around 150 samples. The distribution of the activity concentration level can be discussed in the future using the classification approach discussed in Callegari et al. [18] and Strati et al. [19] according to the percentiles of the dataset.
- The geo-statistical methods are more appropriate for the spatial distribution of the data. However, the variability within chosen cell must be taken into account by increasing the number of samples ($5 \times 5 \text{ km}^2$). A comparison between the two methods is also a topic of interest.
- The high resolution gamma-ray spectrometry technique was considered. The results of the activity concentration of different gamma-ray lines in the ^{238}U and ^{232}Th decay chains are found to be comparable within the uncertainty indicating the quality of the calibration procedure.
- The equilibrium in the ^{238}U and ^{232}Th decay chains was confirmed by $^{210}\text{Pb}/^{226}\text{Ra}$ and $^{228}\text{Ra}/^{228}\text{Th}$ ratios, respectively 1.29 ($R^2 = 0.75$) and 0.99 ($R^2 = 0.95$).
- The average annual effective dose rate was found to be $63 \mu\text{Sv y}^{-1}$ which is comparable with the worldwide average values of $70 \mu\text{Sv y}^{-1}$.

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Identification of Industrial Sectors Involving NORM in Kosovo

Gerti Xhixha^{1, a)}, Erjon Spahiu^{1, b)}, Fadil Hasani^{2, c)}, Manjola Shyti^{3, d)} and Florinda Cfarku^{3, e)}

¹ University of Tirana, Faculty of Natural Sciences, Zogu I Blvd., AL-1001 Tirana, Albania.

² Kosovo Agency for Radiation Protection and Nuclear Safety (KARPNS), Ish-Gërmia - 10000 Prishtinë, Kosovo

³ University of Tirana, Institute of Applied Nuclear Physics, Str. Thoma Filipeu, Qesarakë, Tirana, Albania.

^{a)}Corresponding author: gerti.xhixha@fshn.edu.al

^{b)}erjon.spahiu@fshn.edu.al

^{c)}fadil.226@gmail.com

^{d)}manjolahyti@yahoo.com

^{e)}fcfarku@yahoo.com

Abstract. This study presents a review of the first results on the identification of naturally occurring radioactive materials (NORMs) in the main industrial sectors of Kosovo: (i) the production of chemical fertilizers; (ii) processing of iron-nickel and lead-zinc metallic minerals; (iii) production of cements and (iv) production of electricity by coal-fired power plants. The activity concentrations of raw materials, products and residues was determined by using high-resolution gamma-ray spectrometry. The production of chemical fertilizers (non-active industry in Kosovo), was identified as an industrial sector of enhanced natural radioactivity, containing an average activity concentration of 385 ± 100 Bq/kg for ^{226}Ra and negligible for ^{232}Th and ^{40}K . In general, was observed an increase of NORMs in tailing and slag residues from metallic minerals mining and processing, and in fly/bottom ash from coal-fired power plants. In the last case, both fly and bottom ash, used as raw material in the cement industry were controlled regarding the radiological point of view. In general, the obtained results show that industrial residues can be used as solid materials, recycled or conventionally dumped (conventional landfills). These results will be an important baseline information for implementing in the Kosovo legislation the recommendations of the Council Directive 2013/59/Euratom (2014) regarding the basic safety standards in radiation protection.

INTRODUCTION

The EU Council Directive [1], propose a list of industrial sectors that could potentially lead to the concentration of Naturally Occurring Radioactive Materials (NORM) in by-products, residues or product streams. In fact, natural raw materials contain radionuclides due to the presence of ^{40}K , ^{238}U and ^{232}Th in the Earth. The world average radioactivity content in the upper continental crust is 727 ± 60 Bq kg⁻¹, 33 ± 7 Bq kg⁻¹ and 43 ± 4 Bq kg⁻¹, respectively for ^{40}K , ^{238}U and ^{232}Th [2].

According to the EU Council Directive [1] the concentration values applied to the clearance of solid materials for reuse, recycling, conventional disposal or incineration are discussed in each case. These industrial practices have to be monitored to assess the worker and public exposure to comply with the action levels of 1 mSv y⁻¹. The exemption levels for naturally occurring radionuclides in solid materials in secular equilibrium with their progeny are respectively 10 kBq kg⁻¹ for ^{40}K and 1 kBq kg⁻¹ for ^{238}U and ^{232}Th . In case of recycling such by-products or residues in the construction industry, the reference level applying to indoor external exposure to gamma radiation emitted by building materials, in addition to outdoor external exposure, shall be 1 mSv y⁻¹. The directive proposed a screening tool, for conservatively controlling the radiological hazards due to building materials, using the activity concentration index following the equation:

$$I = \frac{A_{Ra-226}}{300} + \frac{A_{Th-232}}{200} + \frac{A_{K-40}}{3000} \leq 1 \quad (1)$$

where A_{Ra-226} , A_{Th-232} and A_{K-40} are the activity concentrations in $Bq\ kg^{-1}$ for radium (equivalent to uranium under secular equilibrium conditions), thorium and potassium, respectively.

The aim of this paper is to present a review in order to summarize the main results made in the preliminary campaign to build the first national inventory of NORM involving industrial sectors. This control is not yet implemented in Kosovo and no data exist on the radiological quality of raw materials, by-products, residues or products. Therefore, this study aims to control the industrial activities in Kosovo in view of the implementation of the EU Council Directive [1], in the framework of harmonization of the legislations started with the stabilization association act.

REVIEW OF NORM IN KOSOVO INDUSTRIAL ACTIVITIES

The industrial sectors in Kosovo, which are subject to the implementation of the EU Council Directive [1], are identified as specified in the list of the industrial sectors as referred to in Article 23. The strategy for identification of by-products, residues or product streams of potential concern was based on the process analysis (Figure 1). At this stage, only solid materials were taken into consideration and analysis were performed by means of gamma-ray spectrometry technique [3]. In Table 1 are summarized the range and the average values of the activity concentrations of natural radionuclides in different materials.

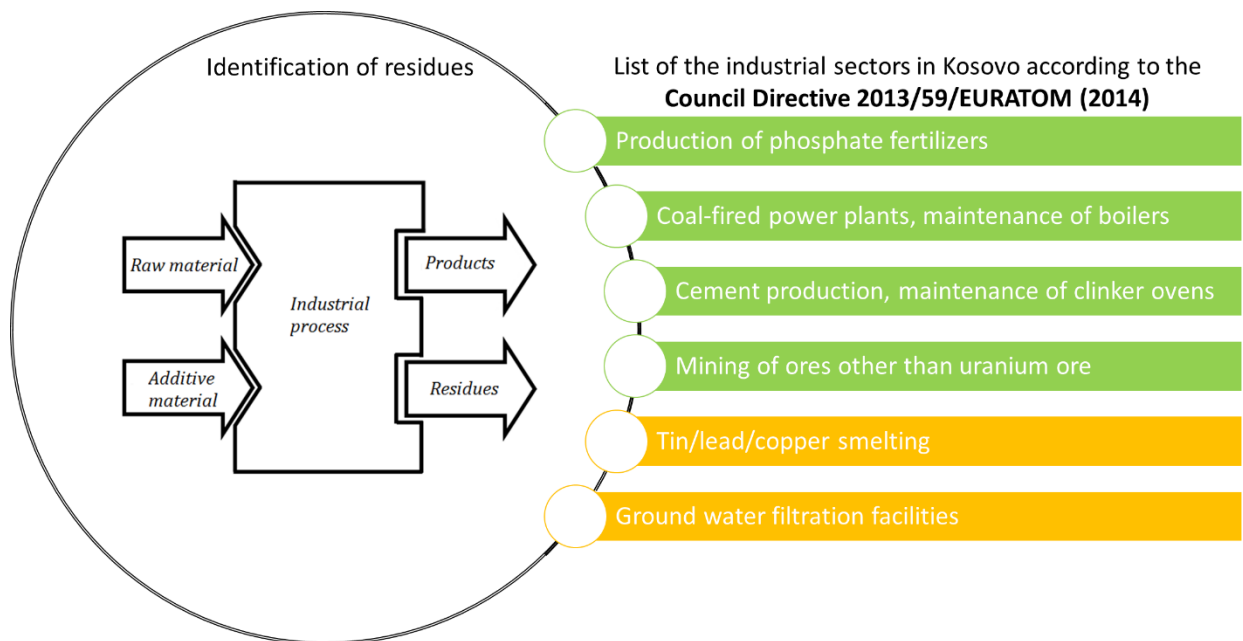


FIGURE 1. The strategy for the identification of by-products, residues or product streams from the industrial sectors in Kosovo.

TABLE 1. The range and the average value of the activity concentrations of natural radionuclides in the raw materials, by-products, residues and products streams in some industrial sectors in Kosovo.

Industrial sector	Sample type	Sample No.	⁴⁰ K (Bq kg ⁻¹)	²³⁸ U		²³² Th	
				²²⁶ Ra (Bq kg ⁻¹)	²²⁸ Ra (Bq kg ⁻¹)	²²⁸ Th (Bq kg ⁻¹)	
Production of phosphate fertilizers	Phosphogypsum	8	(<5-35) 16 ± 10	(310-350) 385 ± 100	(<5-15) 7 ± 4	(<5-15) 10 ± 4	
	Lignite	8	(29-53) 36 ± 8	(7-10) 9 ± 1	(4-12) 9 ± 3		
Coal-fired power plants	Fly ash	9	(104-146) 133 ± 16	(26-35) 30 ± 3	(27-34) 30 ± 3		
	Bottom ash	9	(168-211) 195 ± 13	(22-31) 28 ± 3	(32-38) 34 ± 2		
	Limestone	5	<6	<2	<2		
Production of cements	Marl	5	(129-137) 133 ± 9	(10-17) 13 ± 3	(15-17) 16 ± 2		
	Gypsum	5	<8	(3-5) 3 ± 1	<2		
	Opalite	5	(44-53) 50 ± 7	(101-105) 105 ± 6	(58-71) 65 ± 5		
	Pyrite	5	(108-124) 117 ± 10	(6-7) 6 ± 1	(<2-4) 3 ± 1		
	Clinker	8	(164-200) 189 ± 15	(17-22) 21 ± 2	(24-29) 26 ± 3		
	Fly/Bottom ash	-	-	-	-		
	Cements	15	(158-181) 169 ± 15	(26-34) 29 ± 4	(28-41) 33 ± 6		
	Processing of metallic minerals (Lead-Zinc and Ferro-Nickel)	Pb-Zn ore	2	(118-127) 123 ± 9	(8-10) 9 ± 1	(6-7) 6 ± 1	(6-7) 6 ± 1
Pb Concentrate		2	(11-14) 13 ± 1	(7-9) 8 ± 1	(6-8) 7 ± 1	(6-7) 7 ± 1	
Zn Concentrate		2	(14-15) 15 ± 2	(5-6) 5 ± 1	(3-4) 4 ± 1	(3-4) 4 ± 1	
Tailings Pb-Zn		4	(200-358) 272 ± 82	(16-19) 18 ± 1	(5-15) 10 ± 5	(4-16) 10 ± 6	
Fe-Ni ore		3	(3-6) 4 ± 2	(7-10) 8 ± 1	(4-5) 2 ± 1	(4-5) 2 ± 2	
Electric furnace slag Fe-Ni		5	(17-23) 19 ± 4	(5-11) 7 ± 2	(4-7) 5 ± 1	(4-7) 5 ± 1	
Converter slag Fe-Ni		3	(10-14) 12 ± 3	(6-8) 6 ± 1	(4-5) 5 ± 1	(4-5) 4 ± 1	

Production of Phosphate Fertilizers

In Kosovo the former fertilizer production industry has generated approximately $2.5 \cdot 10^5$ tons of phosphogypsum as by-product of phosphoric acid production. The area of the phosphogypsum deposits amounts to almost 10 ha. The natural radionuclides present in phosphate ores depend on their origin and in particular the concentration of ²³⁸U can vary from 370 to 2470 Bq kg⁻¹ [4]. Therefore, as it is expected, the activity concentration of ²²⁶Ra is relatively high with values ranging from 310 to 530 Bq kg⁻¹ and an average value of 385 ± 100 Bq kg⁻¹ [5]. These values are on average compared to other studies, where the activity concentration of ²²⁶Ra ranging from 24 Bq kg⁻¹ up to 1420 Bq kg⁻¹ [4 and references therein]. The activity concentrations of ²³²Th and ⁴⁰K are found to be respectively <5-15 Bq kg⁻¹ and <5-35 Bq kg⁻¹. The recycling alternatives of phosphogypsum are mainly in the building materials manufacturing industry [6] or in the rehabilitation of agricultural soils [7]. The radiological hazards due to building materials can be conservatively screened by controlling that the activity concentration index (Eq. 1) remains below unity. Regarding the reuse of phosphogypsum for soil amendment and remediation in agriculture, it is common practice to consider as suitable activity concentration lower than 370 Bq kg⁻¹ [8], therefore in our case the control it before any use for agricultural purposes is necessary.

Coal-Fired Power Plants

In Kosovo, lignite combustion (approximately 14.7 billion tons of reserves) is the main energy source for the production of electricity by approximately 97%. The exploited lignite used for electricity production generates a large amount fly ash and bottom ash that residues, estimated to be approximately 50 million tons and covering a total area of about 370 ha, according to the Kosovo EPA [9]. In particular, approximately 1.36 million tons of fly ash and 0.25 million tons of bottom ash are annually generated [10]. Coal generally contains trace amount of radionuclides with a typical range of activity concentrations of 30 - 100 Bq kg⁻¹, 10 - 600 Bq kg⁻¹ and 10 - 200 Bq kg⁻¹ [4], respectively, for ⁴⁰K, ²³⁸U and ²³²Th. The mean activity concentrations of ⁴⁰K, ²²⁶Ra and ²³²Th in lignite are, respectively, 36 ± 8 Bq kg⁻¹, 9 ± 1 Bq kg⁻¹ and 9 ± 3 Bq kg⁻¹. Fly ash and bottom ash samples show higher activity concentrations of ⁴⁰K, ²²⁶Ra and ²³²Th relative to lignite: in fly ash, respectively, the concentrations are 133 ± 16 Bq kg⁻¹, 30 ± 3 Bq kg⁻¹ and 30 ± 3 Bq kg⁻¹; whereas in bottom ash, respectively, the concentrations are 195 ± 13 Bq kg⁻¹, 28 ± 3 Bq kg⁻¹ and 34 ± 2 Bq kg⁻¹ (Table 1) [11]. The generated ashes may either be disposed in landfills or recycled in other applications, such as in cement production [12], agriculture use as a soil ameliorant [13]. According to EN 197-1 [14], the fly ash is classified as type W (or type C according to ASTM C618:12a [15]) with calcareous nature, showing hydraulic properties when used as a cement additive. In Kosovo, approximately 22% of the fly ash and bottom ash produced annually is recycled in cement production. According to the EU Council Directive [1], the activity concentration index (Eq. 1), fly ash is below unity and therefore, from the radiological point of view, is found to be a suitable material for use as cement additive.

Production of Cements

In Kosovo are produced three types of cement (about 1 million tons per year), standardized according to EN 197-1 [14]. The cement CEM I, well known as Portland cement, is produced by grinding clinker (95-100%) and minor constituents e.g. natural gypsum (5-0%) and the other two types of cement, CEM II/B-M (P-W) and CEM IV/B (P-W), are Portland composite cement containing 20-35% fly ash and pozzolanic cement 35-55% fly ash respectively with additional minor constituents. The activity concentrations of ⁴⁰K in raw materials are found from <6 Bq kg⁻¹ (limestone) to 133 ± 9 Bq kg⁻¹ (marl). The activity concentrations of ²²⁶Ra are found to be from <2 Bq kg⁻¹ (limestone) to 105 ± 6 Bq kg⁻¹ (opalite) as for ²³²Th varying from <2 Bq kg⁻¹ (limestone) to 65 ± 5 Bq kg⁻¹ (opalite). Even the activity concentration of ²²⁶Ra and ²³²Th is relatively high in opalite, its contribution to the radionuclide concentration in clinker is very low due to the low mixing factor. Indeed the main contribution to the activity concentration of ⁴⁰K, ²²⁶Ra and ²³²Th is attributed to marl due to its high mixing fraction. The mean activity concentration in clinker is found to be 189 ± 15 Bq kg⁻¹ for ⁴⁰K, 21 ± 2 Bq kg⁻¹ for ²²⁶Ra and 26 ± 3 Bq kg⁻¹ for ²³²Th [16]. The average activity concentration of natural radionuclides of ⁴⁰K, ²²⁶Ra and ²³²Th in different cement types was found to be 169 ± 15 Bq kg⁻¹, 29 ± 4 Bq kg⁻¹ and 33 ± 6 Bq kg⁻¹ respectively. These activity concentrations are comparable with the mean concentrations found in Europe, respectively 216 Bq kg⁻¹ for ⁴⁰K, 45 Bq kg⁻¹ for ²²⁶Ra and 31 Bq kg⁻¹ for ²³²Th [17].

Processing of Metallic Minerals (Lead-Zinc and Ferro-Nickel)

The lead-zinc deposits lie in the northeastern part of the Republic of Kosovo, mainly in the “Trepça metalogenic belt” with the main occurrences in Stantërg complex, Crnac-Bellobërd complex and Hajvali-Badovc-Artana complex [18]. It is estimated to be approximately 33.5 million tons of tailing from flotation process which cover a total area of almost 82 ha, according to the Kosovo EPA [9]. The lead-zinc smelting industry is not currently active, however large amounts of slag waste in Zvecan and Mitrovica are estimated to be about 2.6 million tons and covers an area of about 5 ha. These residue material is not yet investigated and need a detailed study in the future. The average activity concentration of ⁴⁰K, ²²⁶Ra and ²³²Th in Pb-Zn mineral ore is found to be respectively 123 ± 9 Bq kg⁻¹, 9 ± 1 Bq kg⁻¹ and 6 ± 1 Bq kg⁻¹. The activity concentration of ⁴⁰K shows a clear depletion in Pb and Zn concentrates, while ²²⁶Ra and ²³²Th show a comparable activity concentration in Pb concentrate and a slight depletion in Zn concentrate. The tailing residues exhibit a higher concentration of ⁴⁰K, ²²⁶Ra and ²³²Th respectively, 272 ± 82 Bq kg⁻¹, 18 ± 1 Bq kg⁻¹ and 10 ± 5 Bq kg⁻¹ [19]. Few studies on the activity concentration of natural activity in Pb-Zn industry report maximum activity concentrations of ⁴⁰K, ²²⁶Ra and ²²⁸Ra in mineral ore respectively 65 Bq/kg, 21 Bq/kg and 17 Bq/kg and in tailing 194 Bq/kg, 40 Bq/kg and 17 Bq/kg [20]. These results show a general tendency of increase of radioactivity in tailings, however the activity concentrations are found to be relatively low.

The Ferronikeli Smelter Complex located near Drenas, in central Kosovo, treats ores from Kosovo in Gllavica mine and imported from Albania, Philippines and Indonesia. The area of the landfill deposit amounts to almost 24 ha. The average activity concentration of ^{40}K , ^{226}Ra and ^{232}Th in Fe-Ni mineral ore is found to be $4 \pm 2 \text{ Bq kg}^{-1}$, $8 \pm 1 \text{ Bq kg}^{-1}$ and $2 \pm 1 \text{ Bq kg}^{-1}$. The activity concentrations of ^{40}K and ^{232}Th in electric furnaces and converter slags are found to be higher respect to Fe-Ni mineral ore, except for ^{226}Ra showing comparable results. The activity concentration of ^{40}K , ^{226}Ra and ^{232}Th in electric furnace slag is, $19 \pm 4 \text{ Bq kg}^{-1}$, $7 \pm 2 \text{ Bq kg}^{-1}$ and $5 \pm 1 \text{ Bq kg}^{-1}$ respectively. The activity concentration of ^{40}K , ^{226}Ra and ^{232}Th in converter slag is generally lower respect to electric furnace slag, respectively $12 \pm 3 \text{ Bq kg}^{-1}$, $6 \pm 1 \text{ Bq kg}^{-1}$ and $5 \pm 1 \text{ Bq kg}^{-1}$. This can be reasonable since the converter process already concentrated Fe-Ni material.

Based on the calculation of the index of the concentration of activity, ($I < 1$), we conclude that tailing and slag residues do not cause the exceeding of the gamma radiation reference dose level (1 mSv y^{-1}) as defined in Article 75 of the EU Council Directive [1].

CONCLUSIONS

The conclusion from this preliminary inventory of industrial activities in Kosovo shows that:

- The first campaign for the identification of industrial sectors involving NORM surveyed four out of six sectors present in Kosovo. The future studies need to increase the number of data and including liquid materials other than including in the investigation the remaining industrial sectors.
- In general, it was observed an increase of the concentration of natural radionuclides in residues respect to raw material. However, the activity concentrations in the residues are relatively low and comparable to that in soils [21].
- In all cases the solid materials are below the exemption levels of the activity concentration of natural radionuclides and therefore do not pose any radiological issue for conventional disposal or incineration. Regarding the reuse or recycling in the construction industry or agriculture in some cases, attention should be made to the phosphogypsum residues, which need further studies.
- Future studies need to monitor exposures and to evaluate the related doses for each work activity identified in order to implement, if necessary, corrective measures to reduce exposure.

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Dry Eye Symptoms and Screen Exposure Time in the Workplace

Feim Mazreku^{1*} and Ilir Mazreku²

¹Department of Ophthalmology, Regional Hospital, Prizren, Republic of Kosovo

²Biology Department, Faculty of Natural Sciences, Pristina, Republic of Kosovo

*Corresponding Author: Feim Mazreku, Department of Ophthalmology, Regional Hospital, Prizren, Republic of Kosovo.

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Abstract

Introduction: Dry eye syndrome is one of the most common ophthalmology disorders, influenced by many factors and symptoms such as eye irritation, turbulent eyesight, foreign body feeling, burns, etc. Electronic devices have become an indispensable part of our private and professional life, and long-lasting focus on them causes decreased eye blinking, increased tear volume, eye irritation and pain.

Aim: Evaluation of the tear production level in the patients that work at different period with electronic devices.

Methodology: Subjects were patients of the Ophthalmologic ambulance "Guri-Clinic" in Prizren. Their symptomatology included scratches, burns, pain or foreign body feeling in the eye. With Schirmer test (type I, without topical anaesthesia) was measured the level of tear production for 5 minutes.

Results: Participants were 64 patients, respectively 128 eyes, 39% were women (age 41.56 ± 7.76) and 61% males (age 34.62 ± 11.05). According to the results analysis, there is a moderate negative correlation (-0.42 to -0.54) between the level of the tear production and the working years of experience, or the working hours during per day (-0.37 to -0.45) with electronic devices.

Discussion: The findings of our study are in coherence with the literature used, including the significant difference in the level of tear production in females and males, and the moderate negative correlation of the tear production level and years of experience in work or duration of working hours per day with electronic devices which in this study was -0.42 while according to Yang, *et al.* (2015) this correlation rate was -0.65.

Keywords: Ophthalmology; Eye; Computer; Tear; Patients

Introduction

Dry eye syndrome is one of the most frequent disorders in ophthalmology. It is a condition influenced by many factors and with a large number of symptoms such as eye irritation, turbulent eyesight, foreign body feeling, burns, pain, increased osmolarity of tears and changes in the surface epithelium of the eye [1]. Smart electronic devices have become an inseparable part of our private and professional life. Focusing on a smart electronic device screen requires continuous eye contact without blinking for a long period of time. With a reduction of eye blinks, the rate of tear evaporation increases thus the time to accommodate proper eye condition increases, therefore, irritation of the eye and the pain is unavoidable. Recently, there have been concerns over the negative effect of blue light, generally from light diodes or LED screens, on cornea and retina as it stimulates phototoxicity and oxidative stress [2]. This study is primarily concerned with patients who have declared that in their workplace they are required to use a smart device of some sort.

Aim of the Study

Evaluating the rate of tear production at patients who work for a long time with various screens of electronic devices.

Methodology

The subjects of this study are patients who have been treated at the Ophthalmological Center “Guri-Clinic” in Prizren, Kosovo. Their symptomatology included: scratching, burning, pain or feeling of the foreign body in the eye. Using the Schirmer test, we measured the rate of tear production for a duration of five (5) minutes. Other data included the duration of the symptoms, the time that patients stay in front of screens within twenty-four (24) hours and throughout the annual working days. Statistical analysis is conducted with the OneWay ANOVA test, the t-test, correlation coefficient and regression analysis with SD 0.05.

Schirmer test produces four outcomes and those are:

1. Normal ≥ 15 mm, coloring of the ribbon after 5 minutes.
2. Light 14 - 9 mm, coloring of the ribbon after 5 minutes.
3. Moderate 8 - 4 mm, coloring of the ribbon after 5 minutes.
4. Severe < 4 mm, coloring of the ribbon after 5 minutes.

Results

Participants in this study were sixty-four (64) patients, respectively one hundred twenty-eight (128) eyes. Of these, 39% were women (age 41.56 ± 7.76) and 61% males (age 34.62 ± 11.05). From statistical analysis it appears that there are no significant differences in the results of the level of tear production between females and males. On the other hand, the correlation analysis confirms that there is a moderate negative correlation (-0.42 to -0.54) between the level of tear production and working hours, or the daily periodic length of exposure to screen (-0.37 to -0.45) of electronic devices.

Female	Age (years)	Schirmer test OD (mm)	Schirmer test OS (mm)	Work hours (within a day)	Years at work	Early symptoms (after x working years)
Average	41.56 ± 7.76	6.96 ± 4.04	7.40 ± 3.67	6.14 ± 1.63	9.08 ± 4.11	3.15 ± 1.87
Min	23.00	3.00	2.00	3.00	2.00	0.15
Max	56.00	17.00	18.00	10.00	16.00	7.00

Table 1: Statistical summary of female participants' age, Schirmer test results, hours and years exposure to screen and time when early symptoms appeared in reference to the work starting date.

Male	Age (years)	Schirmer test OD (mm)	Schirmer test OS (mm)	Work hours (within a day)	Years at work	Early signs of symptoms (after x working years)
Average	34.62 ± 11.0	8.10 ± 3.73	8.13 ± 4.10	6.97 ± 1.86	7.92 ± 3.99	3.10 ± 1.74
Min	15.00	3.00	3.00	2.00	2.00	0.50
Max	55.00	17.00	18.00	10.00	16.00	7.00

Table 2: Statistical summary of male participants' age, Schirmer test results, hours and years exposure to screen and time when early symptoms appeared in reference to the work starting date.

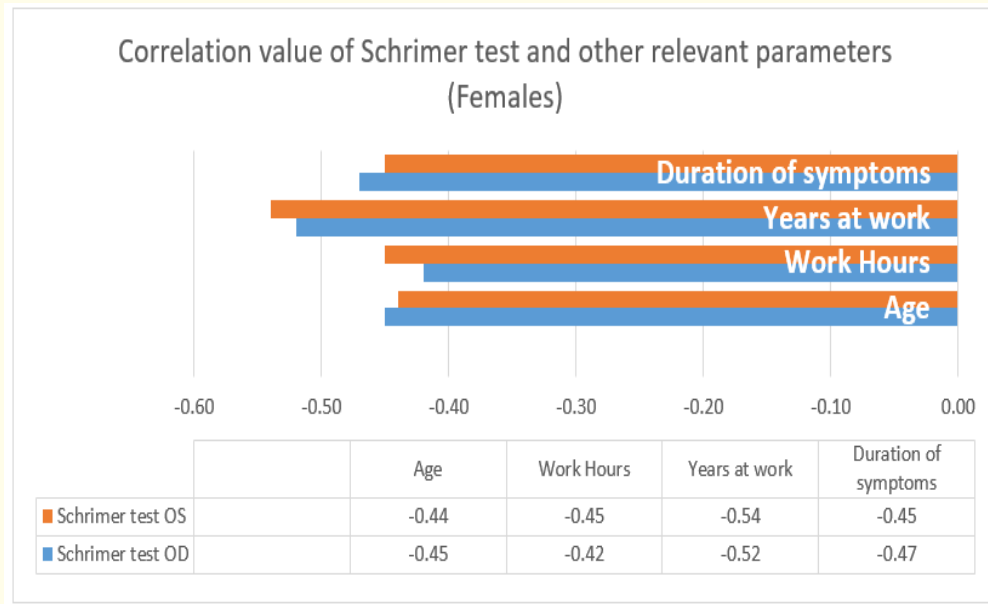


Figure 1: Graphical representations of the correlation between Schirmer test results with age, exposure time, years at work, and duration of symptoms for female patients.

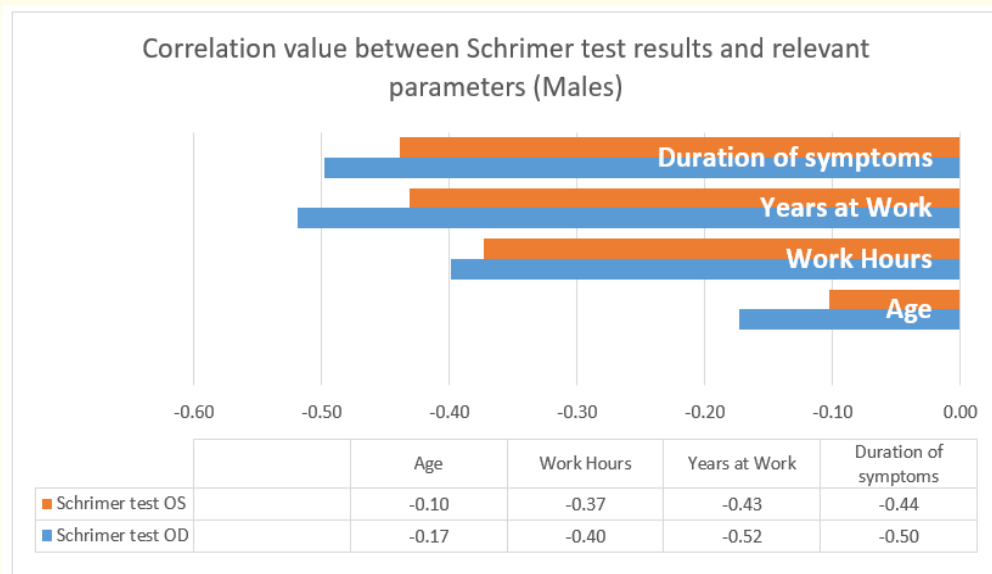


Figure 2: Graphical representations of the correlation between Schirmer test results with age, exposure time, years at work and duration of symptoms for male patients.

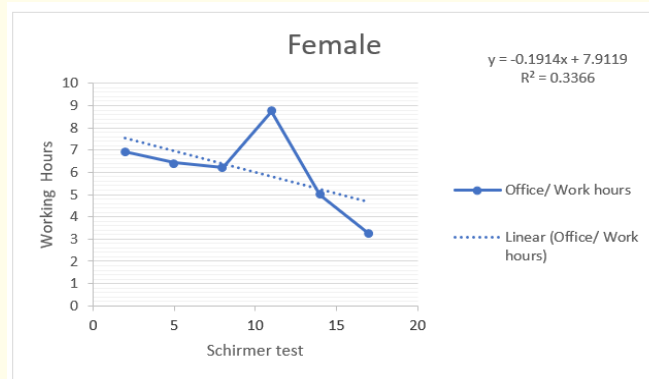


Figure 3: Graphical representation of the regression analysis between Schirmer test results and hours at work (Females).

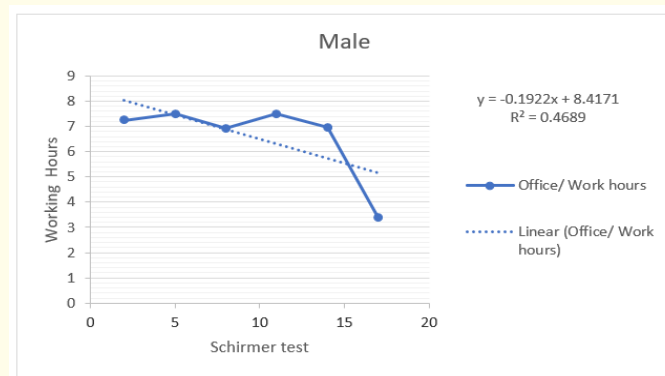


Figure 4: Graphical representation of the regression analysis between Schirmer test results and hours at work (Males).

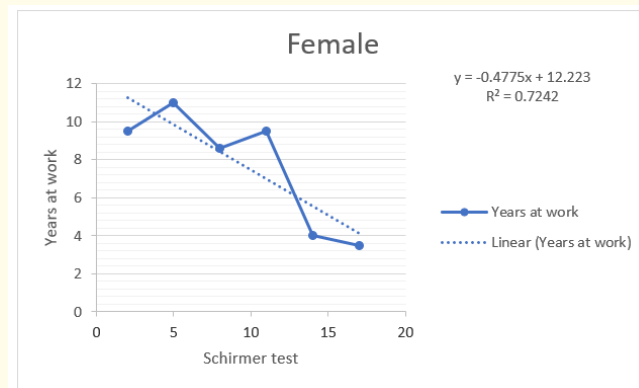


Figure 5: Graphical representation of the regression analysis between Schirmer test results and years at work (Females).

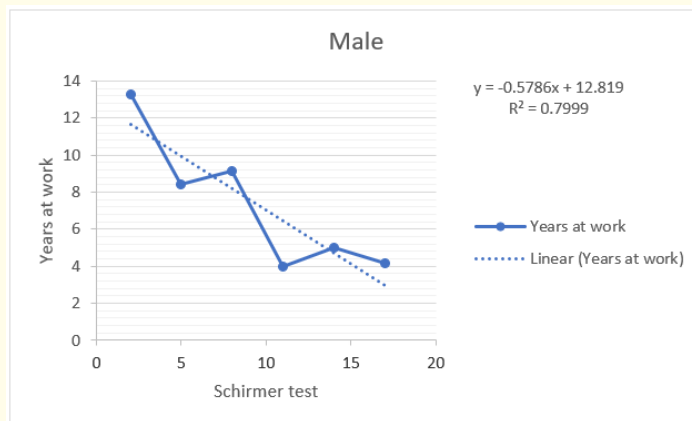


Figure 6: Graphical representation of the regression analysis between Schirmer test results and years at work (Males).

Discussion

The data analyzed from this study are consistent with data from the used literature, including the significant difference in the level of tear production in females and males and the moderate negative correlation between tear production rate and daily periodic length exposure to screens of electronic devices. For the latter, Yang, *et al.* (2015) [5] had correlation coefficient of -0.65, whereas our study concluded a correlation coefficient of -0.42. Yang, *et al.* [5] looked at a Japanese company and found significant difference in the amount of tear production (measured by the Schirmer test) between individuals who work more than six (6) hours a day with computer screens and those under one hour [4-9].

Kim, *et al.* (2016) [10] also found a significant difference between eye tear production rate at subjects who used smart tablets for a constant two hours long exposure and the control group.

Conclusion and Recommendations

This study reaffirms that dry eye symptoms have a connection to the exposure time of patients' eye to various screens such as smart devices in their workplace. Moreover, a slightly higher correlation coefficient for both males and females was the Schirmer test results and the years at the workplace. The surveyed patients stated that they are required to use smart devices in their workplace, and as a result, the amounts of years at work reflects in the exposure time to screens of those smart devices. Therefore, some of our recommendations are:

1. Application of artificial tear without preservatives.
2. After twenty (20) up to thirty (30) minutes of exposure to the screen, it is recommended to look at a further object and blinking the eyes.
3. Blinking twelve to fifteen (12 - 15) times per minute is also recommended.
4. Washing the face with water during breaks.
5. A few minutes of walking during the work breaks will refresh the body and the mind. In addition, it will benefit your eyes due to increased blood flow from walking.
6. Use a screen filter to reduce blue light. Moreover, adjust the position of the screen in a way that light reflection from the screen is minimal.
7. Follow-up with regular examination at your nearest ophthalmological clinic.

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Palatal Foreign Body - A Case Report

**Šumilin Lada¹, Žužul Ivona², Muhaxheri Granita³, Andabak Rogulj Ana⁴,
Vidović Juras Danica⁴ and Lončar Brzak Božana^{4*}**

¹*Polyclinic Aviva, Zagreb, Croatia.*

²*Department of Oral Surgery, School of Dental Medicine, Zagreb, Croatia.*

³*Private Dental Practice, Pristina, Kosovo.*

⁴*Department of Oral Medicine, School of Dental Medicine, University of Zagreb, Zagreb, Croatia.*

Authors' contributions

This work was carried out in collaboration between all authors. Author SL referred the patient to our department and wrote the first draft. Authors ZI and MG searched the literature and helped in writing.

Authors ARA, VJD and LBB examined the patient, established diagnosis and reviewed the manuscript. Author LBB wrote the final version of the manuscript. All authors read and approved the final manuscript.

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Case Report

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ABSTRACT

Aims: Foreign bodies exerted in the palatal mucosa are extremely rare in clinical work. The aim of this case report was to describe a case of foreign body in the palate of an infant and to highlight that foreign bodies must be considered in the differential diagnosis of palatal lesions found in children.

Presentation of Case: A healthy 12-month child was brought by his parents to the Department of Oral Medicine, School of Dental Medicine, University of Zagreb, since the mother accidentally noticed something in the child's mouth, a week before. The child's pediatrician and the doctor of dental medicine had examined the child and referred him for CT scan and examination at department of maxillofacial surgery. The lesion was hard on palpation and it bothered him while feeding. The child's medical history was non contributory and he was not taking any medication. Clinical examination revealed an oval white lesion in central hard palate, smooth and hard on

*Corresponding author: E-mail: loncar@sfzg.hr;

palpation. Surrounding mucosa appeared normal. While mother was holding the child, we opened his mouth and examined the edges of the lesion with the instrument Heidemann spatula. The lesion suddenly started to separate from palatal mucosa until it had completely fallen out. It turned out to be an artificial nail.

Discussion and Conclusion: Besides local trauma, foreign bodies of oral mucosa are potentially lethal because of the risk of ingestion or obstruction of respiratory tract. Differential diagnosis of palatal lesions in children should include a possible foreign body. A thorough clinical examination can prevent unnecessary diagnostic procedures and misdiagnosis.

Keywords: Clinical examination; diagnosis; foreign body; oral mucosa.

1. INTRODUCTION

Foreign bodies exerted in the palate mucosa are extremely rare in clinical work. These cases mostly occur in infants and children and present potential danger of ingestion or obstruction of airways. Characteristic texture of the child's palate, together with thumb-sucking habits which are frequent in children, favors the retention of a foreign object [1,2]. Clinical examination in children is sometimes challenging which can lead to misdiagnosis of a foreign body as some other oral lesion [1,3-5]. The aim of this case report was to describe a rare case of foreign body in the palate of an infant and to highlight that foreign bodies must be considered in the differential diagnosis of palatal lesions found in children.

2. PRESENTATION OF CASE

We report a case of a healthy 12-month male child who was brought by his parents to the Department of Oral Medicine, School of Dental Medicine, University of Zagreb, because the mother accidentally noticed something in the child's mouth, a week before. The lesion was hard on palpation and it bothered the child while feeding. The child's pediatrician and the doctor of dental medicine had examined the child and referred him for a CT scan and examination at maxillofacial surgery, but the parents have come to our Department, as suggested by the doctor of dental medicine, before they made the examinations they were sent to.

The child's medical history was non contributory and he was not taking any medication. The lesion appeared asymptomatic, except while eating or on palpation. Clinical examination revealed an oval white lesion in central hard palate, smooth and hard on palpation (Fig. 1). Surrounding mucosa appeared normal. While mother was holding him, we opened his mouth and examined the edges of the lesion with the instrument Heidemann spatula. The lesion suddenly started to separate from palatal mucosa until it has

completely fallen out. The underlying mucosa was little irritated and erythematous so it was wiped with antiseptic solution (chlorhexidine gluconate 2%). The removed object turned out to be an artificial nail that was pushed inside the palate (Fig. 2). A few days later the palatal mucosa showed complete healing.



Fig. 1. Impacted object prior to removal



Fig. 2. Removed object - artificial fingernail

3. DISCUSSION

Foreign bodies in oral mucosa are very rare, yet we have found some interesting cases in the literature. Besides local trauma, these lesions are potentially lethal because of the risk of ingestion or obstruction of respiratory tract. According to the literature data, foreign body aspirations are most frequent in children younger than five years, and they are the most deadliest in infants younger than one year [6]. Differential diagnosis without a detailed clinical examination may include different malignant tumours and cysts, such as eosinophilic granuloma, melanotic neuroectodermal tumor of infancy, sarcoma, osteolipoma, sphenothmoidal encephaloceles or odontogenic cysts [1,3-7]. Also, leukemic infiltrates, ulcerative and necrotic lesions or fungal infections should be considered, although these are also infrequent diagnoses in paediatric population. In children, a foreign body should always be included in differential diagnosis of palatal lesions. Besides artificial fingernails [3,8,9], foreign bodies reported in the oral cavity are pistachio nut shells [10], plastic stickers [10], buttons, plastic lids, parts of toys [12] etc. These reports mostly refer to very young children who can not speak for themselves so the oral lesion is often an accidental finding by their parents. It should be emphasized that sometimes the object can be attached to oral mucosa for a few months [13], making the establishing of a correct diagnosis difficult. Initial diagnosis often includes some of the previously mentioned differential diagnoses, and the patient is thoroughly processed (CT, exfoliative cytology, fine needle aspiration cytology) [3,4,10,11], but the findings are frequently misleading. For example, in case reports with CT finding of the lesion [3,4,10,11], radiolucent mass or cyst with radiopaque borders or calcified rim was found, but the CT finding did not contribute to setting the right diagnosis. Foreign bodies in these cases were artificial fingernail [3,4], pistachio nutshells [10] and a plastic sticker [11]. A definitive diagnosis was established during examination in general anesthesia, prior to planned surgical removal [3,4,10,11].

In case the patient is uncooperative, this contributes to referring to other specialists and makes it difficult to set up a proper diagnosis [5]. In some examples, patients have been examined by several different doctors until the correct diagnosis is established [10]. In uncooperative patients, clinical examination is difficult and the

lesion is misdiagnosed, which leads to avoidable examinations such as CT scan or examination in general anesthesia.

It is important to carefully plan and conduct a clinical examination. The edges of the lesion should be inspected and, in case of suspicion of a foreign body, the object should be removed in posteroanterior direction, while the patient is in a position that makes swallowing or aspiration difficult. It is recommended that the child is positioned laterally in the parent's lap, with his head lightly down [1].

4. CONCLUSION

Foreign body should always be included in differential diagnosis of palatal lesions in children. It is important to carefully inspect the edges of the lesion during clinical examination. A well conducted clinical examination can prevent unnecessary diagnostic procedures and misdiagnosis.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Multivariate analysis of preoperative and postoperative neutrophil-to-lymphocyte ratio as an indicator of head and neck squamous cell carcinoma outcome

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Multivariate analysis of preoperative and postoperative neutrophil-to-lymphocyte ratio as an indicator of head and neck squamous cell carcinoma outcome

G. Muhaxheri¹, V. Vucicevic Boras¹, A. Fucic², D. Plavec³, M. Sekerija⁴, M. Filipovic⁵, K. Grsic⁶, B. Stubljar⁶, T. Krnic⁶, B. Vrdoljak⁵

¹Department of Oral Medicine, School of Dentistry, University of Zagreb, Zagreb, Croatia; ²Institute for Medical Research, Zagreb, Croatia; ³Childrens Hospital Srebrnjak, Zagreb, Croatia; ⁴Institute for Public Health, Zagreb, Croatia; ⁵School of Medicine, University of Zagreb, Zagreb, Croatia; ⁶Clinic for Tumours, Zagreb, Croatia

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Abstract. Recent publications have highlighted a greater utility of routine blood tests in patients with various cancers than previously assumed. It appears that the neutrophil-to-lymphocyte ratio (NLR) may be a good predictive biomarker for overall survival (OS) and disease-free survival (DFS). Preoperative and postoperative NLR data for patients with head and neck cancers have yet to be established. The aim of this study was to evaluate the preoperative and postoperative NLR in 182 patients with head and neck squamous cell carcinoma and to determine the association of NLR with OS and DFS. The statistical analysis of OS and DFS and their predictors was performed using Kaplan–Meier survival analysis and multivariate Cox proportional hazards regression analysis, with factors including age, sex, alcohol and tobacco use, tumour location, treatment after surgery, and lymphocyte and neutrophil counts. Longer OS was significantly associated with not consuming alcohol, preoperative neutrophil and lymphocyte counts, preoperative NLR, and the difference between the preoperative and postoperative NLR ($P = 0.016$). Longer DFS was significantly associated with not consuming alcohol, preoperative neutrophil and lymphocyte counts, postoperative NLR, and the difference between preoperative and postoperative NLR ($P = 0.028$).

Key words: neutrophils; neutrophil-to-lymphocyte ratio; HNSCC.

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Head and neck cancers are a heterogeneous group of tumours with varying aetiology. More than 90% of head and neck cancers are squamous cell carcinomas^{1,2}. The global incidence of all new head and neck cancer cases has been reported to be between 400,000 and 600,000 per year and the mortality rate between 223,000 and 300,000 deaths per year³. Risk factors for head and neck cancers are tobacco and alcohol intake, poor diet, and infection with human papillomavirus (HPV) or Epstein–Barr virus (EBV). Recent reports have shown an increase in head and neck cancer incidence with significantly higher mortality in developing countries⁴. These cancers are very aggressive and can develop distant metastases even after effective local therapy^{5–7}. For this reason, predictive biomarkers that may direct the clinical decision for follow-up and diagnostics are of crucial significance.

Current models for predicting survival and the efficacy of therapy in patients with head and neck cancer are still inadequate; therefore, available biomarkers are being re-evaluated in order to improve their informativeness. Inflammation is a significant moderator of carcinogenesis, also associated with poorer disease-free survival (DFS) and overall survival (OS)⁸. Recently, it has been shown that the preoperative neutrophil-to-lymphocyte ratio (NLR) may predict a poor response to treatment, DFS, and OS in patients with many cancer types, including small cell lung carcinoma, oesophageal carcinoma, pancreatic adenocarcinoma, and head and neck cancers^{9–18}.

Neutrophils take part in the elimination of pathogens by phagocytosis – the generation of reactive oxygen species – via phagocyte NADPH oxidase, the release of antimicrobial and cytotoxic compounds, formation of neutrophil extracellular traps, and secretion of chemokines and cytokines¹⁹. An increased neutrophil level is characteristic of many cancer types, as they promote disease progression by releasing matrix metalloproteinase 9 (MMP9)²⁰. Neutrophils themselves are also a significant source of hepatocyte growth factor, which has been implicated in the regulation of mitogenesis, motogenesis, and morphogenesis of epithelial and endothelial cells²¹. NLR has recently been associated with the metabolic tumour volume in patients with oesophageal squamous cell cancer²².

In recent years, the cut-off values of the NLR for different cancer types have been estimated on several occasions. The pre-treatment NLR cut-off value for the

prediction of OS in patients with colorectal cancer was determined to be $>5^{23}$. The median NLR cut-off value for the prediction of DFS was suggested to be >4 in a systematic meta-analysis of publications investigating the association of NLR and DFS in solid tumours¹¹.

Currently, preoperative and postoperative NLR data for patients with head and neck squamous cell carcinoma (HNSCC) are lacking. Therefore, the aim of this retrospective study was to establish preoperative and postoperative NLR levels for HNSCC, together with their association with DFS and OS. Additionally, multivariate analysis was used to determine the impact of alcohol and tobacco intake, patient age, tumour location, cancer stage, and therapy on the capability of the NLR to predict the disease outcome.

Subjects and methods

Study population

Data were retrieved from the patient charts at the Department of Otorhinolaryngology, Clinic for Tumours, Zagreb, Croatia. The study population consisted predominantly of male HNSCC patients (156 male patients, 26 female patients). The preoperative and postoperative NLR values were associated with demographic characteristics, lifestyle characteristics, OS, and DFS. For the purpose of the analysis, the TNM (tumour–node–metastasis) stage of disease was dichotomized as follows: stages I and II were designated as early stage, whereas stages III and IV were designated as advanced stage; this was done following classification according to the criteria of the TNM Classification of Malignant Tumours eighth edition²⁴. With regard to treatment, all patients were initially treated with surgery, after which some patients received concomitant radiotherapy and some also chemotherapy. The localization of the cancer was dichotomized as ‘oral cavity’ (anterior two-thirds of the tongue, gingiva and alveolar ridge, hard palate and buccal mucosa) and ‘oropharynx’ (soft palate, pharynx, and tonsils). The treatment type was dichotomized as follows: patients with stage I or II disease were treated only with surgery; patients with stage III or IV disease were treated after surgery with adjuvant radiotherapy. Those with involved resection margins and/or extranodal spread of disease (histologically confirmed) received concomitant cisplatin chemotherapy at a daily dose of 100 mg/m² of body surface, every 3 weeks. The patients’ HPV status was not investigated, as the study period

started 16 years ago, at a time when HPV status was not part of routine diagnostics.

Patients with TNM stage I and II disease were treated by surgery. Those with stage III and IV disease received adjuvant therapy after surgery, using three-dimensional conformal radiotherapy. The target volume encompassed the lymph node regions bilaterally to a prescribed dose of 50 Gy and the tumour bed to a prescribed dose of 60 Gy (with or without a ‘booster’ dose of 6 Gy) and 6 (or 18) MV photons with a linear accelerator (ARTISTE or ONCOR; Siemens Medical Solutions USA, Inc.). Patients were irradiated over the course of 6 to 6.5 weeks with daily doses of 2 Gy.

DFS was defined as the period after surgery during which the patient had no sign of cancer recurrence. OS was defined as the period from the date of surgery to individual death from any cause or the last follow-up.

Laboratory measurements

Blood samples were taken 1 week before surgery and postoperatively 7 days after surgery. The exclusion criteria were patients with missing clinical data (none) and positive surgical margins (none). Cancer patients had no autoimmune disorders or haematological disorders, were not on any ongoing immune-modulating medications, and had no previous history of malignant disease. The Ethics Committee of the Clinical Hospital ‘‘Sisters of Mercy’’, University Hospital Centre approved the study.

Preoperative and postoperative serum neutrophils and lymphocytes were extracted from blood counts of blood samples using a fully automated five-part differential haematology analyzer (Sysmex XN-1000; Sysmex, Kobe, Japan). The time of blood sampling (7 days after surgery) ensured that the process of wound healing did not affect the results. The maximum follow-up period was 202.9 months (mean 102.1 months). The NLR was calculated by dividing the neutrophil count by the lymphocyte count. In addition, the difference between the preoperative and postoperative NLR (DiffNLR) was introduced in order to test its predictive capacity for DFS and OS.

Statistical analysis

The statistical analysis was conducted using Statistica data analysis software system version 12 (StatSoft, Inc., Tulsa, OK, USA) and MedCalc statistical software version 16.8.4 (MedCalc Software, Ostend, Belgium; <https://www.medcalc.org>; 2016). Categorical variables were recorded as numbers and

proportions (%). Quantitative variables were tested for normality of distribution using the Kolmogorov–Smirnov test and recorded as the mean and standard deviation (SD) or median and interquartile range (IQR), depending on the type of data distribution. Kaplan–Meier survival analysis was used to determine OS and DFS. Associations of OS and DFS with possible predictors were analyzed using Cox proportional hazards analysis with a backward stepwise approach. All of the tests were two-tailed, and $P < 0.05$ was used to indicate significance in all analyses.

Results

This study included the results of 182 patients treated for HNSCC (85.7% male), whose mean age was 60.0 years (range 23.4–86.3 years). The baseline characteristics of the study population are presented in Table 1. The analysis included pre- and postoperative measurements of neutrophil and lymphocyte counts, together with the NLR and DiffNLR. DFS was a median of 29.7 months (IQR 11.8–80.9 months) and OS was a median of 31.4 months (IQR 14.8–83.8 months) (Kaplan–Meier survival analysis).

Table 2 presents the preoperative and postoperative neutrophil count, lymphocyte count, NLR, and DiffNLR values.

Significant independent associations with OS and DFS are presented in Tables 3 and 4. As seen in Table 3, not consuming alcohol significantly lowered the risk of

death by 45% ($P = 0.030$), the preoperative neutrophil count lowered the risk of death by 16% per each $1 \times 10^9/l$ ($P = 0.030$), and the preoperative lymphocyte count marginally increased the risk of death by 49% per each $1 \times 10^9/l$ ($P = 0.059$). The risk of death was significantly increased by 38% for a unit change in the preoperative NLR ($P = 0.002$), and was marginally increased by 4% for a unit change in DiffNLR ($P = 0.081$).

As seen in Table 4, not consuming alcohol significantly lowered the risk of disease relapse by 42% ($P = 0.041$), the preoperative neutrophil count lowered the risk of disease relapse by 13% per each $1 \times 10^9/l$ ($P = 0.049$), and the preoperative lymphocyte count significantly increased the risk of disease relapse by 53% per each $1 \times 10^9/l$ ($P = 0.026$). There was a significant increase in the risk of disease relapse of 34% for a unit change in the postoperative NLR ($P = 0.002$), and a significant decrease in this risk by 22% for a unit change in DiffNLR ($P = 0.008$).

Tumour location, tumour stage, smoking, age, and type of therapy were not significantly associated with either DFS or OS.

Radiotherapy or combined radiotherapy with chemotherapy had no impact on the biomarkers measured.

Discussion

This study showed that preoperative and postoperative neutrophil counts and NLR

can be predictive biomarkers for DFS and OS in HNSCC. This study used multivariate analysis, which enabled the testing of neutrophil count, NLR, and DiffNLR as predictive biomarkers for DFS and OS by including alcohol consumption, patient age, smoking, tumour stage, and therapy type as modifying parameters. The finding that the difference in NLR before and after surgery was a significant predictor of OS and DFS appears to be novel. Not consuming alcohol was significantly associated with longer DFS and better OS.

Lymphocytes are the most significant components of the adaptive immune system, which when infiltrated into the tumour indicate the generation of an effective anti-tumour cellular immune response²⁵. Increased NLR has been associated with an increase in the peri-tumoural infiltration of macrophages and an increase in interleukin 17, interleukin 6, interleukin 8, tumour growth promoting factors, vascular endothelial growth factor, hepatocyte growth factor, and matrix metalloproteinases, all of which form tumour microenvironments²⁶.

Previous studies have shown an association between a low peripheral blood lymphocyte count and shorter survival of patients with different types of cancer^{27,28}. However, other cell types also involved in the immunological response have been shown to play a significant role in the progression of cancer. Thus, it has been reported that neutrophils support metastasis by producing leukotrienes, which enable the colonization of distant tissues with cancer cells²⁹. Both neutrophil levels and the NLR have been shown to be prognostic factors in nasopharyngeal cancer independent of OS and DFS³⁰. Tsai et al. reported that neutrophil counts and NLR increased with the advancement of the clinical stage (i.e., T4) and poorer tumour differentiation in patients with oral cancer, which was also accompanied by a decrease in the lymphocyte count³¹.

The location of the tumour was not associated with preoperative NLR, postoperative NLR, DiffNLR, OS, or DFS, which is in accordance with published data³².

The results of this study confirm those of a study by Rachidi et al., which showed that the NLR is a robust predictor of OS in oral, pharyngeal, and laryngeal squamous cell carcinomas³³. Perisanidis et al. analyzed 97 patients with oral cancer undergoing preoperative chemo-radiotherapy in terms of DFS³⁴. These authors reported that a high pre-treatment NLR is a significant independent predictor of shorter DFS in patients with oral cancer

Table 1. Baseline characteristics of the study patients ($N = 182$)^a.

Characteristic	
Age, years, mean \pm SD (range)	60.0 \pm 9.7 (23.4–86.3)
Sex	
Male	156 (85.7%)
Female	26 (14.3%)
Alcohol consumption	136 (74.7%)
Smoking habit	
Non-smokers	20 (11.0%)
Active smokers	120 (65.9%)
Ex-smokers	25 (13.7%)
Missing data	17 (9.3%)
Tumour location	
Oral	132 (72.5%)
Pharyngeal	48 (27.5%)
TNM stage	
1	21 (11.5%)
2	91 (50.0%)
3	48 (26.3%)
4	22 (12.1%)
Treatment after surgery	
No additional treatment	34 (18.7%)
Radiotherapy	143 (78.6%)
Radio- and chemotherapy	5 (2.7%)
Relapse	124 (68.1%)
Death	122 (67.0%)

SD, standard deviation.

^a Results are presented as the number and percentage of patients, unless stated otherwise.

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Table 3. Covariates depicted for the model of overall survival (OS); $P = 0.016$ for the model, Cox proportional hazards model.

Covariate	b	SE	Wald	P-value	HR	95% CI of HR
Not consuming alcohol	-0.601	0.276	4.734	0.03	0.548	0.319 to 0.948
Preoperative neutrophil count	-0.17	0.078	4.691	0.03	0.844	0.724 to 0.984
Preoperative lymphocyte count	0.397	0.21	3.554	0.059	1.487	0.984 to 2.245
Preoperative NLR	0.319	0.101	9.954	0.002	1.375	1.128 to 1.676
DiffNLR ^a	0.043	0.024	3.054	0.081	1.044	0.995 to 1.095

E, standard error; HR, hazard ratio; CI, confidence interval; NLR, neutrophil-to-lymphocyte ratio.

^a Difference between preoperative and postoperative NLR.

Table 2. Neutrophil count, lymphocyte count, and NLR before and after surgery, and the difference in NLR.

Characteristic	Mean \pm SD or median (IQR)	Range
Preoperative		
Neutrophil count, $\times 10^9/l$	5.11 \pm 1.88	1.33 to 10.63
Lymphocyte count, $\times 10^9/l$	2.11 \pm 0.78	0.44 to 5.89
NLR	2.27 (1.77, 3.19)	0.51 to 12.39
Postoperative		
Neutrophil count, $\times 10^9/l$	6.34 (4.77, 8.10)	1.86 to 19.70
Lymphocyte count, $\times 10^9/l$	1.72 (1.25, 2.20)	0.44 to 5.59
NLR	3.80 (2.53, 5.33)	0.00 to 38.41
DiffNLR ^a	1.06 (-0.02, 2.33)	-9.09 to 34.06

NLR, neutrophil-to-lymphocyte ratio; SD, standard deviation; IQR, interquartile range.

^a Difference between preoperative and postoperative NLR.

Table 4. Covariates depicted for the model of disease-free survival (DFS); $P = 0.028$ for the model, Cox proportional hazards model.

Covariate	b	SE	Wald	P-value	HR	95% CI of HR
Not consuming alcohol	-0.546	0.267	4.179	0.041	0.579	0.343 to 0.978
Preoperative neutrophil count	-0.144	0.073	3.845	0.049	0.866	0.750 to 0.999
Preoperative lymphocyte count	0.428	0.192	4.975	0.026	1.534	1.053 to 2.235
Postoperative NLR	0.29	0.096	9.145	0.002	1.336	1.107 to 1.613
DiffNLR ^a	-0.245	0.093	6.943	0.008	0.783	0.653 to 0.939

SE, standard error; HR, hazard ratio; CI, confidence interval; NLR, neutrophil-to-lymphocyte ratio.

^a Difference between preoperative and postoperative NLR.

receiving preoperative chemo-radiotherapy³⁴. Fang et al. analyzed data from 226 patients with oral cancer and reported that elevated NLR was significantly associated with tumour status, nodal metastasis, tumour depth, DFS, and OS³⁵. The effect of the NLR on DFS and OS was shown to exist even after adjusting data for tumour status, lymph node metastasis, and tumour cell differentiation. Song et al. showed that a high preoperative NLR was associated with increased wound complications and poorer survival in patients with hypopharyngeal squamous cell carcinoma after radical resection³⁶. The present study results are in concordance with these previous studies, which have suggested that the preoperative NLR is an independent predictor of head and neck cancer recurrence³⁷⁻³⁹, even after other lifestyle and demographic data are included in the analysis.

A recent study involving a healthy population of adult, non-geriatric subjects esti-

ated normal NLR values to be between 0.78 and 3.53, adding significant value to the application of the NLR⁴⁰. However, the control range obtained did not take into account smoking habit or alcohol intake. It is interesting to note that in the present study, in which 70–80% of the subjects were alcohol consumers and smokers, the mean postoperative NLR was higher (group value) than the suggested control range values. Alcohol intake has been associated with neutropenia, and neutrophils have also been shown to be hypo-responsive in cases of exposure to alcohol due to impaired phagocytosis and superoxide generation in humans and in animal models^{41,42}. Additionally, the heterogeneous impact of alcohol on OS related to treatment and primary site shows the need for further investigation in this study⁴³. However, the effect of smoking on the parameters measured was not of great significance, as it is known that in

contrast to alcohol, smoking causes an increase in neutrophil counts⁴⁴. The present study results confirm the importance of taking alcohol consumption and smoking into consideration in studies assessing the NLR.

This study showed the neutrophil count, the NLR, and DiffNLR to be significantly associated with DFS and OS in HNSCC. Future studies should be performed in order to determine whether the use of focused medical surveillance protocols depending on neutrophil counts, the NLR, and DiffNLR in these patients might increase DFS or OS. Finally, a significant association between alcohol consumption and tumour site with regard to the DFS and OS was noticed. Studies of the same design as the present study should be performed in other cohorts in order to define cut-off values for HNSCC and to include the use of these parameters in clinical practice.

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Competing interests

We wish to confirm that there are no known conflicts of interest associated with this publication.

Ethical approval

Ethical approval was obtained from the School of Dental Medicine, Zagreb, Croatia.

Patient consent

Not required.

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Address:

G. Muhaxheri
School of Dentistry
University of Zagreb
Gunduliceva 5
Croatia
E-mail: muhaxherig@gmail.com

Level of Stress at Nurses Working in Emergency Clinic and Central Intensive Care: University Clinical Centre of Kosovo

Elvana Podvorica^{1,2}, Besnik Rrmoku³, Albulena Metaj⁴, Hasan Gashi⁵

¹Faculty of Medicine, Department Nursing and Midwifery, University of Pristina, Pristina, Kosovo

²Universi College, Pristina, Kosovo

³Emergency Clinic, University Clinical Centre of Kosova; Prishtina, Republic of Kosovo

⁴Faculty of Education, University of Prishtina, Prishtinë, Kosovo

⁵Rezonanca College, Pristina, Kosovo

Email: elvana.podvorica@uni-pr.edu, albulena.metaj@uni-pr.edu, hasan.gashi@rezonanca-rks.com

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Abstract

Background: Emergency Clinic (EC) and Central Intensive Care (CIC) are high-stress environments that directly affect the health status of nurses. **Methods:** The aim of this study was to assess the level of stress at nurses working in the Emergency Clinic and nurses working in the Central Intensive Care. The study included 90 nurses, 45 nurses working in Emergency Clinic and 45 nurses working in Central Intensive Care. The study applied the Emergency Nurse Stress Questionnaire as an instrument to gather the data. This questionnaire was adopted from the Operational Police Stress Questionnaire in order to serve for the function and aim of the present study. **Results:** Nurses' cohort-age ranged from 20 to 62 years. The largest proportion of respondents 40 (44.4%) was in the 20 - 30 age group, 58 (64.4%) were married, 60 (66.6%) hold bachelor degree and (33.3%) were with secondary school educational level. In terms of work-related fatigue, significant difference was found in working hours, participants reported that there was a significant difference in fatigue between 12 hours shift nurses (61 ± 10.5) compared to 8 hours nurses (41 ± 23.6) with $P < 0.001$. Results indicated that a vast majority of participants reported moderate to high levels of stress (81% of participants). There was no significant difference in the level of stress between the two groups of participants. There were also no significant differences compared to their demographic characteristics. **Conclusion:** These findings emphasize the role of using and assuring adequate strategies for ensuring quality management and finding ways of facilitating the increase in the number of nursing staff in these two departments because workplace overload and fatigue are potential

factors that increase nurses' stress levels.

Keywords

Emergency Clinic, Central Intensive Care, Nurses, Workplace Stress, Fatigue

1. Introduction

The first professionals who attend stressful medical situations, providing care to injured people and those who are critically ill, suspended between life and death, are often constituted staff from the Emergency Medical Services (EMS). Thus, these individuals are often exposed to specific conditions in which they experience occupational stress. Findings of various studies have demonstrated that 55.5% of emergency nurses suffer from extreme occupational stress [1]. The environment is known to be stressful when working in an Intensive Care Unit (ICU). For all ICU healthcare workers, more than the physical work, it is the psychological factor of dealing with the seriously ill patients, which leads to huge mental stress [2] [3]. Occupational stress is an emotional and physical condition that can have damaging effects, and often results from an inability to meet the needs, resources and capabilities that are required of an employee [4]. In recent decades, the effect of occupational stress has become an important global issue in health care and management, with stress providing a major challenge for health institutions [5] [6] [7]. A workplace issue in a number of countries has been identified that nursing is an occupation that has been observed to have high level of stress [6] [8] [9]. For example: According to a study in Iran: between 69% - 75% of nurses have experienced stress [6] [10] with 33% having experienced high levels of stress [11]. Occupational stress has a number of negative consequences including burnout which has long-lasting effects on the individual with impacts on workplace performance and culture [12]. Other impacts of stress include but are not limited to intention to leave the workplace, reduced quality life [11], lower job satisfaction [13], and impaired job performance [14]. Several factors have been identified as being associated with occupational stress in nursing [4]. All factors that make nursing a particularly stressful profession are usually difficult working conditions, long working hours, and shift work [15]. Emergency nurses experience more stress than nurses working in other units in hospitals [16]. Similarly, other studies have found that large international studies have shown that emergency nurses are particularly vulnerable to occupational stress [16]. Nurses working in emergency departments experience exposure to traumatizing incidents such as mutilation, aggression and extreme suffering of patients [16]. The aim of this study was to assess the level of stress at nurses to working in the Emergency department and to compare this with the level of stress at nurses working in the Intensive Care Unit.

2. Methodology

2.1. Study Design

A cross-sectional survey design was used to identify the associations between the study variables.

2.2. Identification of Setting and Sample

The setting for this study was University Clinical Center of Kosovo, respectively Emergency Clinic and Central Intensive Care in Pristina of the Republic of Kosovo. A total of 90 respondents, 45 nurses working at the Emergency Clinic and 45 nurses at Central Intensive Care, participated in this study. The Emergency Clinic has 19 beds and serves as the main emergency center in the country. The average number of patients treated per year is about 70,000. Central Intensive Care has a total of 24 beds, serving within the UCKK. About 600 patients are treated at this center within a year.

2.3. Data Collection

The data were collected between March 2019 and June 2019.

The inclusion criteria were: 1) emergency department nurses with at least one-year experience, and 2) nurses providing direct patient care and 3) Central intensive care nurses with at least one-year experience, and 4) nurses providing direct patient care.

The exclusion criteria were: 1) nurses with less than 1-year experience, 2) nurses working in emergency department management 3) nurse educators, 4) traveler nurses, and 5) registry nurses.

2.4. Instrument

The Emergency Nurses Stress Questionnaire was the first tool used in the study. This questionnaire was modified and adopted from the Operational Police Stress Questionnaire [16]. The tool was designed to look at the stress and health dimension for those who work in high stress occupations. The Emergency Nurse Stress Questionnaire consists of 12-item self-report instrument designed to measure stress in an emergency room nursing. Responses are rated on a 7-point Likert scale from 1 - No stress at all to 7 - A lot of stress.

2.5. Ethical Considerations

This research project was approved and applied taking in consideration the Ethical standards of the Ethical Committee within the Centre Clinic of the University Prishtina, Kosovo. Questionnaires were completed anonymously to ensure participants' confidentiality.

3. Results

The demographic characteristics of the sample are summarized in Table 1. The sample consisted of 90 participants, of which 71.11% were women. Nurses' co-

hort-age ranged from 20 to 62 years. The largest proportion of respondents (44.4%) were between 20 - 30 age group with 22.2%, 31 - 40 age group consisted of 16.6%, while in the cohort-age group 41 - 50 and the smallest group was the >50 years age group with 7.7%. The majority were married (64.4%). Regarding their level of education, majority of them were with Bachelor degree level (66.6%) the others reported only secondary school level (33.3%). 50% of nurses worked in Emergency Center Nurses and 50% in Central Intensive Care Nurses. According the stress levels, further classified into low, moderate, and high by taking mean \pm 1 SD as per the suggestion from the author who developed the questionnaire (Table 2).

Table 1. Characteristics of participants (N = 90).

Variable	Number (%)
Gender	
Male	26 (28.88)
Female	64 (71.11)
Age (years)	
20 - 30	40 (44.4)
31 - 40	28 (22.2)
41 - 50	15 (16.6)
>50 years old	7 (7.7)
Marital status	
Married	58 (64.4)
Unmarried/divorced/widowed	25 (35.5)
Level of education	
Secondary school	30 (33.3)
Bachelor degree	60 (66.6)
Work-place	
Emergency Center Nurses	45 (50)
Central Intensive Care Nurses	45 (50)

Table 2. Operational and organisational stress scores.

Average stress rate	
Mean (SD)	78.2
Median	80.7
Mode	76
Minimum	22
Maximum	135
Stress classification,	n (%)
Low	11 (12.22)
Moderate	47 (52.22)
High	32 (35.55)

The causes of operational stress, and their frequency among respondents are given in Table 3 and Table 4. Questionnaire referenced [16] asked questions about operational causes of stress level there were 12 operational based questions in total, and these 12 questions were analyzed in two sectors: Emergency Clinic and Central Intensive Care. The study found that moderate to high stress level of stress was present in both groups of nurses working in Emergency Clinic and Central Intensive Care. Respondents' reported moderate to high levels of stress in 81% of participants. There was no significant difference in the degree of stress between the two groups of participants. There were also no significant differences in their demographic characteristics. For more detailed data, a reference could be found at [Table 3 and Table 4].

Significant differences were found in working hours, where nurses working 12-hour shifts reported higher level stress of stress compared to nurses working only 8 hours per day ($P < 0.002$). Participants also reported that there was a significant difference in fatigue between 12 hours shift nurses compared to 8-day nurses ($P < 0.001$). These data and presented in Table 5.

4. Discussion

The study aimed at assessing the level of stress at nurses who work in the Emergency Clinic and those in the Central Intensive Care. It applied the Emergency Nurse Stress Questionnaire as an instrument to gather the data among 90 nurses, 45 nurses working in Emergency Clinic and 45 nurses working in Central Intensive Care. The distribution of both clinics for the sample was practically equal, enabling thus the adequate comparisons of the level of the stress among the

Table 3. Causes of operational stress with percentage of subjects reporting them.

No.	Emotional stressors at Emergency Centre	Percentages of respondents who reported stress (moderate stress and above)
1	Not enough time available to spend with friends and family	88
2	Fatigue	86
3	Traumatic events	85
4	The risk of being injured on the job	84
5	Occupation related health issues	81
6	Shift in work	74
7	Finding time to stay in good physical condition	71
8	Feeling like always on the job	69
9	Making friends outside the job	59
10	Limitations to social life	56
11	Friends/family feel the effects of the stigma associated with job	54
12	Managing social life outside of work	49

Table 4. Causes of operational stress with percentage of subjects reporting them.

No.	Emotional stressors at Central Intensive Care	Percentages of respondents who reported stress (moderate stress and above)
1	Not enough time available to spend with friends and family	74
2	Fatigue	85
3	Traumatic events	69
4	The risk of being injured on the job	59
5	Occupation related health issues	81
6	Shift in work	84
7	Finding time to stay in good physical condition	71
8	Feeling like always on the job	56
9	Making friends outside the job	54
10	Limitations to social life	86
11	Friends/family feel the effects of the stigma associated with job	49
12	Managing social life outside of work	88

Table 5. Level of stress measurement data between nurses according to shift switch in work and the fatigue.

Variable	Shift in work	Fatigue
	(n = 90) Mean \pm SD	(n = 90) Mean \pm SD
8 hours	40 \pm 22.9	41 \pm 23.6
12 hours	60.9 \pm 11.6	61 \pm 10.5
P value	P < 0.002	P < 0.001

sample. The Emergency Clinic and Central Intensive Care are both challenging work environments as evidenced by high quantitative demands, traumatic events, stress, fatigue, burnout and occupation related health issues, work shift and work pace, and the feeling like being always in charge the job. Results of the present study indicate moderate to high stress levels in both groups of nurses, those working in Emergency Clinic and Central Intensive Care. With regard to the stress level, significant differences were found in the working hours. Hence, from the results, it can be stressed that nurses working in a 12-hours' shifts have reported higher levels of stress compared to nurses working only 8 hours shifts per day. Furthermore, the results have also shown significant difference regarding the work fatigue between nurses who work 12 hours shift compared to those who work 8 hours shift per day. Hence, nurses working on a shift of 12-hour have reported higher level of stress (60.9 \pm 11.6) compared to nurses working only on a 8 hours shift per day (40 \pm 22.9) with P = 0.002. In addition, results indicated that there was a significant difference in work fatigue between 12 hours shift nurses (61 \pm 10.5) compared to 8 hours working shift of nurses (41 \pm

23.6) with $P < 0.000$. Similarly, other studies have found matching results in terms of work-related fatigue, where (77.1%) of respondents have reported adequate recovery between shifts, although prevalence of chronic and persistent fatigue was considerably high (30.3%) [17]. In addition, results from different studies indicate that higher fatigue had negatively affected physical and mental work performances and it has also impacted an increase of medical errors and workers' injuries [18]. Therefore, proper management strategies, duty shifts to minimize work-related fatigue, are among important measures to improve many health problems suffered by nurses [19]. In addition, proper management parameters regarding to duty shift would have an impact in minimizing work-related fatigue in order to improve many health issues encountered at nurses exposed to the high levels of stress [10]. There are also findings which indicate the emotional costs of caring for people who encounter distress, traumatic states, by emphasizing hence the relation between fatigue and job stress, especially in cases of chronic stress as a potential case for burnout [20] [21] [22] [23]. Stathopoulou *et al.* in 2010 [24], have found that female emergency nurses in Greece have reported higher scores of anxiety and higher levels of emotional exhaustion compared to other nurses. In addition, the study indicates that 1/4th of the nurses', exhibit "very severe" depressive mood and sleeping disorders.

Emergency Clinic and Central Intensive Care are known as environments that could be stressful for the health professionals who work there. This was confirmed by the results of the present study. Similarly, findings from other studies have reported that the ED staff ($n = 103$) had experienced stress at work "frequently" or "very frequently", while others (37%) indicated that they've experienced it occasionally. Findings from these studies concluded that 97% of emergency department staff have experienced higher levels of stress and these findings indicated that the emergency care context is stressful [25].

The need to expand the sample, including the older cohort age of nurses working in these Institutions in order to be able to generalize the findings to the entire population of the sample and adequately indicate to the institutional and managerial policies are implied as limitation of the study. Also, in future, in order to accurately measure not just the level of the stress but also other related variables such as: emotional costs, work fatigue, etc, the questionnaire needs to be revised and adopted only for this category of the sample, enabling to specifically measure variables related to the healthcare professionals scope of the work.

5. Conclusions

Workplace stress is a significant problem in healthcare, especially in nursing. Work fatigue, work shift, traumatic events may cause physical and psychological disorders and may indicate towards negative feelings and related to the feelings of being always in charge or on the job, not enough time available to spend with friends, family and as an indicator of minimizing the social interactions and social life.

From the results of the present study related to stress level of nurses working in Emergency Clinic and Central Intensive Care, it can be concluded that:

- The study indicates that moderate to high stress levels were found in both groups of nurses, those working in Emergency Clinic and Central Intensive Care;
- Regarding stress level, significant difference was found in working hours, where nurses working 12-hours' shifts reported higher levels of stress compared to nurses working only 8 hours shifts per day;
- Nurses also reported that there was a significant difference in work fatigue between 12 hours shift compared to 8 hours shift per day.

In conclusion, the initiation of adequate institutional and managerial strategies was recommended as an important means to reorganize the work in a way to alleviate the physical and psychological stressors of the emergency clinic and central intensive care.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Annex 1

Emergency Nurse Stress Questionnaire

Below is a list of items that describe different aspects of being an emergency nurse. After each item, please circle how much stress it has caused you over the past 6 months, using a 7-point scale (see below) that ranges from “No stress at all” to “A Lot of stress”

	No stress At All			Moderate Stress			A lot of Stress			
	1	2	3	4	5	6	7			
1. Shift Work (does the shift you work because you stress?)				1	2	3	4	5	6	7
2. Risk of being injured on the job										
3. Traumatic events of patients (eg. MVA, death, injury)										
4. Managing your social life outside of work										
5. Not enough time available to spend with friends and family										
6. Finding time to stay in good physical Condition										
7. Fatigue (e.g. shift you work, overtime)										
8. Occupation related health issues (e.g. Back pain)										
9. Lack of understanding from family and friends about your work										
10. Making friends outside the job										
11. Limitations to your social life										
12. Feeling like you are always on the job										

Annex 2

Development of the Police Stress Questionnaires

Researchers:

- 1) Dr. Donald R. McCreary (Principal Investigator)
- 2) Dr. Megan M. Thompson (Co-investigator)
- 3) Wendy Sullivan, MA (Research Assistant)

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- 1) Workplace Safety and Insurance Board of Ontario, Research Advisory Council

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- 1) Ontario Provincial Police Academy
- 2) Ontario Provincial Police Association
- 3) Ontario Police College
- 4) Defence R & D Canada-Toronto

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McCreary, D.R., & Thompson, M.M. (2006). Development of two reliable and valid measures of stressors in policing: The Operational and Organizational Police Stress Questionnaires. *International Journal of Stress Management*, 13, 494-518.

Summary:

The relationship between stress and health (i.e., both physical health and psychological well-being) has received much attention over the years, with researchers demonstrating a consistent association between the two; that is, the more stress people experience, the poorer their physical and mental health. People with higher stress levels report significantly lower overall health and well-being, report the presence of significantly more adverse health symptoms (e.g., increased blood pressure, sleep disturbances), are at greater risk for long-term health problems (e.g., hypertension, coronary artery disease, auto-immune disorders, diabetes), are at greater risk for premature mortality, are more likely to experience symptoms of depression, generalized anxiety, post-traumatic stress disorder, and other psychological ailments (e.g., substance abuse), and they utilize significantly more health care resources (e.g., physicians, hospitals, sick days).

Occupational stress also has a negative effect on employers, something which many people (including the employers themselves) often overlook. Direct costs to employers include reduced productivity, as well as increased absenteeism and employee turnover as a result of issues such as stress-related illness, burnout and low levels of job satisfaction (e.g., Spielberger, Reheiser, Reheiser, & Vagg, 2000). Other costs to employers include health insurance payments to individuals and their families for workplace-related psychological disabilities. A recent study by Sauter (1992) revealed that occupational health insurance payouts total more than five billion dollars annually in the US alone. While these costs tend to be borne by the insurers, as opposed to the employers, they are passed onto the employers and employees through higher insurance premiums.

The association between stress and health is particularly worrisome for those who work in high stress occupations. One of the most highly stressful occupations in North America is policing (e.g., Pendleton, Stotland, Spiers, & Kirsch, 1989). But what are the aspects of policing that are most stressful and what impact do these stressors have on the health and well-being of police officers? This is a complex question, and one that has not been adequately addressed by researchers. While many studies have sought to identify the stressors associated with policing, few have actually tried to link those stressors to officer health and quantify the association.

One reason for this is that there is no commonly used measure of police stress. Thus, the purpose of this research is to develop a short, psychometrically sound measure of the stressors associated with policing, which will then be used in a future program of research investigating the associations among stress, physical health, and psychological well-being.

A three-phase development procedure was followed:

1) Focus Groups: A series of six focus groups were conducted with 55 experienced, active duty officers from the Ontario Provincial Police (OPP). The focus groups helped us identify current and commonly experienced stressors associated with policing. Based on these, we determined that there were two general categories of stressors faced by police officers: Operational Stress and Organizational stress. It was decided to use the most commonly mentioned stressors from the focus groups to create two separate police stress questionnaires: the Operational Police Stress Questionnaire (PSQ-Op) and the Organizational Police Stress Questionnaire (PSQ-Org).

2) Phase 1 (Pilot-testing): The PSQ-Op and the PSQ-Org were given to a group of 47 OPP officers to determine whether there were any problems with the wording of the items or instructions. Participants rated each item for both stress and frequency. In addition, the phase 1 pilot-testing served as an initial assessment of the PSQ-Op's and PSQ-Org's reliability. Based on the responses, the wording of three items was altered slightly, as were the instructions. One item from the PSQ-Org was split into two separate questions. Initial psychometric analyses showed that both the PSQ-Op and PSQ-Org had excellent internal consistency (Cronbach alphas > 0.90) and corrected item-total correlations between 0.30 and 0.60. Finally, stress ratings for the PSQ-Op and PSQ-Org were correlated with their respective frequency ratings ($r = 0.70$).

3) Phase II (Reliability and Validity): This was conducted in two parts. In the first part, 197 active duty police officers from throughout Ontario completed the PSQ-Op (20 items), the PSQ-Org (20 items), the Perceived Stress Scale (Cohen et al., 1983), a short version of the Daily Hassles scale (McCreary & Sadava, 1998), and a measure of negative life events (McCreary & Sadava, 1998). Findings demonstrated that both the PSQ-Op and PSQ-Org were highly reliable (alphas > 0.90 ; corrected item-total correlations between 0.40 and 0.60) and both were positively correlated ($r = 0.50$ or less) with the other general stress measures. In the second part, a different group of 188 police officers (mostly from Ontario, but with some officers coming from other Canadian provinces) completed the PSQ-Op, the PSQ-Org, the Job Satisfaction Survey (JSS; Spector, 1997), and the Job-related Affective Well-being Scale (JAWS; Van Katwyn et al., 2000). The results again showed that the two PSQ scales were highly reliable (alphas > 0.90 ; corrected item-total correlations between 0.40 and 0.60). In addition, the PSQ-Op and PSQ-Org scores were negatively correlated with self-ratings from the JSS (-0.19 to -0.56) and the positive work-related emotions subscale from the JAWS (-0.20 to -0.25), but were positively correlated with scores from the negative work-related emotions subscale from the JAWS (0.27 to 0.34).

UPDATE: As of the current update of this page, the Operational Police Stress Questionnaire (PSQ-Op) and the Organizational Police Stress Questionnaire (PSQ-Org) are available for use by researchers interested in exploring police stress. The PSQ-Op and PSQ-Org are both 20-items each and can be used

either separately or together. The short length of each PSQ helps to reduce the burden placed on officers completing them and allows researchers greater flexibility (in terms of focussing on either operational or organizational stress, if they so desire). Each PSQ is scored by summing or averaging the 20 items from each to create separate PSQ-OP and PSQ-Org scale scores.

The PSQ-Op and PSQ-Org have been adopted by researchers world-wide. They also have been translated into numerous other languages.

The PSQ-Op and PSQ-Org can be downloaded from this web page in PDF format or you can contact Dr. Don McCreary (NOTE: this e-mail link takes you to Don's work e-mail address) for either an electronic copy (in MS Word) or a hard copy version. Please note that the PSQ-OP and PSQ-Org can be used freely for academic research, as well as by police departments. For all other purposes, contact Dr. McCreary.

When citing or referencing the PSQ scales in your publications or presentations, please use the reference citation at the top of this page.



ORIGINAL ARTICLE

Relationship Between Lead (Pb) Concentration in Soil, Grass , Blood, Milk and δ -aminolevulinic Acid Dehydratase (ALAD) Activity , Hemoglobin (Hb) and Hematocrit (Hct) in Grazing Cows from Vicinity of Smelter “Trepça” in Kosovo

Igballe Krasniqi-Cakaj¹, Isa R. Elezaj^{*1}, Qerim I. Selimi², Muhamet Zogaj³, Kasum Rr. Letaj¹

¹Department of Biology, Faculty of Mathematical and Natural Sciences, University of Prishtina “Hasan Prishtina”, 10000 Prishtina, Republic of Kosovo

²Faculty of Education, University of Mitrovica “Isa Boletini”, 40000 Mitrovica, Republic of Kosovo

³Faculty of Agriculture and Veterinary, University of Prishtina “Hasan Prishtina”, 10000 Prishtina, Republic of Kosovo

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KEYWORDS

Cows;
Lead;
ALAD;
Blood;
Milk

ABSTRACT: This study aimed to evaluate current lead concentration (Pb) in topsoil, grass, blood and milk, ALAD activity, hemoglobin (Hb) and hematocrit (Hct) in cows (Simmental breed), rearing in three villages situated in different distances from the smelter “Trepça”: Kalemend 2km, Boletin 3km and Zazhë 5km and Koliq 40km. The Pb concentration in samples is measured by atomic absorption spectrometry (AAS), blood ALAD activity is measured according to the CEC standardized method. Pb concentration in topsoil and grass in the three villages from smelter is higher ($P < 0.001$) than Pb concentration in reference. Blood lead level (BLL) in cows from smelter area is higher ($P < 0.001$) than in control. Milk lead level (MLL) in cows from smelter area is higher compared with control. Blood ALAD activity in cows from smelter area is significantly ($P < 0.001$) inhibited compared with control. There is an adverse correlation ($r = -0.812$, $P < 0.001$) between BLL and ALAD activity in cows from Kalemend. A positive correlation ($r = 0.987$, $P < 0.001$) is established between BLL and MLL in cows from Boletin. There is no significant difference of Hb and Hct values among each group of cows. There was a progressive decrease of Pb concentration in topsoil, grass, blood and milk with increasing distance from the smelter. ALAD activity is proved as sensitive and useful biomarker at very low BLLs in cows. The vicinity of smelter still poses a threat for livestock welfare and human health.

INTRODUCTION

Smelting of metal ores cause ambient contamination with different metals [1]. Metals emitted by smelters as ultrafine particle fractions may travel in greater distance before

situated close to the Mitrovicë. The smelter had been in operation intermittently more than six decades. During this period of time a huge amounts of metals were released in town Mitrovicë and surrounding rural area [3]. An environmental audit ordered by the Kosovo authorities, warned that smelter should be closed as an “un-acceptable” source of air contamination with metals especially by lead.

The smelter “Trepça” was closed in 2000 year [4]. Several studies reported high levels of heavy metals concentration in agricultural soils which are located in mining and smelting area in the Mitrovicë region [4, 5, 6 and 7]. The results of studies showed high concentration of heavy metals in agricultural crops and vegetables from Mitrovicë region [8, 9 and 10].

Toxic metals and trace elements are studied in cow, goat, sheep and buffalo milks from different regions such as Spain [11], Iran [12 and 13], India [14 and 15], Turkey

[16], Croatia [17 and 18], Poland [19], China and Japan [20], Argentine [21]. Earlier study - 1968 [22] showed that milk of sheep from vicinity of smelter “Trepça” contained, on average, about 132 γ per 100 g lead (Pb), a much larger quantity than in other districts. Lambs showed paralysis of extremities and tongue, temporary diarrhea, anemia and general weakness. In cattle no marked lead poisoning were observed, although their milk in this area showed about the same concentrations as that in sheep [22]

Blood lead level (BLL) and blood ALAD activity is considered valuable biomarkers for evaluation of Pb toxicity [23].

Lead is a multi-targeted toxicant, with hematotoxicity a primary effect, specifically inhibition of heme synthesis. ALAD is an enzyme a primary of heme synthesis pathway, whose inhibition in humans and animals has served as an important, and sensitive, biological marker for Pb exposure and injury [23]. BLL and ALAD activity are studied in cows environmentally exposed to lead [24, 25, 26, 27, 28 and 29].

sedimentation in the soil [2]. The residual metals in the soil may enter in the food chain. Mitrovicë is the second largest town in Kosovo. Lead and Zinc smelter “Trepça” is

Grazing dairy cows would ingest daily less than 250 g of dry soil under grazing conditions. The majority of Pb consumption through most of the year at moderately and highly contaminated environments with lead relatively higher amounts of soluble soil-Pb can be ingested at rates exceeding safety threshold limits [30].

In Kosovo, cattle are the most numerous of the different kinds of livestock and are fed particularly on grass and locally grown fodder. The aim of this study was to analyze lead concentrations in soil, grass, blood and milk, ALAD activity, Hb and Hct values in grazing cows from vicinity of smelter “Trepça” in Kosovo.

MATERIAL AND METHODS

Study area

Mitrovicë is the second largest town in Kosovo. Lead and Zinc smelter “Trepça” is situated close to the Mitrovicë. The smelter had been in operation intermittently more than six decades. During this period of time a huge amounts of metals were released in town Mitrovicë and surrounding rural area [3]. An environmental audit ordered by the Kosovo authorities, warned that smelter should be closed as an “un-acceptable” source of air contamination with metals especially by lead. The smelter “Trepça” was closed in 2000 year [4].

Sample collection and analysis

This study was conducted during May-June 2018. In total, 59 topsoil, 62 grass samples were collected in land and pastures, 103 blood and 68 milk samples were collected in cows (Simmental breed) from smelter area within a radius of 2-3-5 km from smelter “Trepça”: Kelemd village – 2 km (10 cows), Boletin village - 3 km (19 cows), Zazhë village – 5 km (46 cows) and Koliq village control - 40 km from smelter - 29 cows (Figure 1). All cows reared in the local pastures, they

were also given locally produced hay. Topsoil samples from the pastures of villages (Kelmend, Boletin, Zhahzë and Koliq) were collected according to European guidelines. After surface litter (0 horizons) was removed, top soil samples were collected from each pasture. The weight of soil sample was 1 kg. Soil samples were air-dried in laboratory for two weeks. Air-dried soil samples then gently disaggregated, cleaned of extraneous material and sieved through a nylon sieve of 2 mm. Sieved samples of soil were homogenized and a quarter was milled in an agate mill to an analytical grain size of <0.125 mm. Grass were collected in pastures, dried at 80°C for 48 h and ground to a fine powder.

Soil samples were mineralized with a microwave acid digestion apparatus -Milestone Ethos with internal temperature sensor, 640-260 terminal with easy CONTROL software installed and HPR1000/105 high pressure segmented rotor. Soil samples were solubilized by acid digestion adding in Teflon bombs 3 ml HNO₃ 65%, 9 ml of HCl 37 % to 500 mg of powdered samples. Solutions were collected in flasks and adjusted to 25 ml with distilled water.

Grass samples were mineralized with a microwave acid digestion apparatus - Milestone Ethos. Grass samples were solubilized by acid digestion adding in Teflon bombs 6 ml HNO₃ 65%, 2 ml of H₂O₂ 30 % to 500 mg powder samples. Solutions were collected in flasks and adjusted to 25 ml with distilled water.

Milk samples were collected grazing cows between 7.00 to 9.00 a.m. in clean polyethylene bottles. Each milk sample was stored at - 20 °C until analysis. Milk samples were mineralized with a microwave acid digestion apparatus - Milestone Ethos. Samples were solubilized by acid digestion adding in Teflon bombs: 6 ml HNO₃ 65%, 2 ml of H₂O₂ 30 % to 500 mg powder samples. Solutions were collected in flasks and adjusted to 25 ml with distilled water. Solutions were collected in flasks and adjusted to 25 ml with distilled water. A 5.0 ml of blood samples were obtained by jugular vein puncture from all cows by a veterinary trained phlebotomist. The blood was stored in BD vacutainer (LH 170 I.U; UK.). Blood samples during transportation were stored at 4°C. The total content of Pb in soil, grass, milk and

blood samples was analyzed by graphic furnace atomic absorption spectrometry GF-AAS (Varian spectra AA 640Z Zeeman AAS, equipped with a GTA 100 graphite furnace – Varian USA and PSD 100 auto-sampler –Varian, USA).

Blood ALAD activity was measured according the European standardized method [31], Hematocrit values (Hct) were determined in the heparinized capillary tubes, centrifuged for 8 minutes at 10.000 rpm (Heamofuge Heraues). Blood hemoglobin (Hgb) concentrations were measured by standard cyan-methemoglobin method.

km) from former smelter “Trepça” are significantly higher

($P < 0.001$) compared with (184.9 ± 114 ; 75 ± 15 ; 83 ± 45 ; 40 ± 16 mg kg⁻¹, respectively) their concentrations in a topsoil from control locality-Koliq. There was also found significant concentration of lead in the topsoil of

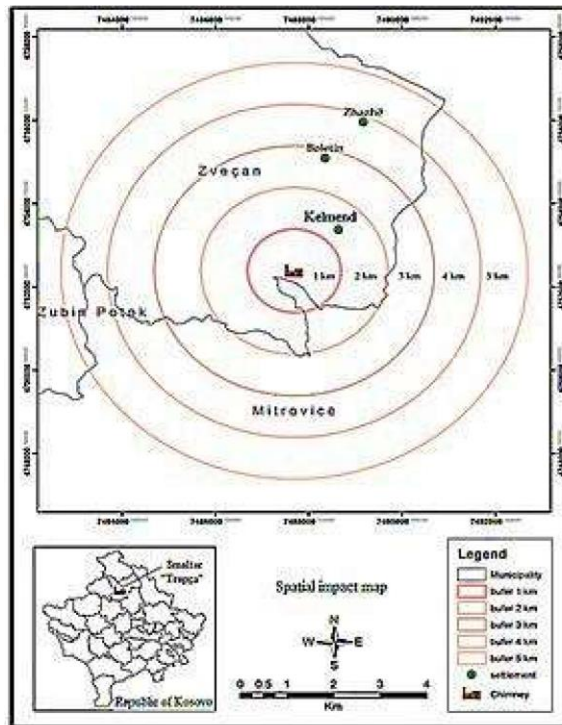


Figure 1. Map of the spatial impact in the study area: Kelmend, Boletin and Zhazhë (smelter area).

RESULTS AND DISCUSSION

The results of lead (P) concentration in topsoil, grass from three different contaminated localities (Kelmend, Boletin and Zhazhë), and control locality, Koliq village, blood lead level (BLL), milk lead levels (MLL), ALAD activity, Hb and Hct contents in grazing cows from these localities are presented in the Table 1 and Figure 2). The results of correlations between Pb concentration in the soil, grass, blood and milk and ALAD activity are presented in Table

2.

The results (Table 1) show that Pb concentrations in the topsoil (PbS) in three localities situated at different proximity (Kelmend 2 km, Bolerin 3 km and Zhazhë 5

Kelmend

(2 km) compared with lead in the topsoil's from Boletin ($P < 0.05$; 184.9 ± 114 ; 75 ± 15 mg kg⁻¹, respectively) and Zhazhë ($P < 0.001$; 184.9 ± 114 ; 83 ± 45.0 mg kg⁻¹, respectively). Lead concentration in grass (PbG) form Kelmed, Boletin and Zhazhë is higher ($P < 0.001$; $P < 0.006$; $P < 0.03$; 37 ± 10.7 ; 34 ± 8.3 ; 27.3 ± 14.07 ; 24.8 ± 5.2 mg kg⁻¹, respectively), compared with concentration in grass form control locality-Koliq. Blood lead level (BLL), in the grazing cows from Kelmend, Boletin and Zhazhë is significantly higher ($P < 0.001$; 0.0403 ± 0.0277 ; 0.0268 ± 0.0113 ; 0.0248 ± 0.0168 ; 0.0128 ± 0.0041 mg kg⁻¹, respectively). There is higher BLL in grazing cows form Kelmed compared with BLL in the grazing cows from

Zhazhë ($P < 0.02$; 0.0403 ± 0.0277 ; 0.0268 ± 0.0113 ; 0.0248 ± 0.0168 mg kg⁻¹, respectively).

Table 1. Mean value of lead concentration (Pb mg kg⁻¹) in soil, grass, blood, milk and δ -aminolevulinic acid dehydratase (ALAD) activity, hemoglobin (Hb) and hematocrit (Hct) in cows (Simmental breed) from vicinity of former smelter “Trepça” in Kosovo.

Parameters	Distance from smelter			Control
	2 km (Kelmend)	3 km (Boletin)	5 km (Zhazhë)	40 km (Koliq)
Soil: Pb mg kg⁻¹	184±114 ***	75±15 ***	83±45 ***	40±16
Grass: Pb mg kg⁻¹	37.0±10 (15)	34±8.3 (5)	27±14 (22)	24±5.0 (20)
Blood: Pb mg kg⁻¹	0.040±0.027 (10)	0.0268±0.011 (19)	0.0248±0.016 (46)	0.0128±0.0041 (28)
Milk: Pb mg kg⁻¹	0.045±0.033 (5)	0.052±0.032 (36)	0.054±0.058 (20)	0.033±0.016 (7)
ALAD: U/LE	3.7±1.3 (11)	4.6±1.6 (19)	7.3±2.9 (46)	6.0±2.9 (29)
Hb: g/l	104±5.0 (11)	104±9.0 (19)	104±22 (46)	99±25 (29)
Hct %	30.7±5.0 (11)	30.4±8.0 (19)	31.6±4.0 (46)	30.7±6.0 (29)

Note: The results are presented as Mean - X: Standard deviation - Sd±: * P<0.05; ** P<0.01; *** P<0.001. Number of samples in parenthesis.

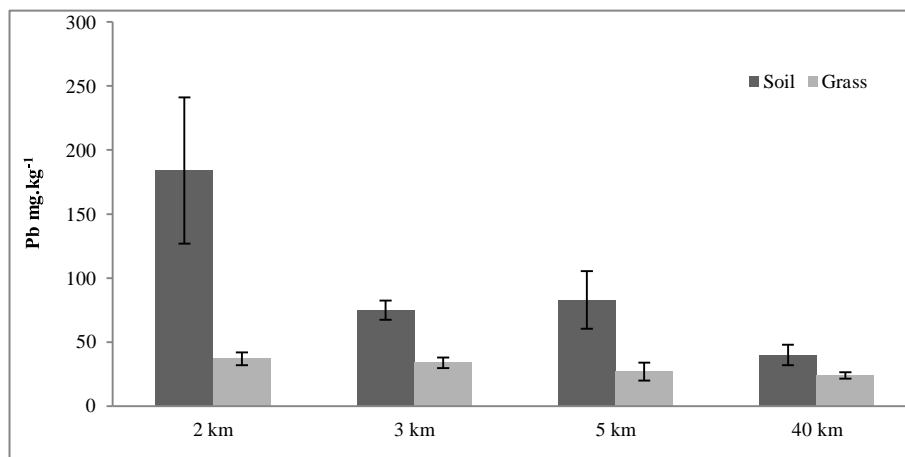


Figure 2. Lead (Pb) concentration (mg/kg) in the soil and grass from smelter Trepca – Mitrovicë

Table 2. Spearman correlation (r) between Pb soil-Pb grass, Pb soil-Pb blood, Pb grass-Pb blood, Pb grass-Pb milk and Pb-blood –milk concentrations (mg kg⁻¹) in three localities from vicinity of former smelter “Trepça” in Kosovo.

Area	Pb: Soil/Grass	Pb: Soil/Blood	Pb: Grass/Blood	Pb: Grass/Milk	Pb: Blood/Milk
Kelmend (2 km)	-0.315	0.675	-0.265	-0.177	0.099
Boletin (3 km)	-0.353	0.716	-0.303	-0.025	0.987***
Zhazhë (5 km)	0.159	0.171	0.319	0.319	-0.031
Koliq (40 km)	-0.153	-0.132	--	0.008	-0.439

*P<0.05, ***P<0.001

MLL in grazing cows from three localities from smelter contaminated area: Kelemnd, Boletin and Zhazhë although is higher compared with MLL in the grazing cows from control locality-Koliq (0.045±0.33; 0.052±0.32; 0.054±0.58; 0.033±0.16 mg kg⁻¹, respectively), a statistically significant higher MLL was found (P<0.05) only between MLL in a grazing cows from Boletin and grazing cows from control locality-Koliq. The results of ALAD activity in the blood of grazing cows from Kelmend and Boletin is inhibited (P<0.001) in comparison of blood ALAD activity of grazing cows from Zhazhë (3.7±1.3; 4.6±1.6; 7.3±2.9, respectively). Blood ALAD activity in the cows from Kelemnd and Boletin is also significantly inhibited (P<0.01; 3.7±1.3; 4.6±1.6; 6.0±2.9 U/LE, respectively) compared with ALAD activity

in the blood of grazing cows from control area-Koliq. There is adverse correlation between BLL and ALAD activity (r=-0.812; P<0.004) in grazing cows from locality of Kelemnd situated 2 km from former smelter “Trepça”.

Significant positive correlation (P<0.001) was found between BLL and MLL in grazing cows with BLL above >0.026 mg L⁻¹ – Boletin village. The Hb content and hematocrit values did not show significant difference between grazing cows from different localities.

Our results of mean values of Pb concentrations in the topsoil from contaminated area: Kelmend, Boletin and Zhazhë (184.9±114; 75±15; 83±45 mg/kg, respectively), are several times higher compared with worldwide average of soil Pb concentration [32] and the upper limit of Pb concentration for unpolluted soils [33]. A study results undertaken in 2009 in soils of Mitrovicë region- northern Kosovo [3],

showed the Pb concentration mainly between 100 and 300 mg/kg, 5% below 100 mg/kg, and 10% above 2000 mg/kg. Studies in the soil from the narrower vicinity of Mitrovicë [4] showed 20-fold higher Pb concentration compared with the European median soil Pb concentration. The above authors note that concentrations of some metals (Pb, Cd, As, Hg, Zn and Cu) in the soil from towns of Mitrovicë and Zveçan, are higher than the intervention values, according to the New Dutch list [4].

Our results of decrease of Pb concentration in topsoil with proximity from smelter “Trepça” are in accordance with results of Nanoni [5], who also found decrease of Pb concentration in soil with proximity from smelter “Trepça”, Trepça battery factory and urban center of Mitrovicë.

Study results of heavy metal concentrations in agricultural soils in Kosovo [6] established that soil Pb concentrations vary from 15.5 to 2206.3 mg kg⁻¹, with mean 163.3 mg kg⁻¹.

Decrease of Pb concentration in topsoil with distance from the smelter “Trepça” area (Kelmend, Boletin and Zhazhë villages) are consistent with results of other authors [7], who reported a progressive decrease of mean Pb soil concentration in villages of Kelemend, Boletin and

Zhazhë in order: 1309.20 > 496.22 > 366.39 mg kg⁻¹. Higher Pb concentrations in the soils from the same villages [7] compared with Pb soil concentrations in our study can be as a result that we collected the topsoil samples from 0-5 cm while they collected up to 20 cm. Our results of Pb concentration in topsoil, grass and BLL in grazing cows within 1-5 km around smelter “Trepça” are consistent with results of Zadnik [25], who during long period of study (1975-2002) of Pb concentration in topsoil, forage and Pb concentration in the blood, liver and kidneys in cows from farms situated in the distance from 1-10 km from the lead mine and smelter (Meža River Valley) after lead filtration in 1978, found progressive decrease of Pb lead concentration. Results of this study showed that filters influenced on prominent decrease of lead concentration from 1975-2002 in the soil (192-1,558 to 347.5±300 mg kg⁻¹), forage (584.0±324.9; 5.6±3.3 mg kg⁻¹) and mean blood lead level (1.251±0.580 to 0.069±0.041 mg kg⁻¹) [36]. Our results

of decrease of Pb concentration in grass with from smelter area (Kelmend 37 mg kg⁻¹; Boletin 34 mg kg⁻¹ and Zhazhë 27 mg kg⁻¹) and Koliq (12.8) control area, are lower than Pb concentrations founded in crops from of different locations (Rudare: Lucerne 52.50 mg kg⁻¹, Hay 62.50 mg kg⁻¹ and Spinach 100 mg kg⁻¹ and in Shupkocv: Lucene 46.25, Hay 46.25 and Spinach 31.73) in the vicinity of “Trepça” plant in Mitrovicë [8].

On the other hand results of Pb concentration in leaves of onion plants grown (in laboratory) in soil samples with Pb concentrations 2516.54 mg/kg from Shupkocv and 2122.09 Mitrovicë town showed mean values of lead concentration 8.35 mg kg⁻¹ and 5.69 mg kg⁻¹ respectively [10].

Studies in cattle from lead-zinc smelter in Guizhou, China, showed Cd and Pb concentration in bovine kidney and liver slightly over Chinese standard [34]. The results of study [35] found that grazing cows can ingest 1% to nearly 18% of their dry matter as soil [35]. When grazing conditions are severe, dry soil intake in cows grazing in intensive rearing systems can increase up to 1kg/day and individual intakes can even exceptionally reach 1.3 kg/day [30].

Our results of progressive decrease of blood lead level (BLL) in grazing cows within 2-5 km around smelter

“Trepça” are consistent with the results established in the 25-year long systemic investigation (1975-2002) in the blood of cows from the vicinity smelter in Meza Valley – Slovenia [36]. The results of their study showed progressive decline of BLL in cows from mining and smelter area from 1975 – to 2002. The BLL in 2002 was 18.13 times lower compared with blood level in 1975 (BLL in 1975: 1.251±0.580 mg kg⁻¹; BLL in 2002: 0.069±0.041 mg kg⁻¹, respectively) [36].

A 16 year retrospective study (1998-2013) of acute Pb poisoning in Canadian cattle [37] showed that mean Pb concentrations in the blood, liver and kidney were several times higher (1.30±1.70 mg kg⁻¹ w.t., 33.5± 80.5 mg kg⁻¹ w.t., 55.5 ± 39.7 mg kg⁻¹ w.t. respectively), compared with mean “normal” Pb concentrations a liver and kidney (0.03±0.003 mg kg⁻¹, w.t. 0.16±0.63 mg kg⁻¹, w.t., and 0.41±0.62 mg kg⁻¹ w.t.) [37].

Results of study [38], found significantly higher Pb values in the blood and hair in cattle from lead-zinc smelter area in India compared with control (blood: 0.097 mg kg⁻¹, 0.07 mg kg⁻¹, respectively. hair: 156 mg kg⁻¹ and 3 mg kg⁻¹, respectively). In cattle from lead-zinc area the accumulation of Pb in the hair compared with Pb level in blood was 160.8 times higher, while in the control group of cattle it was 42.8 times higher. Results of herds clinically poisoned with Pb (39) proved that 7-40% of A previous study [39] of herds with clinical cases of Pb poisoning found that 7-40% of asymptomatic cattle had BLL $\leq 10 \mu\text{g dl}^{-1}$.

Our results of BLL and ALAD activity in the grazing cows from smelter "Trepča" are consistent with results of PrpicMajic [24], who in a 5 year study (1976, 1978, 1982, 1984, 1988) in cows smelter in Mezica Valley-Slovenia recorded progressive decrease of BLL from 1976 to 1980 (1976: 3.09 $\mu\text{mol/L}$, 1978: 1.44 $\mu\text{mol/L}$, 1982: 1.44 0.27

$\mu\text{mol L}^{-1}$, 1984: 0.75 $\mu\text{mol L}^{-1}$, and in 1988: 0.66 $\mu\text{mol L}^{-1}$, respectively), while blood ALAD activity was increased from 0.6 U/LE in 1976 to 1.5 U/LE 1982, 2.7 U/LE $\mu\text{mol/L}$ in 1984 and 3.4 U/LE in 1988, meanwhile the EP was progressively decrease: 1976: 14.55 $\mu\text{m L}^{-1}$, 1982: 3.47 $\mu\text{m L}^{-1}$, 1984: 3.48 $\mu\text{m L}^{-1}$ and in 1988: 2.66 $\mu\text{m L}^{-1}$

[24].

Study results of in the cows from the vicinity of lead and zinc plant showed [23], higher BLL (155 $\mu\text{g L}^{-1}$, 30 $\mu\text{g L}^{-1}$), inhibition of ALAD activity (2.4 U/LE, 6.4 U/LE respectively; $P < 0.01$), and lower Hb amount (99.8 g l⁻¹, 104.0 respectively; $P < 0.001$). The authors of this study showed that the relationship between ALAD activity and BLL, in cows is not linear but is exponential (log

ALAD/Pb). After them could be related to the fact that ALAD is more sensitive than BLL in reflecting the chronic Pb exposure and accumulation in bone as a

"critical organ" of lead exposure. On the other hand results of study in cow-calf herds found an exponential, rather than linear concentration of Pb in milk with respect to the BLLs in cows [39].

Results of study on the cattle and sheep from mining polluted area Spain, showed BLL 160 times higher in cattle (15.45 $\mu\text{g dl}^{-1}$;

0.1 $\mu\text{g dl}^{-1}$ respectively), in sheep's d 35 times higher compared with their respective controls (2.41 $\mu\text{g dl}^{-1}$; 0.06 $\mu\text{g dl}^{-1}$ respectively). The blood ALAD activity of cattle and sheep from mining area were significantly inhibited compared with ALAD activity in respective control groups (cattle ALAD: 7.95 nmol ml⁻¹ RBC; 3.280 nmol ml⁻¹ RBC, respectively) [26].

Our results of milk lead levels (MLL) in the cows from smelter contaminated area are consistent with results [12] recorded in the milk of livestock animals from different regions of Iran [12]. Analyses of lead level in the buffalo, cows from Township: Urmia. Khoy, Salmas, Naghadeh and Miandoab showed the highest MLL in buffalo

0.018 \pm 0.002, moderate in cow 0.008 \pm 0.01 mg kd⁻¹ and the lowers in ewe 0.002 \pm 0.001 mg kg⁻¹ [13], after them the concentration of lead in the milk do not exceed the safety limits [13].

Results of study of Pb contents in Chinese commercial and raw milks from China was several times higher than in Japanese commercial milks [20].

Results of study [15] of cows environmentally exposed to higher level of lead and cadmium around different industrial units in India, found the higher lead and cadmium in their milk. The authors of this study argue that increased blood and milk Pb or Cd level as a result of natural exposure of lactating cows significantly influences trace minerals compositions of milk [15].

The results of study of metals (Pb,As, Zn, Co and Fe) in raw milk from industrial, rural and a heavy traffic intensity region from Bursa, in Turkey [41], found highest metal content in the milk samples collected from industrial region, followed by traffic intensive region and rural region [41].

Results of cow's milk from fifteen farms near Knjž in Zagreb region – Croatia showed [42], Pb and Cd mean values (0.27 \pm 0.06 mg kg⁻¹ and 0.037 \pm 0.007 mg kg⁻¹ DM). The results of study in six individual cows accidentally exposed to lead showed that BLL ranged from 0.8822 to 1.216 mg l⁻¹ BLLs of these samples ranged (from six individual cows) from 0.8822 to 1.216 mg l⁻¹. The BLL of 22 samples collected within the first 90 days showed decrease of BLL $> 0.025 \text{ mg l}^{-1}$. On the first day of testing MLL was 0.0362 mg l⁻¹ while

12 days later MLL was 0.0406 mg l^{-1} [43]. The MLL of cows from smelter area is more than two times higher compared with the permissible concentration ($0.02 \text{ } \mu\text{g mL}^{-1}$) of the raw milk given by the standards of the European Commission Regulation [44].

CONCLUSIONS

Soil, grass, blood and milk lead content in the grazing cows from smelting area progressively decreased with the proximity of smelter “Trepça”. ALAD activity is proved as a sensitive and useful biomarker at a relatively low blood lead levels in cows. Milk lead levels in cows from smelter area are more than two times higher than given standards by the European Commission Regulation. The vicinity of smelter still poses threat for livestock welfare and human health

CONFLICT OF INTERESTS

The authors declare that there are no competing interests associated with the manuscript. Manuscript was self-funded by authors.

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Molecularly imprinted polymer modified glassy carbon electrodes for the electrochemical analysis of isoproturon in water

Imer Sadriu^{a,b}, Sarra Bouden^a, Jimmy Nicolle^a, Fetah I. Podvorica^b, Valérie Bertagna^a, Catherine Berho^c, Laurence Amalric^c, Christine Vautrin-UI^{a,*}

^a ICMN Interfaces, Confinement, Matériaux et Nanostructures, UMR7374 - Université d'Orléans-CNRS, 1b rue de la Férollerie, 45071, Orléans Cedex 2, France

^b Chemistry Department of Natural Sciences Faculty, University of Prishtina, rr. "Nëna Tereze" nr. 5, 10000, Prishtina, Kosovo

^c BRGM, Bureau de Recherches Géologiques et Minières, Laboratory Division, 3 avenue Claude Guillemin, 45060, Orléans Cedex 2, France

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ABSTRACT

Isoproturon-imprinted polypyrrole films were electrochemically synthesized onto glassy carbon (GC) electrodes in an ethanol/aqueous solution of pyrrole as a monomer, isoproturon as a template molecule and LiClO₄ as supporting electrolyte. Electropolymerization was performed by cyclic voltammetry and chronoamperometry. The isoproturon template molecules were successfully trapped in the polypyrrole film where they created artificial recognition cavities. After the electrochemical extraction of the template, the polypyrrole film acted as a molecularly imprinted polymer (MIP) for the selective recognition of isoproturon whereas the non-imprinted polymer (NIP) film, made in the same conditions except for the presence of isoproturon, did not exhibit any interaction. The MIP and NIP films were characterized by cyclic voltammetry in the presence of redox probes and the thickness of the polymer layers was estimated by EQCM (Electrochemical Quartz Crystal Microbalance) and calculated using Faraday's law. The isoproturon-imprinted polypyrrole films were found to selectively detect isoproturon even in the presence of the interferents carbendazim and carbamazepine. Its limit of detection (LOD) in milli Q water, achieved via square wave voltammetry was as low as 0.5 μg L⁻¹, whereas in real water samples it was found to be 2.2 μg L⁻¹.

1. Introduction

The use of many herbicides in agriculture, although beneficial for food production and prevention against nuisance organisms, can lead to their release into the environment where they have a negative impact on the ecosystem and human health. Furthermore their degradation products and metabolites are toxic toward living organisms. Systematic quality monitoring of groundwater and surface water showed that many aquifers have been contaminated by these pollutants. Isoproturon (3-(4-isopropylphenyl)-1,1-dimethylurea or 3-*p*-cumenyl-1,1-dimethylurea), a herbicide belonging to the phenyl urea family, is widely used to kill weeds in soils [1]. Recent studies indicated that isoproturon has become an ecosystem contaminant due to its intensive use [2] and in 2016, the EU decided to ban its use. Isoproturon has been detected worldwide in soil, groundwater, surface water, or even in drinking water, exceeding the threshold values (e.g., the European Union threshold is 0.1 μg L⁻¹). Raw waters may become contaminated with isoproturon from production plant discharges and diffuse agricultural sources [3].

According to the World Health Organization (WHO), isoproturon has demonstrated toxicity to the liver system and appears to be a tumor promoter [4]. Considering its hazardous effects, the European Union (EU) has classified it as a priority micro-pollutant and has regulated its maximum level at 0.3 μg L⁻¹ for inland surface waters [5]. It is therefore necessary to develop sensitive and selective techniques to monitor its presence in natural aquatic environments. Sophisticated analytical techniques based on chromatography are frequently used for isoproturon detection in environmental samples [3,6–10] with detection limits between 10 and 100 ng L⁻¹ reported [3,5]. However, these techniques, in spite of their high sensitivities, remain expensive and difficult to implement for on-site and *in-situ* analysis. In addition, on-site sampling and its treatment involves a longer analysis time and complex additional steps to overcome matrix effects and interferences [3,5,7]. There is an increasing need to ensure efficient and continuous on-site and *in-situ* monitoring of environmental waters, and to reduce the cost of analysis, which implies developing novel sensors.

Efforts are thus ongoing to develop rapid and inexpensive sensing devices for the detection of pesticides in general and particularly

* Corresponding author.

E-mail address: christine.vautrin-ul@cnrs-orleans.fr (C. Vautrin-UI).

isoproturon. Among all the conventional techniques, electrochemical analysis is one of the simplest and least expensive. Voltammetric-based methods have been widely reported for the determination of many pesticides and isoproturon more specifically using various carbon electrode materials such as glassy carbon [11,12], graphene [13], wall-jet glassy carbon electrode [14], carbon paste [15,16], carbon fiber [17], doped diamond [18], and multiwalled carbon nanotubes (MWCNT) [19]. More recently, numerous studies have focused on modified electrodes [20] and various assemblies have been investigated for applications in isoproturon electrochemical sensors such as clay-modified GC electrodes [21,22], or enzyme-modified [19,23–25] and conducting polymer modified electrodes [26]. The attachment of the organic film on the electrode surface makes it possible to increase the sensitivity and the selectivity of these modified electrodes toward a targeted analyte. Still, one of the most ingenious methods to achieve this goal is to prepare molecularly imprinted polymers [27–30].

A molecularly imprinted polymer (MIP) is obtained by polymerization of a monomer in the presence of a template molecule and a cross-linker agent. After polymerization, the template molecules, trapped in the polymeric matrix, are removed leaving behind cavities that are complementary not just in shape but also in functionalities. The cavities contain functional groups of the polymer that were left in a “frozen” conformation/orientation that leads to the specific uptake of the targeted analyte. High affinity and selectivity are based on the shape of the print together with the specific interactions between the functional groups from the MIPs and the target micro-pollutant [31–33].

For sensing issues, the selective recognition of the analyte by the MIPs can be achieved through concentration of the samples via: i) MIP Solid Phase Extraction (SPE) cartridges or membranes [34–36] or ii) coated electrodes by *ex-situ* prepared MIP or MIP films deposited *in-situ* at the electrode [37–39]. After this concentration step, the analysis can be achieved by several techniques (electrochemical, chromatographic, optical, etc.).

MIPs of different compositions and for different purposes can be prepared in several ways: photo-polymerization [40,41], free radical [42] and controlled radical polymerization [43,44], polymerization by evanescent waves [45] and *in-situ* electropolymerization. For the MIPs used in electrochemical sensing, those prepared by the latter method are more numerous because it is simple, fast and inexpensive. Moreover, electropolymerization allows a better thickness control and adhesion of the polymer layer on the surface of substrates and shows the ability to attach selective films of any shape and size compatible with commercial and industrial implementation [46]. Various types of electro-synthesized polymers based on molecular imprinting have been reported including polyphenol, polymethacrylic-acid [47], polyaniline [48–50] and polypyrrole [51–54]. Recently the use of some Metal Organic Frameworks (MOFs) based on the electropolymerization of monomer-functionalized gold nano-particles was reported [55].

Among the abovementioned polymers, polypyrrole films are most advantageous for environmental applications thanks to their easy *in-situ* electropolymerization at the electrode surface with or without the presence of the template molecule [55]. Moreover, the formation of the polypyrrole film is fast and it is well known to be a partially cross-linked polymer so that there is no need to add a cross-linker agent [55]. Extraction and rebinding of the template can be conducted electrochemically since polypyrrole is a conducting polymer, to some extent [55]. In addition, the electropolymerization of polypyrrole leads to chemically and mechanically robust films [56,57].

Therefore, thanks to all these properties, MIPs have inspired researchers in analytical chemistry to address the massive demand for monitoring molecules of interest in different fields such as biology [58–61], the environment [56,62], food science and technologies [63,64], pharmaceuticals and controlled drug release [65,66].

While the number of studies on the use of MIP-based sensors in pesticide analysis is increasing [67–71], only a few have focused on

isoproturon detection, which has been classified as a priority pollutant by the WFD [4].

Li et al. [71] developed a new molecularly imprinted poly-aminophenol electrochemical luminescence sensor (MIP-ECL sensor) for isoproturon determination based on the competition reaction between isoproturon and glucose oxidase [72]. However, even though the method has provided very low detection limits (0.8 ng L^{-1}), the use of additional reagents, the equipment required and the complex preparation of the samples remain incompatible with *in-situ* measurements.

Singh et al. (2013) developed an *ex-situ* polymethacrylic imprinted polymer solid phase extraction for isoproturon detection [73] and reached a detection limit of 0.2 mg/L which is unfortunately too high in light of current standards [4]. In addition, an *ex-situ* MIP synthesis involves additional steps, which complicates the implementation of the sensor.

In this context, the aim of the present work was to develop an electrochemically polypyrrole MIP based electrode for the sensitive and selective detection of isoproturon. The influence of the experimental parameters on the characteristics of the polymer matrix and its ability to recognize and to concentrate isoproturon were studied. In order to maximize isoproturon signal detection we adjusted several parameters during the MIP electrosynthesis. The analytical performances of the modified electrodes were then assessed.

2. Experimental

2.1. Reagents and apparatus

Pyrrole, lithium perchlorate, LiClO_4 , isoproturon (ISO), tetrabutylammonium tetrafluoroborate, acetonitrile, ferrocene, NBU_4BF_4 , ethanol, carbamazepine (Cbd), diuron, carbendazim (Cbmz) and sulfuric acid were purchased from Sigma-Aldrich. Electrochemical measurements were carried out using a potentiostat/galvanostat PGSTAT 204 and PGSTAT 128N Autolab Metrohm interfaced with a PC under Nova 2.2 software and three electrodes in a one-compartment cell. The working electrode was a glassy carbon (GC) disc (area of 0.07 cm^2). Ag/AgCl saturated with KCl was used as a reference electrode and a curved platinum wire was used as a counter electrode. The working electrodes were polished before each experiment by using decreasing sizes of diamond paste, rinsed and sonicated with Milli-Q water, dried and stored in a clean place. The EQCM measurements were performed with a Mextek RQCM micro-balance on Mextek 5 MHz Au-Cr crystals.

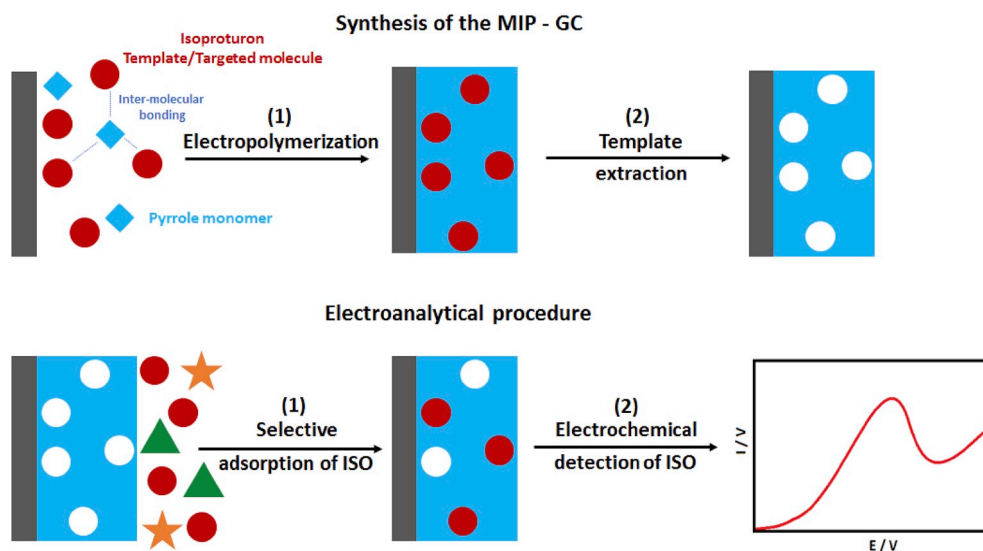
2.2. Synthesis of NIP-GC and MIP-GC electrodes

The electro-synthesis of molecularly imprinted polymer (MIP) films and that of non-imprinted polymer (NIP) films was performed on glassy carbon electrodes, respectively named MIP-GC and NIP-GC, in an ethanol and water (20/80) solution containing 10 mM of pyrrole and 0.1 M of LiClO_4 as electrolyte. For the preparation of MIP-GC, the template molecule, 1 mM of isoproturon, was added. Electropolymerization was accomplished either by applying cyclic voltammetry (CV), 5 scans in the potential range from 0 to 1.4 V vs Ag/AgCl at a scan rate: $v = 10 \text{ mV s}^{-1}$, or by chronoamperometry (CA), with a potential set at 1.1 V vs Ag/AgCl for a duration of 600 s .

The MIP-GC electrodes, once prepared, were rinsed with Milli-Q water and immersed in an ethanol/water (70:30 v/v) solution of 0.1 M sulfuric acid. They were biased to a potential excursion between -0.4 V and 1.5 V vs Ag/AgCl for several scans until complete extraction of the embedded isoproturon molecules. After extraction the MIP-GC electrode was used for isoproturon detection.

2.3. Detection of isoproturon by MIP-GC electrodes

The MIP free of isoproturon and NIP coated electrodes were immersed in an aqueous solution containing isoproturon as template



Scheme 1. Schematic representation of the procedure used for the preparation of imprinted polypyrrole films on GC substrate MIP GC and the electroanalysis of isoproturon.

molecule, under stirring, during an optimized time. Both electrodes were then rinsed with Milli-Q water in order to remove the isoproturon just deposited on the MIP-GC and NIP-GC surface. The electrochemical detection of isoproturon was achieved by square wave voltammetry (SWV), in an ethanol/water (70:30 v/v) solution containing 0.1 M of sulfuric acid. The preparation of the MIP-GC and the detection steps are presented on [Scheme 1](#).

3. Results and discussion

The electropolymerization of pyrrole in the presence of isoproturon leads to the inclusion of the template molecule in the polymer matrix during polymer growth. Isoproturon extraction by CV creates cavities within the MIP, which are complementary in shape and functionalities to the template molecule. These prints should therefore allow selective rebinding by isoproturon, as the artificial receptors are shaped by the template [55], and finally the use of SWV will enable to confirm the presence and to determine isoproturon concentration.

3.1. Electropolymerization of pyrrole

As mentioned in section 2.2, the electrochemical polymerization of pyrrole on the GC electrode surface was performed by cyclic voltammetry with and without the presence of the template isoproturon in the monomer solution, see [Fig. 1](#).

In both cases, we can observe the anodic current that increase after 0.75 V vs Ag/AgCl due to the oxidation of the pyrrole on GC electrode with the current peak at 0.95 and 1.15 V vs Ag/AgCl respectively in the solutions without and with 1 mM of the ISO template. The potential shift is probably due to the presence of isoproturon in the solution which itself is oxidized at a potential around 1.0 V, see below, [Fig. S1](#).

In the second scan, voltammograms of MIP and NIP electropolymerization show that the pyrrole oxidation peak decreases drastically due to the change in the nature of the electrode surface after the deposition of a polypyrrole layer that is less conductive than the carbon itself [74].

3.2. Extraction of the template molecule

The template extraction was achieved by the procedure described in section 2.2 by cyclic voltammetry (voltammograms in [Supplementary](#)

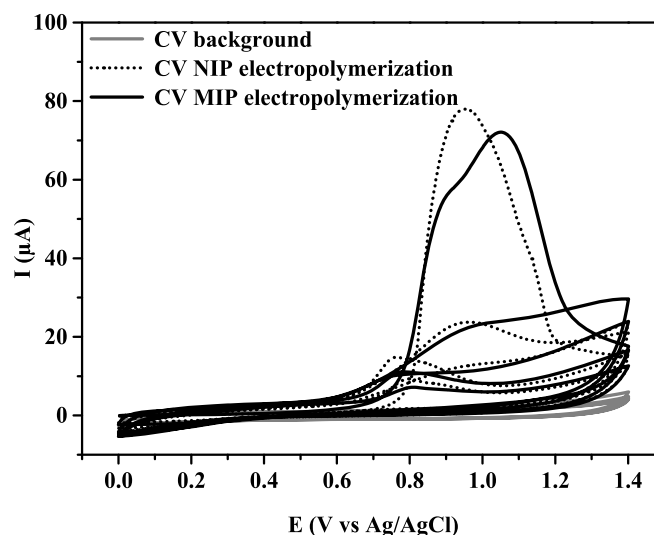


Fig. 1. Cyclic voltammograms taken during electropolymerization of pyrrole (0.01 M) in absence of ISO (1 mM) (NIP) and in presence of ISO (1 mM) (MIP) onto GC electrode, electrolyte: 0.1 M LiClO₄ in ethanol/water (20:80 v/v). Scan rate 10 mV s⁻¹.

[Material Fig. S1](#)). We observed an oxidation peak, at around 1 V vs Ag/AgCl, corresponding to the isoproturon trapped within the polymeric matrix.

Several scans were made until the oxidation peak was no longer observed due to the release of the isoproturon.

The well-known reversible oxidation-reduction behavior of polypyrrole [75] is also clearly observed at 0.4 V vs Ag/AgCl for the polypyrrole oxidation process and -0.25 V vs Ag/AgCl for the reduction process in our MIP-GC electrode.

On the voltammogram of the NIP electrode (not shown), no peak at 1.0 V was observed while the polypyrrole signal was present in the same region as for the MIP.

This method of template extraction is easy to achieve and avoids the use of a variety of chemical reagents. Moreover, the voltammograms obtained validated the removal of the template.

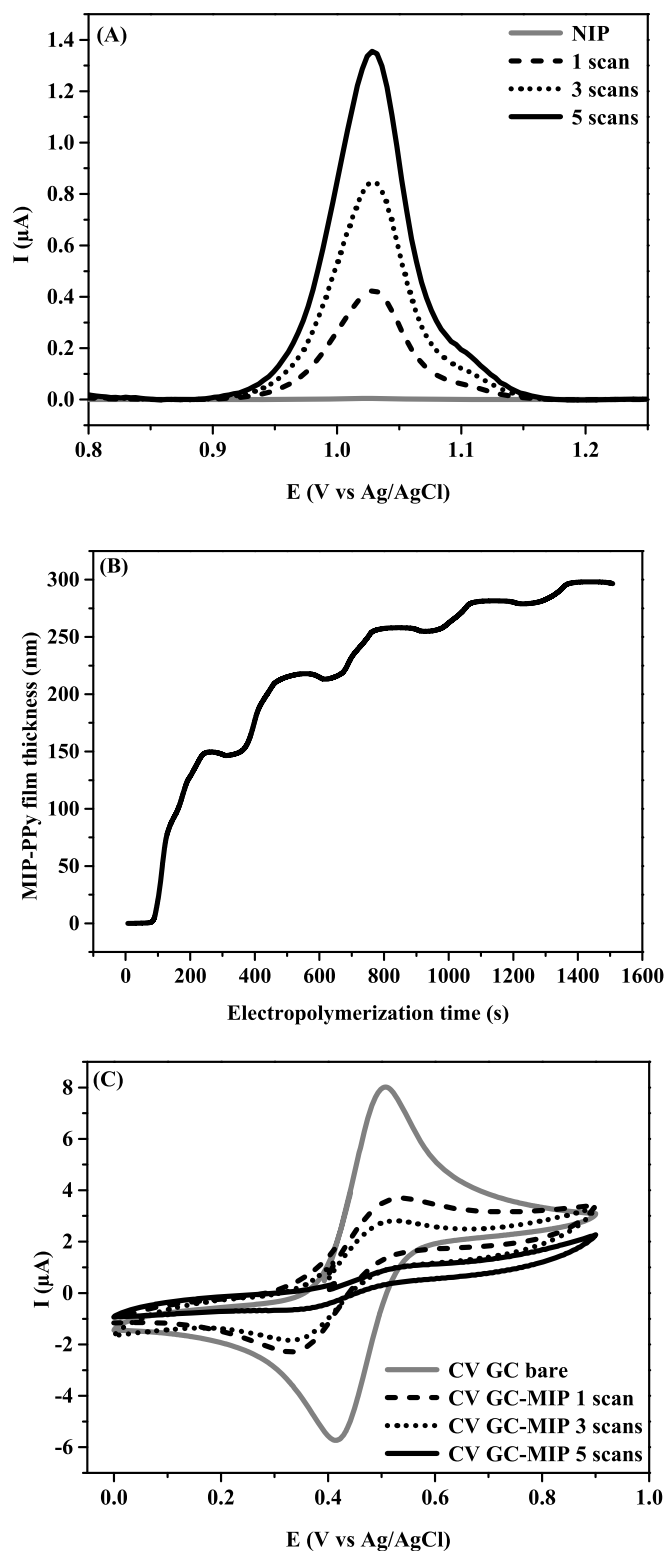


Fig. 2. Influence of the scan number during electropolymerization of the MIP on GC electrode (A) on isoproton response on MIP-GC electrode (B) on MIP film thickness estimated using Faraday's law from the voltammogram (C) on CV ferrocene response (2.5 mM) in NBu_4BF_4 (0.1 M) acetonitrile solution on bare and MIP-GC electrodes - scan rate 100 mV s^{-1} .

3.3. Optimization of the eletropolymerization procedure for the MIP-GC electrode

In this section, the detection signal of isoproton served as a tool

for the electrochemical synthesis optimization of the MIP-GC electrode. The ISO detection was performed as follows: after the electrochemical release of ISO, MIP-GC electrodes were immersed in a $5 \times 10^{-7} \text{ M}$ isoproton water solution during 15 min and the analyses were performed by SWV in an ethanol/water solution (70:30 v/v) of 0.1 M sulfuric acid.

The sensing properties of MIP-GC electrodes obtained by CV and by CA were compared after optimizing the impact of the scan number for CV and the electrolysis time for CA and establishing the influence of the ratio of ethanol in the electrolyte solution.

3.3.1. Preparation of isoproton MIP-GC electrodes by cyclic voltammetry (CV): influence of the scan number

In order to determine the effect of the number of cycles during electropolymerization on the response of isoproton detection by MIP-GC, the film was prepared with 1, 3 and 5 cycles, see Fig. 2A.

It clearly shows that the response of isoproton is improved with the number of scans due to the increase in the thickness of the polymeric matrix and consequently the number of cavities. In order to verify this assumption, the theoretical thickness of the layer was estimated from the electrical charge (Fig. 2B). To correlate polypyrrole thickness x (cm) and electrical charge, Faraday's law was used assuming 100% current efficiency for polypyrrole:

$$x = qM/\rho AzF$$

where q (C) is the electrical charge associated with polypyrrole formation, M (g/mol) is the molar mass of the monomer, F is Faraday's constant (C mol^{-1}), A (cm^2) is the area of the working surface, ρ (g cm^{-3}) is the polymer density and z (mol) is the number of electrons involved in the electropolymerization process of pyrrole which in this case is 2.25 [76,77]. The nominal density for polypyrrole films (ρ) was taken as 1.5 g cm^{-3} [75]. By taking into consideration all the above-mentioned parameters, the required charge density to grow a film with an average thickness of 290 nm is 10.4 mC cm^{-2} [76]. We can see, in Fig. 3B, that the estimated thickness increases with the number of cycles to reach 290 nm after 5 scans in the case of MIP-GC.

To confirm this result, the behavior of a ferrocene 2.5 mM redox probe in acetonitrile solution containing 0.1 M NBu_4BF_4 on MIP layers obtained at 1, 3 and 5 cycles was studied (Fig. 2C). It shows that the blocking effect for the ferrocene signal increases with the number of cycles due to the growth of the insulating polymer layer because of the over-oxidation conditions [73].

Finally, in order to determine the electropolymerization efficiency, one experimental thickness of the MIP layer deposited by EQCM assuming a density of MIP 1.5 g cm^{-3} [74,75] and the corresponding charge was measured. The electropolymerization yield was calculated from the ratio between the experimental and the theoretical thicknesses and was found to be close to 63%.

3.3.2. Preparation of isoproton MIP-GC electrodes by chronoamperometry (CA)

3.3.2.1. Effect of solvent: the role of the ethanol/water ratio. During the electropolymerization of a MIP film (see section 2.2), knowing that the solubility of isoproton is limited in water (70.2 mg/L at 25 °C) we added ethanol in water at different quantities. Fig. 3A shows the film thickness calculated from chronoamperometry curves for a constant electropolymerization time using Faraday's law for different ratios of ethanol. It is shown that the thickness of the MIP film produced is directly dependent on the amount of ethanol, hence when the amount of ethanol is greater than 40% the film thickness is less than 50 nm. Below 40% of ethanol, the film thickness increases linearly until it is six-fold the initial thickness.

The same MIP-GC electrodes were used for ISO detection. Fig. 3B shows the ISO peak current as a function of the ethanol ratio. The best results were achieved with MIP-GC electrodes prepared in water-ethanol solutions containing lower amounts of ethanol. The thickest

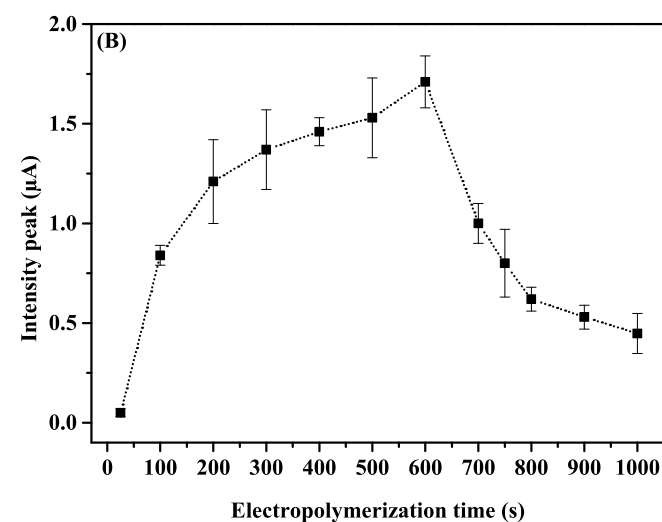
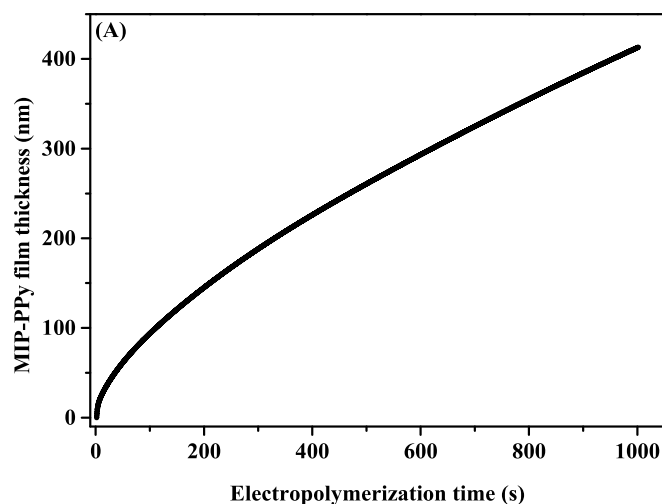
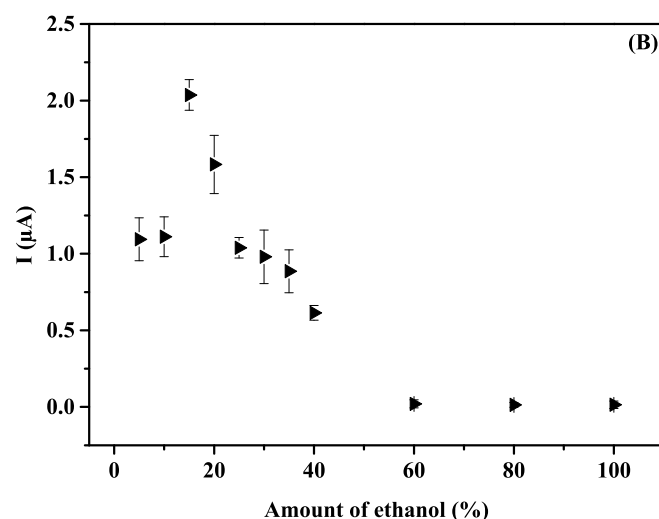
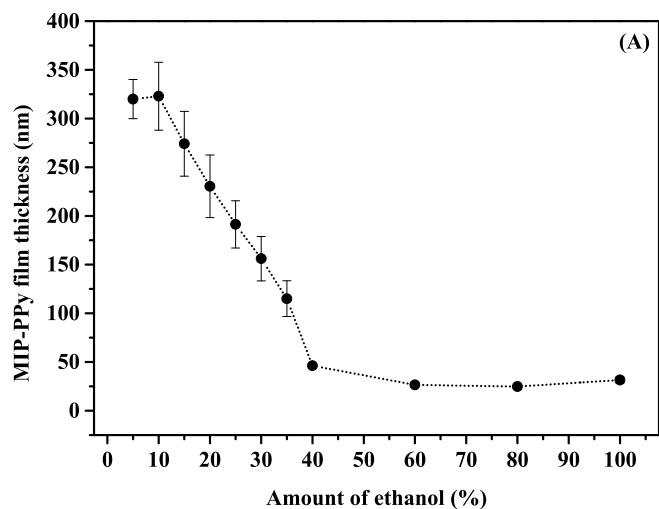
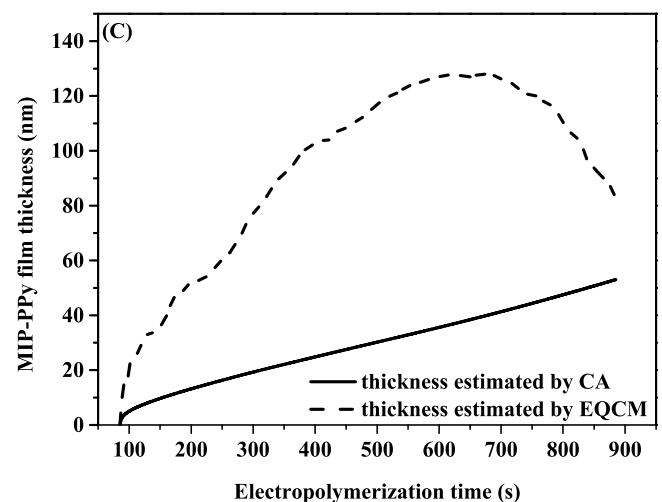


Fig. 3. Electropolymerization of MIP-GC electrode for different ratios of ethanol/water with LiClO_4 0.1M (A) film thickness calculated from chronoamperogram and (B) ISO peak intensity electropolymerization: CA 1.1 V during 600 s; pyrrole 0.01 M in presence of ISO 1 mM.

MIP films were produced with a maximum between 15 and 20% of ethanol. This value was therefore chosen for further study.

3.3.2.2. Influence of electropolymerization time. The influence of the electropolymerization time was studied for the MIP films prepared by CA at 1.1 V vs Ag/AgCl at different times that were used later for ISO detection. The theoretical thickness of the growing layer was estimated from the chronoamperograms on Fig. 4A and a rapid increase in this thickness up to 100 s was observed. Thereafter, its further growth is slowed down, reaching an almost linear increase after 200 s. This is most probably because of the decrease in the electronic transfer due to the thickness of the layer and to the partially insulating character of the MIP.

The electrochemical detection ability of isotroturon on MIP-GC electrodes prepared by CA at different electropolymerization times is presented in Fig. 4B. These results are in agreement with those presented in Fig. 3A. During the first 100 s, the ISO detection signal was very significant, corresponding to the film thickness growth. From 100 s to 600 s, the signal of the target analyte increased more slowly. We can assume that the blocking effect of the charge transfer limits the oxidation of isotroturon during detection. Moreover, the thicker the film is, the more difficult it is for isotroturon molecules to access the cavities and to reach the conducting surface. The sharp signal decrease after



(caption on next page)

Fig. 4. Effect of electropolymerization time on the electrochemical response of 5×10^{-7} M of ISO at the MIP-GC electrode in ethanol/water (70:30 v/v) of H_2SO_4 0.1 M, (A) the thickness of the MIP film estimated from the chronoamperogram (B) isotroturon electrochemical response, MIP film was formed by chronoamperometry setting the potential at 1.1 V, incubation time 15 min (C) comparative study of the MIP film thickness estimated by Faraday's law calculations and by EQCM experiments in order to follow the chronoamperometry electropolymerization process (potential applied 1.1 V, time duration 885 s) of ISO (1 mM) and pyrrole (0.01 M) on gold quartz used as a working electrode, electrolyte: 0.1 M LiClO_4 in ethanol/water (80:20 v/v).

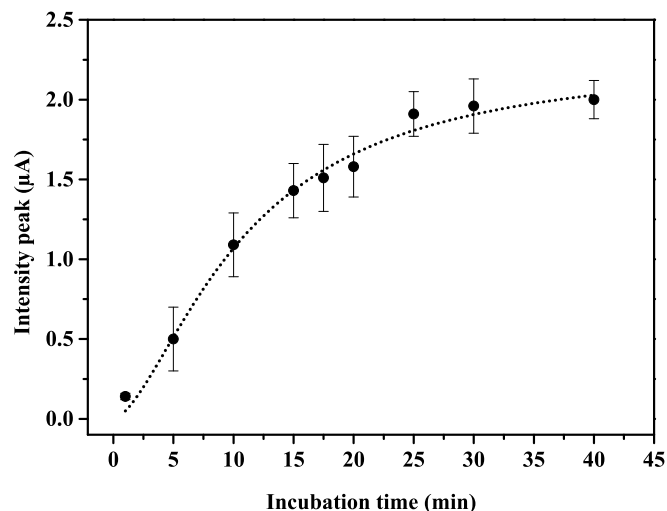


Fig. 5. Isotroturon (5×10^{-7} M) response on the MIP-GC electrode for different incubation time.

600 s is probably due to the film breaking during its growth. This assumption was validated with measures made by EQCM, Fig. 4C. The sharp mass decline also started after 600 s. It should be noted that the sharp decrease both in the ISO peak detection and in the MIP layer mass was observed for the thickest MIP films but not always at the same time.

This fact is due most probably to mechanical constraints in the thick films.

As for CV MIP preparation, see below, the electropolymerization yield obtained from CA with EQCM measurement was calculated and the corresponding charge was found to be close to 66%. This value is of the same order as that obtained with CV electropolymerization. For both methods, the charge loss is consistent with the high potentials applied during the MIP-GC preparation.

These results show that the optimal electropolymerization time for the isotroturon response is 600 s. In addition, the sensitivity and the reproducibility of the MIP-GC (results not shown) are better for films prepared by chronoamperometry, which is consistent with the literature [53].

3.4. Electrochemical detection of isotroturon by the GC-MIP electrode

3.4.1. Optimization of incubation time

The incubation time is an important factor in the analytical procedure since it will impact the overall time of the analysis. The influence of the incubation time in the range of 0–40 min on the isotroturon signal responses was evaluated in aqueous media containing 5×10^{-7} M of isotroturon. As illustrated in Fig. 5 the isotroturon oxidation peak intensity increases proportionally with the incubation time in the first 15 min due to its rebinding to the created cavities. Beyond 15 min, the rebinding kinetic slows down, suggesting the beginning of saturation of the cavities or a more difficult access of isotroturon. An incubation time of 15 min was therefore chosen to obtain the best analytical performances for this study. This parameter impacts the quantification: in the linearity domain, the longer the time, the more the lower limit of quantification (LOQ) diminishes. Moreover, a lower incubation time leads to less accurate values because monitoring the immersion time is less easy and the signal is lower.

3.4.2. Electroanalytical performances

3.4.2.1. Calibration curve and determination of LOD/LOQ. In this subsection, the optimal conditions to achieve the best sensitivity for isotroturon detection were selected: the MIP-GC film obtained by CA at 1.1 V vs Ag/AgCl during 600 s was used and isotroturon detection by

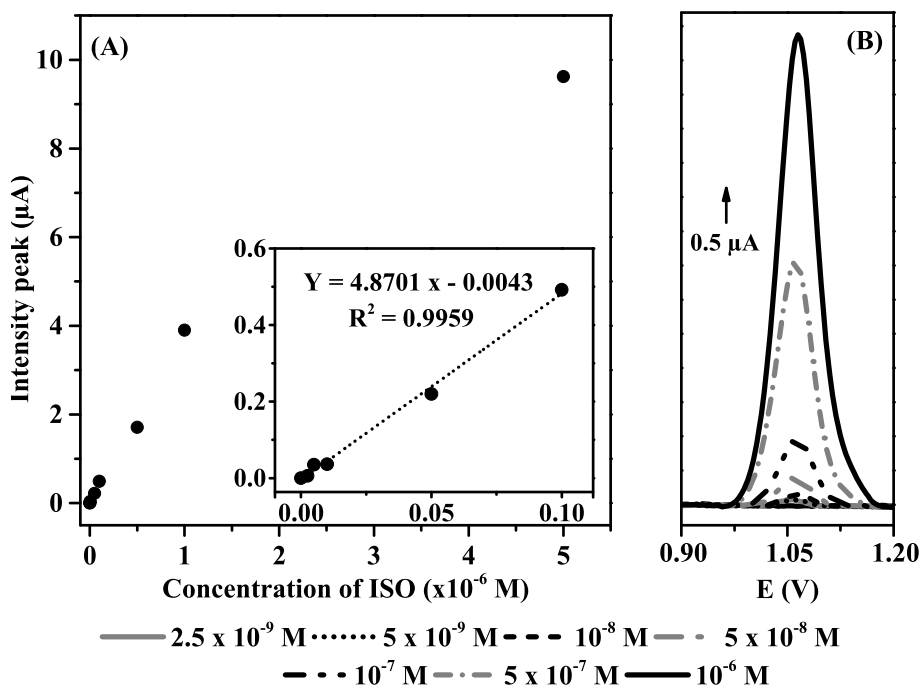


Fig. 6. The calibration curves of isotroturon detection obtained at MIP-GC electrode in Milli-Q water. Current-concentration calibration curve (A) includes linear region for ISO concentration, and calibration linear equation (B) square wave voltammograms obtained during electrochemical determination of isotroturon.

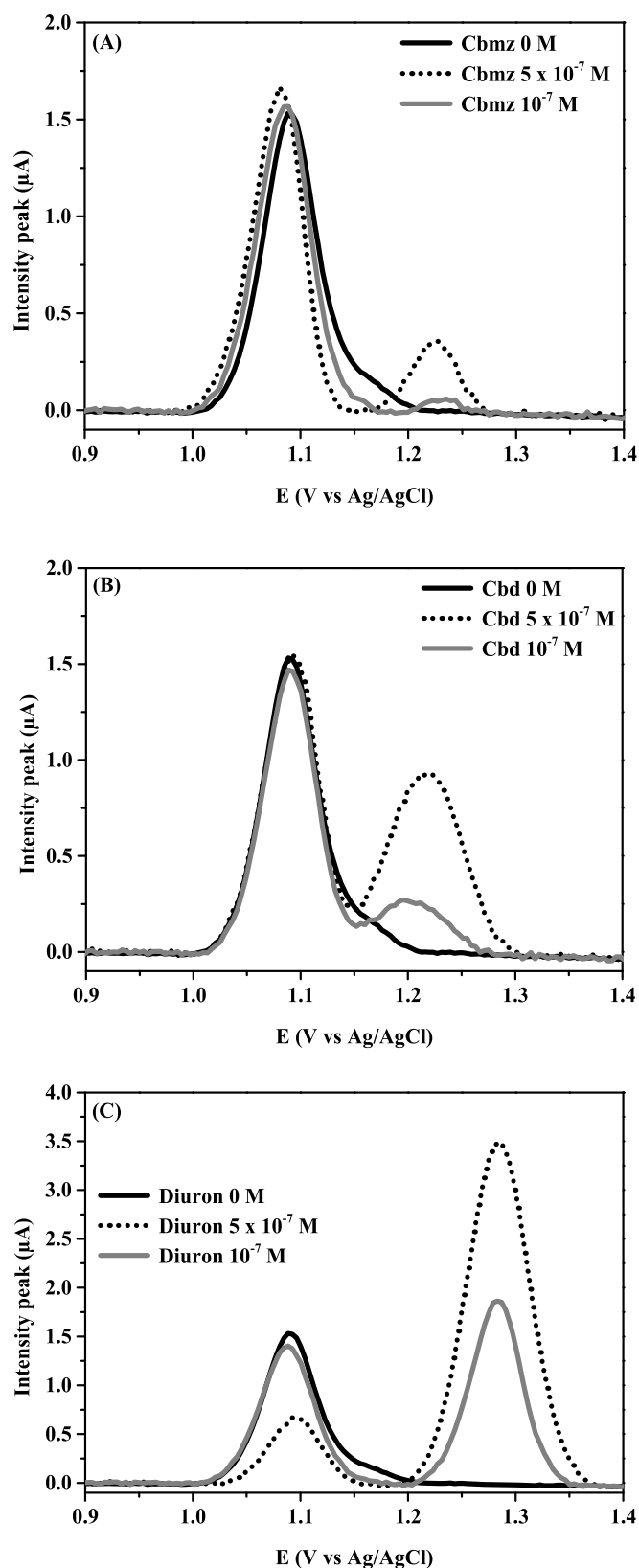


Fig. 7. Effect of the interferences on the determination of ISO (5×10^{-7} M) using the MIP-GC electrode: (A) carbamazepine (B) carbendazim and (C) diuron.

SWV was carried out in an ethanol/water solution (70:30 v/v) of 0.1 M sulfuric acid free of isotroturon after 15 min immersion of the film in a milli Q water solution containing isotroturon at concentrations in the

range of 2.5×10^{-9} - 5×10^{-6} M. The calibration curve is presented in Fig. 6A. The square wave voltammograms clearly showed a current peak increasing with concentration during ISO detection in Fig. 6B.

The sensitivity was obtained from the slope of the calibration curve; its value was 4.8701 A/M. The calibration plot was found to be linear between 0 and 10^{-7} M and obeyed the following relation: $I (\mu\text{A}) = 4.8701 [\text{Isotroturon}] - 0.0043$ ($R^2 = 0.9959$).

The isotroturon limits of detection (LOD) and quantification (LOQ) that were calculated statistically [78] were 2.76×10^{-9} M ($0.5 \mu\text{g L}^{-1}$) and 9.2×10^{-9} M ($1.9 \mu\text{g L}^{-1}$) respectively.

Repeatability was evaluated after seven analysis replicates of isotroturon 5×10^{-7} M solution with a single electrode. Well-shaped voltammograms were obtained for all experiments: the calculated relative standard deviation (RSD) was 7.6%. Reproducibility of the procedure was evaluated by performing a series of analyses with five different electrodes. The RSD obtained was 12%. These results indicate that isotroturon MIP-GC electrodes are reliable tools for isotroturon detection and quantification. Each MIP-GC electrode can be used for 25 cycles of analyses with a change of the isotroturon peak intensity less than 20%. Then a decrease of the signal is observed during the 20 next analysis with an extinction of the signal comprising between 50 and 90% depending on the electrode.

3.4.2.2. Interference study. The selectivity of the prepared MIP-GC electrodes toward isotroturon was tested for three interfering molecules (Fig. 7): carbendazim, diuron and carbamazepine, at two concentrations, 5×10^{-6} M, 5×10^{-7} M and 10^{-7} M, for each molecule, in the presence of isotroturon at 5×10^{-7} M. These molecules are widely found in environmental waters and have a structure and oxidation potentials quite close to those of isotroturon. Thus, these molecules may interfere with the determination of isotroturon.

The results, presented in Fig. 7A and B shows that according to the RSD found (see previous sub-section), neither carbendazim nor carbamazepine seem to affect the MIP-GC sensitivity toward isotroturon, whereas the presence of diuron (Fig. 7C) at the same concentration or at a concentration 10 times higher as isotroturon lowers its signal by 50% and 80% respectively. However, in natural environments, diuron is found at concentrations up to 17 times lower than that of isotroturon [79]. Thus, we can reasonably assume that diuron does not exhibit major interferences with isotroturon.

3.4.2.3. Application of the electrochemical sensors in real water samples. The electrochemical methods developed were applied to the detection of isotroturon in a real water sample. A groundwater sample located in France, Région Centre was chosen in accordance with the funding project (CAPEL MIP, see Acknowledgements). The site is located in the commune of Saint-Rémy-Du-Val (Sarthe, province of Loire-Bretagne region). The monthly monitoring performed in this aquifer revealed that the groundwater is contaminated by several pesticides and notably triazines and chloroacetanilides and their metabolites, but does not contain isotroturon ($< 0.005 \mu\text{g L}^{-1}$). The samples were prepared with this natural groundwater, adding a known quantity of isotroturon to obtain concentrations from 1×10^{-8} to 5×10^{-7} M. The calibration plot obtained with these samples was found to be linear between 0 and 5×10^{-7} M (shown in Supplementary Material Fig. S2) and obeyed the following relation: $I (\mu\text{A}) = 4.1853 [\text{Isotroturon}] + 0.0056$ ($R^2 = 0.999$). The LOD and LOQ were 1.1×10^{-8} M ($2.2 \mu\text{g L}^{-1}$) and 3.7×10^{-8} M ($7.6 \mu\text{g L}^{-1}$), respectively. The sensitivity obtained from the slope of the calibration curve was 4.1853 A/M. The oxidation current of the preconcentrated isotroturon in the MIP film is easily visible in the solutions that contain concentrations higher than 1×10^{-8} M of isotroturon.

4. Conclusion

In this work, a novel electrochemical sensor for the sensitive and selective SWV determination of isoproturon, a priority micro-pollutant, was developed. It is shown that ultra-sensitive MIP films for its detection can be prepared in a simple way by CV or CA on GC electrodes. The key parameters of MIP electro-synthesis such as electrodeposition time, scan number, solvent ratio and incubation time were optimized. The extraction step of the template molecule was conducted electrochemically in aqueous solution, thus avoiding the use of toxic organic solvents.

The MIP-GC sensor was able to detect isoproturon in nano-molar concentration with good reproducibility and repeatability. The functionalized electrode surfaces showed good robustness during several analyses.

The performances in terms of LOD, LOQ and selectivity are satisfactory for the contaminated natural waters of the Loire-Bretagne region, where isoproturon concentrations up to $1.2 \mu\text{g L}^{-1}$ have been measured in 67% of the stations [79]. For other sites, this LOQ is not low enough as worldwide occurrence in the environment showed for effluent a maximum concentration of $0.27 \mu\text{g l}^{-1}$ with a 51% frequency of detection in the effluent of conventional wastewater treatment plants in Europe [78], and for surface water of inland seas a maximum concentration of $0.06 \mu\text{g l}^{-1}$ with a 100% frequency of detection in estuaries on the Baltic coast [79]. But in spite of these good performances, they are still higher than the maximum allowed levels set by the WFD. Thus, investigations are currently being carried out into the nano-structuration of the polypyrrole path in order to enhance the sensitivity of these sensors and to increase the number of accessible cavities.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.talanta.2019.120222>.

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Reumatologjia

Autor

Prof. Asoc. Dr. Ismet H. Bajraktari

ALMA MATER EUROPAEA
Campus College “Rezonanca”
Prishtinë, Kosovë

Recensentë

Prof. Dr. Sylejman Rexhepi

Universiteti i Prishtinës “Hasan Prishtina”
Fakulteti i Mjekësisë
Prishtinë, Kosovë

Prof. Dr. Gani Bajraktari

Universiteti i Prishtinës “Hasan Prishtina”
Fakulteti i Mjekësisë
Prishtinë, Kosovë

Prof. Dr. Cen Bytyqi

Universiteti i Prishtinës “Hasan Prishtina”
Fakulteti i Mjekësisë
Prishtinë, Kosovë

Redaktor profesional

Prof. Asoc. Dr. Agim Krasniqi

ALMA MATER EUROPAEA
Campus College “Rezonanca”
Prishtinë, Kosovë

Redaktor gjuhësor

Prof. Salih Bytyqi

Instituti Albanologjik
Prishtinë, Kosovë



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Parathënie

Reumatologjia është një lëndë sa tërheqëse, aq edhe e ndërlikuar, por gjithsesi e domosdoshme të mësohet, ngase në vete ngërthen qindra sëmundje të pranishme në punën e përditshme të profesionistëve shëndetësorë. Megjithatë, mësimi i reumatologjisë është i lehtë, nëse lexuesi ka njohuritë bazë nga lëndët paraklinike dhe klinike, e sidomos nga anatomia, fiziologjia, biokimia, radiologjia, imunologjia, gjenetika dhe ortopedia.

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Për autorin janë shumë të mirëseardhura të gjitha vërejtjet dhe propozimet që na dërgohen nga lexuesit, ashtu që në botimet e ardhshme libri të jetë edhe më i begatshëm.

Shpresojmë që libri të plotësojë nevojat për literaturë bazë dhe do t’ju shërbejë si studentëve, mjekëve, profesionistëve ashtu dhe pacientëve, që reumatologjinë e kanë detyrë, pasion apo fushë të interesimit.

Nga recensioni

Libri “*Reumatologjia*” i autorit Dr. Ismet H. Bajraktari, i dedikuar për student, mjek dhe specialist kryesisht në lëmin reumatologji, por edhe të disiplinave të përafërta si fiziatri, ortopedi dhe më gjerë, përmban kapitujt nga sëmundjet reumatizmale inflamatore, degjenerative, rrethartikulare dhe sëmundjet e rralla. Ky libër është në vazhde të teksteve të ngjashme të botuara nga autorët tjerë paraardhës vendor, por dhe i përbërë me risitë nga etiologjia, patogjeneza, metodat diagnostike dhe terapeutike.

Libri “*Reumatologjia*” është redaktuar në mënyrën më të mirë nga literatura e autorëve vendor, rajonal dhe në veçanti e autorëve ndërkombëtar të shumtë, por edhe me elemente të përvojës personale të autorit, si rezultat i punës me pacientë, me student, por edhe nga pjesëmarrja në takimet dhe konferencat profesionale vendore dhe ndërkombëtare.

Libri përmban disa kapituj të përbërë nga pjesa e përgjithshme, pjesa speciale, të cilat përmbajnë informatat e hollësishme rreth anamnezës, ekzaminimit fizik të pacientëve, informatave rreth analizave laboratorike hematologjike, biokimike, serologjike dhe imunologjike kryesore që ndërliken me sëmundjet reumatizmale, por edhe me informatat për metodat rëntgenologjike, edhe të tjera imazherike si tomografia kompjuterike, rezonanca magnetike, ultratingulli, për qëllimin e fundit, diagnostikimin sa më të mirë të sëmundjeve reumatizmale.

Kapitulli i mjekimit të sëmundjeve reumatizmale përmban zgjidhjet më të mira terapeutike me analgjetikë, antireumatikë josteroidë, kortikosteroide, me barnat që modifikojnë sëmundjet reumatizmale (DMARDs), terapinë tjetër simptomatologjike dhe në veçanti substancat biologjike dhe të bazuara në udhëzuesit e Shoqatës së Reumatologëve Amerikan dhe Evropian dhe me informata precize të përdorimit të tyre, në kuptim të indikacionit, interaksionit dhe efektetve anësore të tyre.

Rëndësi të veçantë, autori i kushton rëndësisë së dietës në të sëmurë reumatik, ekuilibrit të ushqimit, të pasur me proteina, vitamina dhe minerale, kur të merret parasysh rëndësia e tyre në ecurinë, prognozën dhe ndikimin e tyre në kompletimin e mjekimit modern të sëmundjeve reumatizmale.

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Në përmbyllje të recensionit, me vështrim të shkurtër të disa specifikave, duke përgëzuar autorin për angazhimin dhe përkushtimin, gjuhën e pasur dhe të pastër dhe pedanterinë e tij gjatë përpilimit të librit, dëshiroj të theksoj profesionalizmin, e autorit, durimin me punë në hulumtime shkencore dhe në veçanti, koordinimin e informatave dhe sintezën e të dhënave shumë aktuale nga literatura botërore, rajonale, vendore dhe të përvojës personale, gjatë redaktimit të librit.

Në këtë mënyrë, autori Dr. Ismet Bajraktari, me librin e botuar ka bërë një kontribut shkencor dhe paraqet një sukses të pamohueshëm jo vetëm për reumatologji, por edhe në mjekësinë e përgjithshme kosovare.

*maj 2019,
Prishtinë*

*Prof. Dr. Sylejman Rexhepi
Prof. Dr. Gani Bajraktari
Prof. Dr. Cen Bytyqi*

Reumatologjia e përgjithshme

1 HYRJE ²

2 EPIDEMIOLOGJIA E SËMUNDJEVE REUMATIKE ⁷

3 PROPEDEUTIKA REUMATOLOGJIKE ⁸

4 EKZAMINIMET LABORATORIKE ²⁷

5 EKZAMINIMET RADIOLOGJIKE ³⁹

6 MJEKIMI I SËMUNDJEVE REUMATIKE ⁴⁹

7 NOZOLOGJIA E SËMUNDJEVE REUMATIKE ⁷¹

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Reumatologjia

Libri *Reumatologjia* është një përmbledhje e shkurtër nga fusha e gjerë e shkencës së reumatologjisë, e shtjelluar në mënyrë të thjeshtë dhe praktike për studentët, mjekët e përgjithshëm dhe secilin që dëshiron të lexoj më shumë mbi këto sëmundje të gjata e të mundimshme, diagnostikimi sa më i hershëm i të cilave do të thotë kualitet më i mirë i jetës për pacientin.

Ky libër ka për synim orientimin e studentit dhe mjekut në diagnostikimin e saktë të gjendjes, pa e ngarkuar pacientin me analiza të pa nevojshme dhe të kushtueshme. Përmbajtja e librit ndahet në 17 kapituj që përfshijnë 154 sëmundje reumatologjike, të sistematizuara në dy pjesë:

- ▶ **Reumatologjia e përgjithshme**, ku janë prezantuar të dhënat për anatominë, fiziologjinë, patofiziologjinë, epidemiologjinë, semiologjinë dhe farmakoterapinë e sëmundjeve reumatike, si dhe
- ▶ **Reumatologjia speciale**, ku janë shtjelluar sëmundjet reumatike.

Ilustrimet e shumta, tabelat me sistematizimin e të dhënave, orientimet për ekzaminimet relevante laboratorike të sëmundjet e veçanta, imazhet radiografike, si dhe të dhënat për terapinë dhe mjekimin, e bëjnë librin një doracak të dobishëm gjatë studimit dhe në përditshmërinë profesionale.

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Sero-Prevalence Survey of Small Ruminant Lentivirus (SRLV) Infections in Kosovo

Armend CANA^{1,a} Nick TAYLOR^{2,4} Naomi HONHOLD² Valdet GJINOVCI¹ Arton OSMANI³
Stefano NARDELLI^{2,5,b} Bafti MURATI¹ Jeton SPAHIU¹ Dafina MEHMETUKAJ¹ Mentor ALISHANI^{2,6,c}

¹Kosovo Food and Veterinary Agency, Industrial Zone, 10 000 Pristina, KOSOVO

²KAHL Project, Kosovo Animal Health and Laboratory, EUROPEAID/133795/DH/SER/KX, Str. Zejnel Salihu 28, Nr. 12, 10000 Pristina, KOSOVO

³European Union Office in Kosovo/European Union Special Representative in Kosovo, P.O. Box 331, 10 000 Pristina, KOSOVO

⁴Veterinary Epidemiology and Economics Research Unit (VEERU) & PAN Livestock Services Ltd., University of Reading, School of Agriculture, Policy and Development, Reading, RG6 6AR, UK

⁵Istituto Zooprofilattico Sperimentale Delle Venezie (IZSve), Legnaro (PD), ITALY

⁶Faculty of Agriculture and Veterinary, University of Prishtina, Str. Bill Clinton, n.n., 10 000, Pristina, KOSOVO

^a ORCID: 0000-0001-6034-4903; ^b ORCID: 0000-0002-0344-3101; ^c ORCID: 0000-0002-6853-4792

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Abstract

The objectives of this cross-sectional study were to detect the presence of small ruminant lentiviral infections in Kosovo and estimate the serological prevalence for the year of 2016. A total of 5.272 sheep and 435 goats were tested using a commercially available indirect enzyme-linked immunosorbent assay (ELISA) for Maedi-Visna/Caprine Arthritis-Encephalitis, giving an overall individual sero-prevalence in sheep of 34.8% (95% confidence interval 31.8% to 38.0%), and a flock prevalence of 85%, and in goats an overall individual sero-prevalence of 15.6% (95% confidence interval 7.2% to 25.6%) and flock prevalence of 35%. Sero-prevalence in sheep was higher in the South and West of Kosovo, whereas in goats was higher in the East and South. There were no statistically significant differences in sero-prevalence between sheep in different age groups <2 year to ≥4 year. There were statistically significant differences between the age groups in goats: chi square 25.74 (3d.f.) with P-value <0.0001. Increasing sero-prevalence observed in goats up to 4 years old with a sharp drop in goats older than four years highlights the need for further investigation based on clinical impact and genotype characterization. While retained sero-positive sheep in the population beyond 4 years old may suggest mild clinical impact of small ruminant lentiviral infections in Kosovo sheep.

Keywords: *Small ruminant lentivirus, Maedi-Visna, Caprine Arthritis-Encephalitis, ELISA, Kosovo*

Kosova'daki Küçük Ruminant Lentivirus (SRLV) Enfeksiyonlarının Sero-prevalansının Araştırması

Öz

Bu kesitsel çalışmanın amacı, Kosova'da küçük ruminant lentiviral enfeksiyonların varlığını saptamak ve 2016 yılı için serolojik prevalansı tahmin etmektir. Toplam 5.272 koyun ve 435 keçi ticari olarak temin edilebilen dolaylı enzim bağlı immünosorbent test (ELISA) kullanılarak Maedi-Visna/Keçi Arthritis-Ensefalitis hastalığı yönünden kontrol edildi. Koyunlarda bireysel sero-prevalans %34.8 (%31.8 ila %38.0; %95 güven aralığı), sürü prevalansı % 85 bulunurken keçilerde %15.6'lık bireysel sero- prevalans (%7.2 ila %25.6; %95 güven aralığı) ve %35'lik sürü prevalansı belirlendi. Hastalık sero-prevalansı koyunlarda Kosova'nın güneyinde ve batısında, keçilerde ise doğusu ve güneyinde daha yüksekti. Koyunlarda <2 ila ≥4 yaş aralığındaki farklı yaş gruplarının sero prevalansında istatistiksel olarak anlamlı bir fark yoktu. Keçilerdeki yaş grupları arasında ise istatistiksel olarak anlamlı farklılıklar vardı: ki-kare 25.74 (3d.f.) P değeri <0.0001. Dört yaşına kadar olan keçilerde sero prevalansının artması ancak 4 yaşından büyük keçilerde görülen keskin düşüş, klinik etki ve genotip karakterizasyonuna dayanarak daha fazla araştırma yapılması gerektiğini vurgulamaktadır. Populasyondaki dört yaşından büyük sero-pozitif koyunların sürüde tutulması, küçükbaş hayvan lentiviral enfeksiyonlarının Kosova koyunlarında hafif klinik etkisi olduğu yönünde değerlendirilebilir.

Anahtar sözcükler: *Küçük ruminant lentivirüs, Maedi-Visna, Keçi Arthritis-Ensefalitis, ELISA, Kosova*

✉ İletişim (Correspondence)

✉ +383 44 16 10 60

✉ mentor.alishani@uni-pr.edu

INTRODUCTION

Maedi-Visna (MV) in sheep and Caprine Arthritis-Encephalitis (CAE) in goats are caused by two closely related viruses, commonly referred as small ruminant lentiviruses (SRLVs). SRLVs share many features, but are genetically heterogeneous as shown by phylogenetic analysis [1,2]. Cross-infection between small ruminant species is possible [3,4]. The main routes of transmission are via colostrum/milk and horizontal transmission through aerosol via the natural close contact between the dam and her progeny, especially under intensive housing or grazing conditions. Intrauterine transmission occurs infrequently [5,6]. Some resistance to lentivirus infection may be related to host genetics [7]. Once established, infection is lifelong and persistent. Infected animals are a constant reservoir of infection [8]. Incubation period is long and highly variable. Most infected animals will remain asymptomatic during their productive lifespan. Often it takes years before clinical infection becomes apparent and 30% of infected animals will develop slow progressive multi-systemic chronic disease [1,5]. MV is clinically manifested as chronic progressive interstitial pneumonia (maedi) and/or progressive neurologic form of the disease (visna) usually in adult sheep, although it has been reported in older lamb and encephalitis in 4-6 months old lambs [9-13]. The most common manifestation of CAE infection in goats is polysynovitis-arthritis in adult goats and encephalomyelitis, which is generally seen in kids 2-4 months old but has been described in older kids and adult goats [14,15]. Chronic indurative mastitis is seen in both species [16,17].

Small ruminant lentivirus infection causes underestimated substantial losses in the small ruminant industry due to reduced animal production and increased replacement rates [5]. There are no specific treatments for any of the clinical syndromes associated with MV or CAE virus infection and to date, no vaccines are available [18]. Diagnosis is based on clinical signs, flock history and confirmed by serological tests, PCR, western blot, radio-immunoassay and radioimmuno-precipitation assay. Both agar gel immune-diffusion (AGID) and enzyme-linked immuno-sorbent assay (ELISA) are

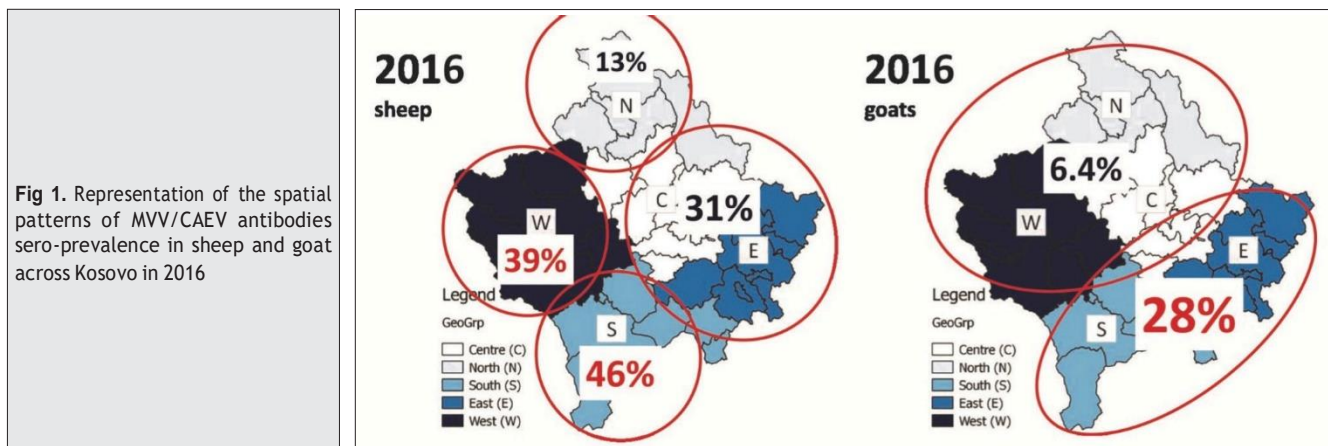
considered referent tests, according to the 2008 OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* [18,19]. However, delayed and intermittent seroconversion, maternal antibodies, high antigenic and genotype variations represent major drawbacks for use of serological tests in control strategies [1,19-21]. SRLV infections are reported in neighbouring and other countries around Kosovo, including Macedonia [22-28]. There is record of samples from a goat flock in Kosovo testing positive (ELISA) for CAE in 2009 [29]. Because there was no recent evidence of existing SRLV infection in Kosovo the initial aim of the surveys reported here was to detect evidence of infection and to perform the first structured and country based serological survey for SRLV in Kosovo.

MATERIAL and METHODS

Kosovo is a landlocked country in South-eastern Europe with a humid continental climate. The total area is 10.908 km², divided into 37 municipalities that for convenience can be grouped into five geographical areas: North, east, central, west, and south (Fig. 1).

Survey Design and Implementation of Sampling Activity

Because there was no recent evidence of existing SRLV infection in Kosovo the initial aim of the surveys reported here was detection of evidence of infection. The survey sample was determined for multiple purposes which were to detect the presence of SRLV and to estimate the sero-prevalence of brucellosis in small ruminants in Kosovo. The survey design set out to collect 8.000 samples from sheep and goats on 500 premises. This number of samples, divided between sheep and goats in similar proportion to that in the whole population (slightly above 10% goats), was calculated to provide at least 95% probability of detecting the both infection if present at a minimum individual prevalence of 0.1% in sheep and 0.5% in goats. In the final analysis, having established the presence of both infections, the results obtained were sufficient to provide estimates of sero-prevalence of SRLV infection with 95% confidence intervals of $\pm 3\%$ in sheep and $\pm 10\%$



in goats. The sampling frame of premises was derived from the official identification and registration (I&R) database maintained by Kosovo Food and Veterinary Agency (KFVA). A list of 2.666 premises with recently registered sheep or goats was extracted. Premises with fewer than 10 registered sheep and goats recorded were omitted from the sampling frame because these flocks would not have sufficient numbers of eligible animals to make sampling viable. These smaller flocks were 22% of all flocks but contained less than 1% of the registered population so their exclusion will have had little impact on the representative nature of the sample. Based on the registrations in the I&R database, the resulting sampling frame included about 270.000 sheep and 28.000 goats held on 2.078 premises (297 goats only, 1.202 sheep only and 579 mixed species). Distribution of samples according to geographic area are shown in *Table 1*.

Sampling was conducted in two stages. At the first stage, a random selection of premises was made from the sampling frame. The selection of premises from the sampling frame was stratified by flock size and intentionally biased towards larger flocks to ensure that the resulting sample of individual animals matched the whole population in terms of the flock sizes in which individuals exist.

At the second stage a pre-defined number of animals was set for sampling by the field staff during the visit to each selected premises. The target animals for sampling were adult animals, 12 months old or over (almost all were female). The number of animals to be sampled per premises was defined according to the total number of small ruminants registered in the premises (5 samples in flocks of 10-39, 10 samples in flocks of 40-99 and 20 samples in flocks of 100 or more). The sampling instructions for field teams included a breakdown of samples to take by species, based approximately on the proportions of sheep

and goats registered in each selected premises.

Field sampling was organised by a single contracted private veterinary company. Sample collection for the survey was carried out between May and September 2016. Bleeding of animals in the field was carried out by locally sub-contracted licensed private veterinary practitioners (PVPs). Blood samples were taken into plain vacutainer tubes. Each sample was given a unique ID and the species, sex and estimated age in years of each sampled animal was also recorded. Instructions were issued that samples were kept cool in transit. Samples were gathered by the contracted veterinary company in Pristina, checked and recorded and then forwarded to the Kosovo Food and Veterinary Laboratory (KVFL) for storage and testing.

Laboratory Diagnostic Testing

On receipt at KFVL the serum was separated and stored at -20°C. Samples were tested using an indirect ELISA based on the use of an immunogenic peptide of transmembrane protein (TM, ENV gene) and of the recombinant p28 protein which enters into the composition of the viral capsid (GaG gene) (Maedi-Visna/CAEV Antibody Test Kit REF: P00303-10 IDEXX MVV/CAEV p28 Ab Screening). Anti-p28 antibodies can appear slightly later than the antiviral envelope protein antibodies. The use of this stable protein allows the serological detection of a wide spectrum of serological variants. The cut-off point was calculated according to the manufacturer's instructions. Samples with sp% ≥ 120 of the control positive were classified positive and samples with sp% < 120 negative. Samples with sp% > 110 but < 120 could be classified as 'inconclusive'.

Statistical Analysis

Asymmetric Wilson score confidence intervals taking into

Table 1. Distribution of samples received and tested by geo-spatial group			
Geographic Area	Total Samples Tested (MV/CAE ELISA)	Registered Population	% of Registered Population Sampled
SHEEP SAMPLES			
Centre	918	46660	2.0%
East	1137	54198	2.1%
North	503	32195	1.6%
South	1339	72196	1.9%
West	1375	68452	2.0%
Overall	5272	273701	1.9%
GOAT SAMPLES			
Centre	104	7091	1.5%
East	124	8661	1.4%
North	47	2820	1.7%
South	61	3987	1.5%
West	99	7087	1.4%
Overall	435	29646	1.5%

account the sample size and the total population (sampling fraction) were calculated for prevalence estimates using the online statistical toolbox at *OpenEpi.com* [30]. This method provides exact, non-symmetrical confidence intervals for estimates based on simple random samples that are robust even when sample size is small and/or the prevalence is close to 0% or 100% [31-33]. The data were analysed using the method described by Bennett et al. [34] so that the effect of two-stage sampling could be taken into account, using premises as the unit of sample clustering. This analysis included a calculation of the overall sampling design effect. To take account of the two-stage sampling design, the lower and upper bounds of the calculated intervals were inflated by a factor of the square root of the design effect.

Calculation of the overall sero-prevalence estimates also took into account the distribution of samples by geographic area. The proportions of the registered sheep or goat population contained within each geographic area are used as weighting factors to adjust the overall prevalence estimate for Kosovo according to the relative population in each geographic area. Sero-prevalences for different age groups (by year cohort) where information on estimated age was provided

were calculated using Wilson score 95% CI as for simple random sample (no accounting for possible design effect).

Differences in sero-prevalence between groups were assessed for statistical significance using the chi-square statistic where more than two groups were involved and the Fisher exact test for 2-way comparisons only (Table 2).

RESULTS

Of the planned 8.000 samples from 500 premises, 6.013 were collected from 356 premises. This difference in sample size reflects the degree of uncertainty in the I&R database which was expected. The total planned sample size had been increased to take this problem into account. Out of 6013 samples collected, 306 samples were classified as haemolysed or did not fulfil other criteria to be tested. The total number of sheep samples tested was 5.272 from 318 premises (average just over 16 per premises) and the total number of goat samples tested was 435 from 54 premises (average just under 8 per premises). These samples represent an overall 1.9% of sheep population and 1.5% of goat population.

Table 2. Maedi-Visna sero-prevalence (ELISA) in sheep and Caprine Arthritis-Encephalitis sero-prevalence (ELISA) in goats across Kosovo in 2016, by age

SHEEP SAMPLES			
Estimated Age (years)	n Samples Tested	n MV ELISA Positive	%MV Positive With 95%CI
1 to <2	899	332	36.93% (33.84% to 40.13%)
2 to <3	1718	567	33.00% (30.82% to 35.26%)
3 to <4	1311	467	35.62% (33.08% to 38.25%)
≥4	1052	352	33.46% (30.67% to 36.37%)
not specified	292	137	46.92% (41.27% to 52.64%)
GOAT SAMPLES			
Estimated Age (years)	n Samples Tested	n CAE ELISA Positive	%CAE Positive With 95%CI
1 to <2	116	12	10.34% (6.02% to 17.21%)
2 to <3	86	17	19.77% (12.72% to 29.40%)
3 to <4	105	30	28.57% (20.81% to 37.85%)
≥4	128	8	6.25% (3.20% to 11.85%)

Table 3. Maedi-Visna sero-prevalence (ELISA) in sheep across Kosovo in 2016, by geographic area

Geographic Area	Number of Premises Sampled (and Number of Samples Tested)	Sero-prevalence (%) with 95% CI
Centre	53 (918)	30.83% (24.87% to 37.13%)
East	68 (1.137)	31.05% (24.96% to 37.43%)
North	33 (503)	12.92% (7.95% to 18.95%)
South	84 (1.339)	45.93% (40.89% to 51.01%)
West	80 (1.375)	39.20% (32.10% to 46.47%)
Overall	318 (5.272)	35.19% (32.13% to 38.29%)
Kosovo (Weighted for geographic area)		34.84% (31.79% to 37.94%)

Table 4. Caprine Arthritis-Encephalitis sero-prevalence (ELISA) in goats across Kosovo in 2016, by geographic area

Geographic Area	Number of Premises Sampled (and Number of Samples Tested)	Sero-prevalence (%) with 95% CI
Centre	12 (104)	4.81% (2.10% to 10.73%)
East	20 (124)	30.65% (8.05% to 56.79%)
North	4 (47)	4.26% (0.58% to 16.30%)
South	7 (61)	21.31% (8.00% to 40.06%)
West	11 (99)	9.09% (4.12% to 17.67%)
Overall	54 (435)	15.40% (7.05% to 25.40%)
Kosovo (Weighted for geographic area)		15.55% (7.20% to 25.55%)

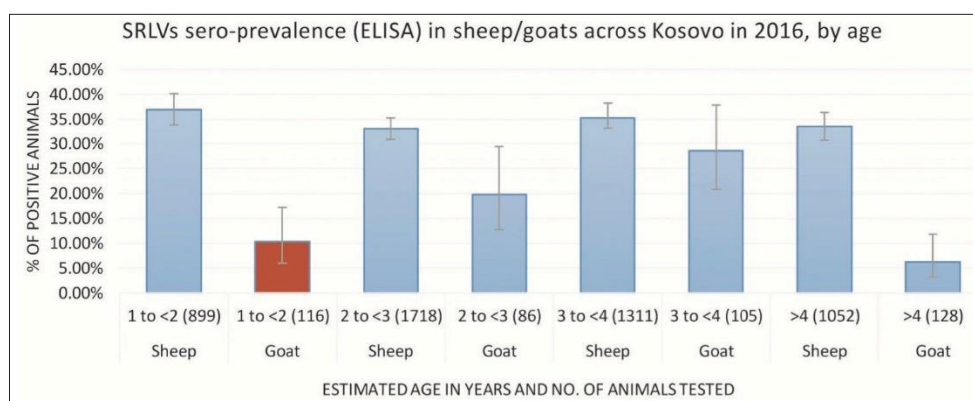


Fig 2. MWV/CAEV antibodies sero-prevalence (ELISA) in sheep and goats across Kosovo in 2016, by age. Estimated age in years and no. of animal tested (in brackets) are shown. The error bars indicate the Wilson score 95% CI calculated as for a simple random sample

The percentage sampling was similar in all geographic areas except the North, where a slightly lower percentage of sheep and higher percentage of goats were sampled. Any possible bias in the overall sero-prevalence estimate as a result of this was accounted for by using a weighted estimate.

Overall individual prevalence in sheep was 35% and flock prevalence was 85%. Average within flock prevalence for sheep was 40%. In total, 67 goat samples were positive giving an overall individual prevalence of 15% and flock prevalence of 35%. Average within flock prevalence for goats was 29%. Table 3 and Table 4 show the sero-prevalences calculated for each geographic area and the overall sero-prevalence both unweighted and weighted to account for differences between geographic distribution of the sample and the target population.

Sero-prevalence for SRLV antibodies in sheep is highest in the South and West geographic areas. Sero-prevalence for SRLV antibodies in goats is highest in the East and South geographic areas. There are statistically significant differences in SRLV sero-prevalence in sheep between geographic areas as indicated by an overall chi-square value of 203 (4d.f.) with a P-value of <0.00001. In pairwise comparisons, all geographic areas are significantly different from one another except the Centre and East (Fig. 1). There are statistically significant differences in SRLV sero-prevalence in goats between geographic areas as indicated by an overall chi-square value of 40.2 (4d.f.) with a P-value of <0.00001. Not all geographic areas are significantly different from one another. The distinct spatial groupings

are the South and East together, with an aggregated prevalence of 27.6% and the Centre, North and West together, with an aggregated prevalence of 6.4%. The difference between these two aggregated prevalences is statistically significant (chi-square value of 34.95 (1d.f.) with a P-value of <0.00001), while differences within these two groupings are not significant (Centre vs North vs West: chi-square p-value 0.3683; South vs East: chi-square P-value 0.2465) (Fig. 1).

The sero-prevalences for different age groups, where information on estimated age was provided with the sample are shown in Table 2 and Fig. 2, with Wilson score 95% CI calculated as for a simple random sample (not accounting for possible design effect).

There are no statistically significant differences between the four age groups in sheep (from <2 year to ≥4 year): chi square 5.285 (3d.f.) with P-value =0.1521. There are statistically significant differences between the age groups in goats: chi square 25.74 (3d.f.) with P-value =0.00001082. The sero-prevalence of the age group 1 to <2 years is significantly lower than the sero-prevalence of the age group 3 to <4 years. Also, the sero-prevalence of the age group ≥4 years is significantly lower than the sero-prevalence of the age groups 2 to <3 years and 3 to <4 years.

DISCUSSION

Although KFVL has previously tested samples from small ruminants for SRLV and found positive results, this had

not been as part of a formal survey and these results had not been reported internationally. The results reported in this paper are resulting from the first structured and country-based survey assessing the SRLV infection across Kosovo. Sero positive sheep and goats were found in all five geographic areas, which means that the headline result of the survey is that there is clear evidence that SRLV infection is present in sheep and goats throughout Kosovo. It is difficult to compare and elaborate the results with neighboring countries due to the extended period of time in reports and differences in study design. Nevertheless, the present data as well as those from neighboring countries suggest that SRLV infection at least in sheep must be endemic in the region. Although, the survey was not designed to estimate flock prevalence with any specified accuracy or precision, flock-level statistics were calculated and reported in the results but only succinctly even though the sample sizes are small. In particular, when analysing the sheep and goat samples separately the number of goat samples available per flock tended to be much fewer than the number of sheep samples. The probability of detecting goat flocks affected by SRLV would therefore be quite low. The flock-level estimates should be viewed cautiously, with the possibility in mind that the true percentage of flocks affected, particularly goat flocks affected by SRLV, could be higher than the percentage apparent from the survey results.

In order to avoid interference of maternal antibodies, only adult >1 year old sheep and goats were included in this study. It is recommended that serologic or molecular testing of lambs and kids occur at least 4-6 months following weaning^[18]. Previous studies have shown that if lambs are allowed to suckle naturally from positive dams and weaned at 8 months, maternal SRLV antibody is detectable starting the first day after suckling and up to 52 weeks of age in some lambs^[29]. In addition, under the same conditions, the SRLV provirus may be detectable in the peripheral blood mononuclear cell of lambs up to 24 weeks old^[29]. Present data suggest that different epidemiological scenarios might apply for sheep and goats. There are no statistically significant differences in SRLV sero-prevalence between sheep in different age groups from <2year to ≥4 year. The fact that sero-prevalent sheep appear to be retained in the population beyond 4 years old may suggest that the clinical impact of SRLV infection is mild in Kosovo sheep. This is in accordance with the fact that mortality due to SRLV infection in sheep may be low in enzootic areas^[5]. In contrast, for SRLV sero-prevalence in goats, there are statistically significant differences between the age groups. There is a steady increase in sero-prevalence from age group 1 to <2 years to age group 3 to <4 years, followed by a sharp decrease, with sero-prevalence in the age group ≥4 years being significantly lower than the sero-prevalence of the age groups 2 to <3 years and 3 to <4 years. This highlights the need for further investigation based on clinical impact of infection and genotype

characterization. However, this could also be explained as an artefact of the lower sample size per flock. This should be further explored through investigations with farmers and PVPs which can be focussed on those flocks where antibodies have been detected.

Kosovo has a very long tradition of sheep production. Sheep production is one of the sectors within Kosovo agriculture that suffered the most severe decline in the post-war period (after 1999). By November 2001, sheep populations were at 56% of their pre-war levels^[35]. Contrary, in former Yugoslavia, the number of goats dramatically dropped after the law of banning goats from fields had been passed in 1954 and lasted till 1989. Since then goat production was never important in Kosovo. After post-war (1999) small ruminant population bottleneck, there was a steady increase of sheep and goat population with a replacement from imported animals as a donation, and from other different sources (frequently uncontrolled). Therefore, multiple-source infections should be expected.

SRLV are heterogeneous, the strains circulating in different areas may differ from each other and thus the performance of diagnostic tests in these areas might vary accordingly. Clear differences have been found among the different ELISA tests in analytical and diagnostic sensitivity and overall diagnostic performance, whereas no significant differences in specificity were found^[19,36,37]. Interestingly, genotype A derived antigens seem more suitable than genotype B antigens to detect heterologous infection^[19,38]. On the other hand, sero-prevalence against genotype E may be underestimated using commercially available ELISAs^[39]. Although, IDEXX MV/CAE p28 Ab screening Kit used in this study is designed to detect a wide range of serological variants, some sensitivity issues might be expected^[37]. Therefore, before any strategy planning, further genotyping of the SRLVs is pivotal. Parturition in small ruminants in Kosovo is exclusively during winter months. Bleeding of animals was done during summer. Therefore, false negative results due to the fluctuation of antibodies during periparturient period^[20] are ruled out. In the absence of vaccination, test and slaughter is the only currently available strategy for control and elimination of these infections. However, this is not financially feasible under current conditions in Kosovo.

Moreover, any attempts of control strategies on a large scale will be hampered by uncontrolled and illegal movements of animals. Small ruminants are confined for long periods due to the harsh Balkan winter and that increases the risk of horizontal transmission. Some control of infection is possible with MV and CAE by separating lambs/kids from infected dams at the time of birth and running separate herds/flocks of uninfected and infected animals but this is complicated and probably not practical for many livestock keepers under conditions in Kosovo. However, at present, it is the only applicable measure to control the prevalence of SRLV infections in Kosovo. Owners are usually unaware

of the role of SRLV infections in animal welfare and economics of sheep and goat farming. Existing authorities should pursue and encourage an active information policy through pre-existing animal health information channels and private veterinarians. Regional cooperation is a must in order to ensure a successful control program.

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First evidence of Schmallenberg virus infection in domestic ruminants in Kosovo and Albania

Agim Rexhepi¹, Kerstin Wernike², Kristaq Berxholi³, Nue Marku³,
Jeton Spahiu¹ and Kurtesh Sherifi^{1*}

¹University of Prishtina “Hasan Prishtina”, Faculty of Agriculture and Veterinary, Kosovo.

²Friedrich Loeffler Institute, Riems, Germany.

³University of Agriculture, Faculty of Veterinary Medicine, Tirana, Albania.

*Corresponding author at: University of Prishtina “Hasan Prishtina”, Faculty of Agriculture and Veterinary, Kosovo.

E-mail: kurtesh.sherifi@uni-pr.edu.

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Schmallenberg virus,
Simbu serogroup,
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Summary

Schmallenberg virus (SBV), a novel Orthobunyavirus, emerged in European domestic ruminants in 2011 causing abortions and malformations in newborns and none or mild clinical symptoms in adult animals. Here, a total of 364 bovine, ovine and caprine serum samples were collected in Kosovo and Albania between May 2014 and August 2016 and analyzed for the presence of anti-SBV antibodies. Sera were tested using an enzyme-linked immunosorbent assay (ELISA), and 48 ELISA-positive samples were subsequently analyzed by serum neutralization test (SNT). The overall percentage of ELISA positive results was 17.9%; 23.1% (53/229) was the prevalence observed in Kosovo (cattle 45.5%, sheep 19.2% and goat 6.8%), while 8.9% (12/135) was that observed in Albania (cattle 11.1%, sheep 0% and goat 20.0%). SNT confirmed the presence of neutralizing antibodies against SBV in all samples tested. This is the first study reporting SBV circulation in domestic ruminants in Kosovo and Albania, with indication that this virus has been present in Kosovo and Albania at least since 2014 without being detected.

Introduction

In November 2011, a novel infectious agent named Schmallenberg virus (SBV) was reported for the first time in adult dairy cows from farms located in Northwest Germany and the Eastern region of the Netherlands (Hoffmann *et al.* 2012). SBV is a member of the genus *Orthobunyavirus* within the family *Peribunyaviridae*. Since its emergence, circulation of SBV has been confirmed from continental Europe to Scandinavia and the British Isles. From the initially most affected countries the following seroprevalences were reported: Netherlands 72.5% in cattle (Elbers *et al.* 2012), Belgium 90.8% in cattle (Garigliany *et al.* 2012a), in France 90% in cattle and 30% in goats (Gache *et al.* 2014), and in Germany 61% in cattle, 24.7% in sheep and 26.4% in goats (Wernike *et al.* 2014). Clinical signs of SBV infection include reduced milk yield, inappetence, and diarrhea in adult ruminants lasting for a few days (Hoffmann *et al.* 2012), and multiple malformations in newborns or aborted ruminants when dams are infected during a critical phase of gestation (Peperkamp 2015). The main fetal malformations

induced by SBV are arthrogyrosis, torticollis, ankylosis, kyphosis, scoliosis, brachygnathia inferior, hydrocephalus, and various malformations of the central nervous system, including porencephaly and hydranencephaly (van den Brom *et al.* 2012, Garigliany *et al.* 2012b, Bilk *et al.* 2012, Herder *et al.* 2012). The incidence of malformation on farms where SBV infection was confirmed varies from 2% in goat kids and 0.5%-3% in calves (Veldhuis *et al.* 2014, Dominguez *et al.* 2014) to 3%-19% in lambs (Harris *et al.* 2014, Meloni *et al.* 2017). SBV is transmitted by hematophagous biting midges (*Culicoides*) (De Regge *et al.* 2012). The presence of species of *Culicoides* of the *Obsoletus* and *Pulicaris* Complexes are reported in Kosovo (Berisha *et al.* 2010). Direct horizontal virus transmission in sheep and cattle by contact seems highly unlikely (Wernike *et al.* 2014); however there is evidence for the presence of SBV in bull semen (Ponsart *et al.* 2014, Schulz *et al.* 2014). SBV infections can be detected by molecular methods or by serology (Beer *et al.* 2013, De Regge *et al.* 2013, Wernike *et al.* 2016). The aim of this study was to investigate

the exposure to SBV of cattle, sheep, and goats in Kosovo and Albania.

Materials and methods

Serum samples were collected during the years 2014 (March, May, July, August, September, December), 2015 (February, July, September), and in 2016 (August) and were stored at - 20 °C until use. The animals originated from 102 different ruminant farms (36 cattle, 63 sheep, and 3 goats) located in 16 different municipalities in Kosovo and 6 municipalities in Albania. Serum samples were collected from a total of 364 animals including 154 cattle, 161 sheep and 49 goats. Animals were from different breeds of cattle (Simmental, Holstein, Busha and mixed breed), sheep (Bardhoka, Sharri sheep) and goat (mixed breed), and their age ranged from 1 to 10 years. Abortions were reported at least in two dairy cattle farms in Kosovo.

All sera were tested using a commercially available enzyme-linked immunosorbent assay (ID Screen® Schmallenberg virus competition multispecies, ID.vet, Montpellier, France) according to the manufacturer's instructions. As a confirmatory test, 48 ELISA-positive sera were additionally analyzed by a serum neutralization test (SNT) performed as described previously (Wernike *et al.* 2013a). All samples were tested in triplicate and the neutralizing titers were calculated as the reciprocal of the serum dilution that still inhibited > 50% of cytopathogenic effect (ND₅₀) according to Behrens and Kaerber.

Results

Antibodies against SBV were detected by ELISA in 17.9% (65/364) of the samples collected from domestic ruminants. Since the nucleocapsid protein based ELISA reacts not only with anti-SBV antibodies, but also with antibodies against closely related viruses from the Simbu serogroup (cross-reactivity) (Bréard *et al.* 2013), a subset of 48 samples that scored positive in the ELISA was additionally analyzed by the more specific SNT. All samples tested positive, the neutralizing titers against SBV ranged from 1/6 to 1/572.

The percentage of ELISA positive results in samples from Kosovo was 23.1% (53 of 229 animals) and 8.9% (12 out of 135) in Albania scored positive (8.9%) indicating the recent circulation of SBV in both countries. The highest percentage of seropositive results was found in cattle 36/154 (23.4%), followed by sheep 25/161 (15.5%) and then goats 4/49 (8.2%). The total number of seropositive farms was 40/102 (39.22%) and 14 of the 25 municipalities (56%) were affected, their geographical location is depicted in Figure 1. The individual numbers of

SBV antibody positive samples per municipality and animals species are given in Table I.

Discussion

Sampled dairy cattle farms with reported abortions tested positive for SBV antibodies, therefore, future

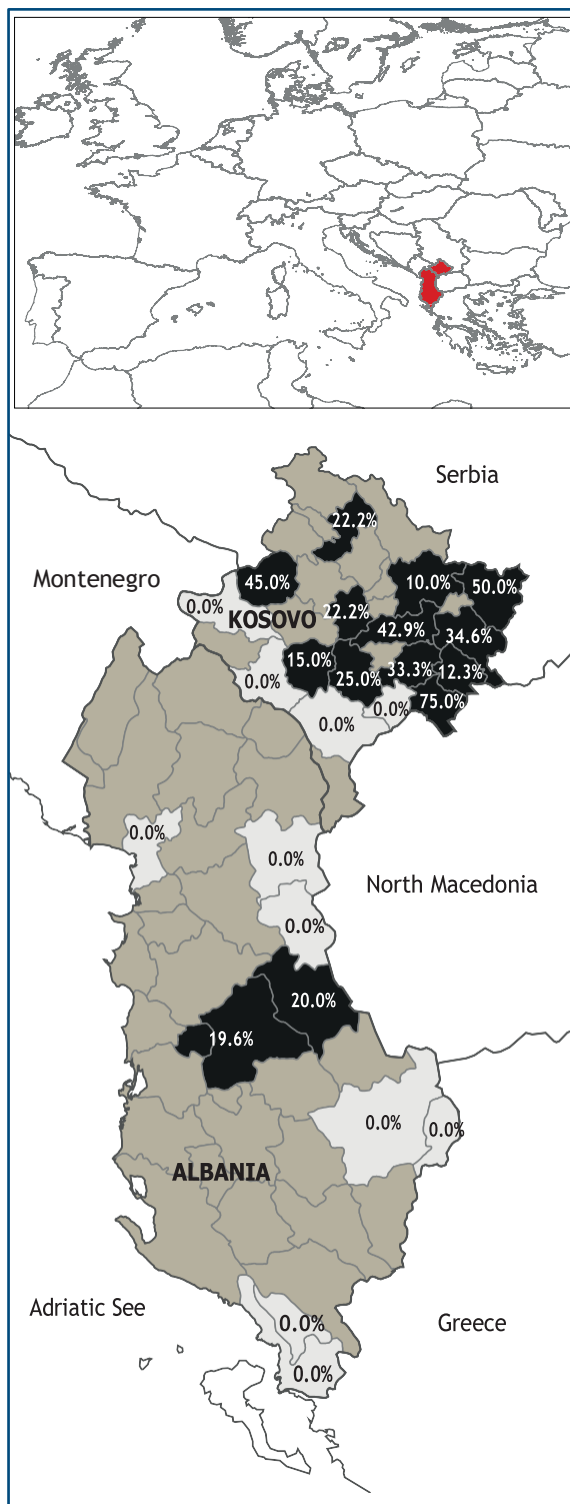


Figure 1. Geographical location of SBV in Kosovo and Albania.

Table 1. SBV seroprevalence in domestic ruminants in Kosovo and Albania.

SBV seroprevalence: sample no. (positive samples)					
Municipalitie:	Sample no.	Cattle	Sheep	Goat	%
Drenas (KOS)	9	-	9(2)	-	22.22%
Ferizaj (KOS)	3	-	3(1)	-	33.33%
Gjakovë (KOS)	1	-	1(0)	-	0.00%
Gjilan (KOS)	26	2(2)	24(7)	-	34.62%
Istog (KOS)	20	19(9)	1(0)	-	45.00%
Kaçanik (KOS)	4	2(2)	2(1)	-	75.00%
Kamenicë (KOS)	14	10(7)	4(0)	-	50.00%
Lipjan (KOS)	7	-	7(3)	-	42.86%
Mitrovicë (KOS)	9	7(1)	2(1)	-	22.22%
Pejë (KOS)	2	-	2(0)	-	0.00%
Prishtinë (KOS)	20	12(2)	8(0)	-	10.00%
Prizren (KOS)	4	-	4(0)	-	0.00%
Rahovec (KOS)	40	-	40(6)	-	15.00%
Shtërpcë (KOS)	1	-	1(0)	-	0.00%
Suharekë (KOS)	4	-	4(1)	-	25.00%
Viti (KOS)	65	3(2)	18(3)	44(3)	12.31%
Elbasan (ALB)	56	49(11)	7(0)	-	19.64%
Dibër (ALB)	11	11(0)	-	-	0.00%
Korçë (ALB)	14	14(0)	-	-	0.00%
Lezhë (ALB)	48	24(0)	24(0)	-	0.00%
Librazhd (ALB)	5	-	-	5(1)	20.00%
Sarandë (ALB)	1	1(0)	-	-	0.00%
Total	364	154(36)	161(25)	49(4)	17.9%

studies should focus on the detection of the virus itself in addition to antibodies against it. However, the viremia in adult animals is very short-lived. It lasts 3 to 6 days only (Hoffmann *et al.* 2012, Wernike *et al.* 2013b) making serological analysis a more appropriate tool for detecting SBV infection. In addition, not every malformed fetus suspected of SBV infection tested positive by PCR (De Regge *et al.* 2013, van Maanen *et al.* 2012).

The overall percentage of seropositive results in the three sampled years were: 24.75% (25/101) in 2014, 17.76% (19/107) in 2015, and 13.46% (21/156) in 2016. This apparent decrease in percentage of

seropositive results has been previously observed in other affected countries as well (Meroc *et al.* 2013, Wernike *et al.* 2015). It might suggest that the virus was firstly introduced into Albania and Kosovo in 2014 or earlier and in the following years it has circulated only to a limited extent. The reduced virus circulation in 2015 and 2016 could have led to a decline in herd seroprevalence caused by a missing infection of the young livestock.

The differing percentage of seropositive results in the tested animal species, i.e. 23.4 % in cattle, 11.5% in sheep and 8.2% in goats, and their range between the municipalities are consistent with studies performed in other European countries such as the Netherlands, Belgium, France, Germany, Turkey or Greece in which SBV has already circulated. Ranges of 8.0% to 95% have been reported in cattle, 1.6% to 89% in sheep and 2.0% to 50.8% in goats (Elbers *et al.* 2012, Garigliany *et al.* 2012a, Veldhuis *et al.* 2013, Azkur *et al.* 2013, Chaintoutis *et al.* 2014, Wernike *et al.* 2014). This observation and the likewise wide range of percentages of seropositive results in the farms included in the present study could be related to the age of the animals, the landscape (availability of breeding sites of the insect vectors) or to the husbandry system as e.g. grazing has been identified as a risk factor for SBV infections when compared to herds in which animals are kept indoors (Veldhuis *et al.* 2014). The reasons for the spatio-temporal distribution of SBV seropositivity in Albania and Kosovo as well as the impact on animal production in these two countries should be evaluated in future studies.

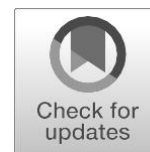
Conclusions

This is the first report on the presence of SBV in Kosovo and Albania. Our results indicate that SBV has been circulating in domestic ruminants in Kosovo and Albania at least from the year 2014 without being detected. Further introductions of vector-borne diseases have to be expected in these two countries, therefore strengthening of the regional surveillance and control strategies for emerging pathogens in animals as a part of global scientific efforts is required.

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Presence of veterinary antibiotics in livestock manure in two Southeastern Europe countries, Albania and Kosovo

Dritan Topi¹ · Jeton Spahiu²

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Abstract

Nowadays, veterinary drug application has become an integral practice in livestock farming. Veterinary antibiotics (VAs) are administered onto animals for therapeutic use; meanwhile, in some countries, they are used for growth promotion. To indicate the level of VAs use in livestock breeding in two countries, Albania and Kosovo, their presence was studied in the animal manure. In total, 38 manure samples, 22 from Kosovo and 16 samples from Albania, belonging to cattle, pig, and poultry, were collected and investigated for the presence of VAs. Seven VAs and 2 metabolites, from the groups of sulfonamides and tetracyclines, were identified by ultra-high pressure liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). The detected antibiotics were sulfadiazine (SDZ), sulfathiazole (STZ), sulfamethazine (SMZ), oxytetracycline (OTC), tetracycline (TC), chlortetracycline (TC), and doxycycline (DOY). VAs were detected in 27% and 31.2% of the manure samples, from Kosovo and Albania, respectively, and the levels ranged from 0.04 to 10.1 mg kg⁻¹. VAs were widely detected (100%) in poultry manure from Kosovo, as well as poultry manure from Albania. The contamination rate ranged from pig manure (25%) to cow manure (66.6%). Sulfonamides were the most commonly detected VAs with maximum concentration of sulfadiazine (10.1 mg kg⁻¹) in poultry manure. Tetracyclines were most widely detected in poultry manure, as well as other animal manures. When it comes to the comparison between the two countries, VAs residues are more frequent per analyzed sample and higher in concentrations in the manure samples from Albania. Therefore, an environmental impact of VAs on both countries may be expected. These results indicate that VAs may enter the local ecosystem through manure application to agriculture and potentially may bring ecological risks.

Keywords Veterinary antibiotics · Tetracyclines · Sulfonamides · Livestock manure · Albania · Kosovo

Introduction

Application of veterinary pharmaceuticals has become an integral part of animal husbandry, with antibiotics the most widely spread class (Sarmah et al. 2006; Baynes et al. 2016). Veterinary antibiotics (VAs) have been applied for therapeutic use, as well as growth promoters in food animals (Chee-

Sanford et al. 2009; Marshall and Levy 2011). In some parts of the world, they are used at sub-therapeutic levels, as additives to animal feed as growth promoters. In contrast, the use of antibiotics as growth promoters is banned in European Union (European Parliament and the Council 2003); meanwhile in the USA, since January 2017, the veterinary feed directive (VFD) has been expanded to antibiotics used in feed for prevention control (Baynes et al. 2016). As a result of a poor metabolism in the animal gut, their pharmacokinetics, and specific physical processes, the applied VAs and their metabolites reach the soil and water compartments via manure application (Boxall et al. 2004; Spielmeyer et al. 2015; Wohde et al. 2016; Hamscher and Bachour 2018). Significant amounts of VAs, their metabolites, or degradation products reach agroecosystems through their fertilization with antibiotic-contaminated manures, or irrigation with contaminated surface waters, and lesser proportion through their aerial transport (Wohde et al. 2016; Kim et al. 2011; Liu 2012), where they can persist for many years or even decades

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* Jeton Spahiu
Jeton.Spahiu@rks-gov.net

¹ Faculty of Natural Sciences, University of Tirana, Blvd. Zogu 1, No. 25, Tirana, Albania

² Food and Veterinary Agency, Prishtina, Kosovo

(Baguer et al. 2000; Spielmeier 2018). Extensive use of VAs on intensive livestock farms has imposed large amounts to reach agricultural fields through manure fertilization practices (Jechalke et al. 2014).

More than 2000 manufactured veterinary pharmaceuticals, with approximately 400 active chemical ingredients, have been in use for over 60 years (Tasho and Cho 2016). The annual global application of antimicrobials in animal food production was estimated at 63,000 t, only during 2010. Published data project an impressive rise, by 67% or up to 106,000 t until 2030. This projection mainly will be driven by the growth in consumers' demand for animal proteins in middle-income countries, as well as the projected population growth in five major emerging economies (Van Boeckel et al. 2015). According to the European Surveillance Agency on Veterinary Antimicrobial Consumption (ESVAC), the overall antibiotics' sale in 30 European countries has reached 7860 t for food-producing animals, where tetracyclines, penicillins, and sulfonamides, together, account for 70% of the total VAs (ESVAC 2018). One study conducted in 25 European states, referring to the total sales of veterinary antimicrobial agents during 2011, accounted for tetracyclines and sulfonamides, 37% and 11%, respectively (Grave et al. 2014). The analysis of veterinary drug residues is not regulated in animal manure within the European Union while regulated in products of animal origin under Commission Decision 2002/657/EC (EU 2002). In the USA, there are approximately 122 tetracycline approvals, which include various salts of tetracycline, oxytetracycline, and chlortetracycline, with about 30 of these products approved for therapeutic use. The sulfonamides, one of the oldest groups of antimicrobials, have been used in food animal production for over 60 years (Baynes et al. 2016). The global average annual consumption of antibiotics per kilogram of animal produced was estimated to be 45 mg kg⁻¹, 148 mg kg⁻¹, and 172 mg kg⁻¹ for cattle, chicken, and pigs, respectively (Van Boeckel et al. 2015). Continuous use of a wide variety of VAs has led to concern on the potential for the development of antibiotic-resistant bacteria (Kim et al. 2011). It is recognized as a global health problem by major health organizations and identified as one of the top health challenges in human and veterinary medicine in the twenty-first century (Marshall and Levy 2011).

The VAs metabolites formed inside the animal during their metabolism are different from the transformation products developed, from excreted parent compounds and metabolites, in the environment (Wohde et al. 2016). Transformation rates of antibiotics are affected by different environmental conditions and medium matrix. A wide range of VAs half-lives are reported, varying from 3 days for chlortetracycline in composted manure in 53 to > 77 days for doxycycline in biosolids storage (Massé et al. 2014). Generally, it is accepted for faster degradation rates of VAs and their metabolites under aerobic

compared with anaerobic conditions (Wohde et al. 2016). Plants cultivated on soils fertilized with animal manure might potentially take up antibiotics from the ground and translocate them in the plant or fruit (Kumar et al. 2005). Significant fractions of most VAs that enter the soil compartment via manure usually are retained in the surface (Kuppusamy et al. 2018), while a portion may leach into groundwater and surface water bodies by infiltration and runoff of agricultural fields (Kim et al. 2011; Pan and Cu 2017; Kivits et al. 2018).

There is a growing public health concern related to the increase in antimicrobial resistance. An essential contribution to this increase originates from antibiotics applied to husbandry animals. The animal manure, contaminated with VAs, applied to the agroecosystems may affect the soil microbial community by creating an ideal selective pressure to the bacterial strains carrying antibiotic-resistant genes (Gullberg et al. 2011). It is not the only way of spreading the bacteria carrying resistance genes. It occurs through direct or indirect contact, through the food, water, or animal excrements to the food (European Commission 2005; Marshall and Levy 2011; WHO (World Health Organization) 2014; Van Boeckel et al. 2015). Because of antimicrobial resistance concerns, FDA has also discouraged the use of antibiotics as growth promoters in livestock (Baynes et al. 2016).

Livestock breeding represents an integral part of the agriculture economy in both countries, with notable differences in livestock production. Albania features small cattle and pig farms, whereas intensive livestock farming plays the most crucial role in poultry production. Annual statistical reports covering the period from 2012 to 2017 indicate a decrease of 9.6% for the livestock's total number, including cattle (4.6%) and poultry (13.2%), respectively. In contrast, a reverse trend was evidenced in pigs and sheep/goat livestock, resulting in increased numbers by 13.2% and 9.2%, respectively (Supplementary Material, Table 1) (MARD 2018). Current data on livestock economy in Kosovo show that cattle and poultry are the main breeding species. Statistics for the period 2012–2017 indicate decreasing trends on the pig (21.9%), cattle (19.5%), and sheep and goat (11.5%) production, while a notable increase is considered in poultry economy (29.8%) leading to an overall rise of the country' livestock with 19.9% (Supplementary Material, Table 2) (FVA (Food and Veterinary Agency) 2019).

This study aims to describe the current situation regarding VAs residues in animal manure obtained from livestock breeding in two Southeastern European countries, Albania and Kosovo. Considered as developing countries, they are facing significant changes in their agricultural production, with livestock breeding as a vital economic resource. Data on VAs residue will allow for an assessment of the environmental impact produced by animal breeding and manures used as fertilizers in arable lands.

Material and methods

Sampling and field sites

The sampling procedure was accomplished during October 2018, by taking into consideration the regional distribution and livestock species in Albania and Kosovo. The sampling procedure was adopted, according to Martin and Beegle (2009). Briefly, a composite fresh manure sample of 10 kg was prepared from multiple subsamples, taken with clean steel shovels, and placed in a clean plastic bucket to mix thoroughly. A subsample of 100 g from the mixed composite samples was placed in plastic bottles. Samples were kept in the icebox and transferred in frozen conditions to the laboratory.

In total, 22 manure samples were collected from Kosovo: four from poultry, six from cattle/calf, and twelve from pig, respectively. From Albania, 16 samples were collected: ten from pig, two from chicken, three from cattle/calf, and one from sheep. The sampling sites strategy consisted of the selection of regions with the main contribution to intensive livestock breeding. In the case of Albania, these regions were Fieri, Durrësi, and Lezha, as well as Korça, an agricultural district in the Eastern part of the country (Fig. 1a). In the case of Kosovo, manure sampling was limited to the regions of Gjilani and Gjakova (Fig. 1b). Detailed information on manure samples regarding the type of animal and the region is provided in the Supplementary Material (Table 3).

Reagents and standards

The sulfonamides and tetracycline standards (96.1% purity) were purchased from Sigma-Aldrich. The sulfonamides used in our investigation were sulfathiazole (STZ), sulfamethiazine (SMZ), and sulfadiazine (SDZ), while for tetracycline, oxytetracycline (OTC), doxycycline (Dox), chlortetracycline (CTC), iso-chlortetracycline (iso-CTC), and tetracycline (TC) were used. All chemicals used were of analytical and HPLC grades, while ultrapure water used in the analysis was produced from Milli-Q system (Millipore, Eschborn, Germany). The sulfonamides and tetracycline stock solutions were prepared by dissolving 10.0 ± 0.1 mg of each standard in 10 mL methanol resulting in 1 mg/mL stock solutions. Working dilutions were prepared freshly on the day of use while stored at -80 °C until the day of analysis.

Liquid chromatography-tandem mass spectrometry and quantification

Samples were prepared and analyzed according to Spielmeier et al. (2014). In brief, 1 g sample material was extracted with methanol/ethanol/dichloromethane (1:3:1, v/v/v). Analysis of the VAs extract was performed in the multiple reaction monitoring modes by UHPLC-MS/MS using two transitions per



a



b

Fig. 1 Region locations of collected manure samples in (a) Albania and (b) Kosovo (<http://www.encyclopædiabritannica.com>)

compound. Due to a new MS system (QTrap 3200, ABScienc, Darmstadt, Germany), lower limits of detection (LOD) and limit of quantifications (LOQ) were obtained (Table 1). LODs for veterinary antibiotics belonging to the sulfonamides group were below or equal to 10 g kg^{-1} , while the LOD values for VAs to tetracyclines group were higher, with LOD of doxycycline 75 g kg^{-1} . This method is able to screen the presence of 14 VAs and one metabolite from two groups: sulfonamides and tetracyclines.

Mass spectrometry was carried out using a LCQ ion trap with an electrospray ionization source (Finnigan Mat, San Jose, CA). Standard compounds ($10 \text{ ng/}\mu\text{L}$) were infused through an integrated syringe pump at a flow rate of

Table 1 Limits of detection (LOD), limits of quantifications (LOQ), recoveries, and RSD of the 14 investigated antibiotics and a metabolite of chlortetracycline in manure

Substance	LOD (mg kg ⁻¹)	LOQ (mg kg ⁻¹)	Recovery (&)	RSD (%)
Sulfonamides				
Sulfachlorpyridazine	0.008	0.025	101.5	9.4
Sulfadiazine	0.008	0.025	98.1	8.1
Sulfadimethoxine	0.003	0.008	84.6	7.8
Sulfaguanidine	0.010	0.050	85.6	8.9
Sulfamerazine	0.005	0.025	78.1	6.1
Sulfamethazine	0.008	0.025	98.4	3.2
Sulfamethoxazole	0.010	0.025	96.1	4.5
Sulfamethoxypyridazine	0.005	0.025	88.1	6.6
Sulfapyridine	0.005	0.025	90.5	8.9
Sulfathiazole	0.010	0.075	88.7	9.1
Tetracyclines				
Chlortetracycline	0.025	0.100	102.3	3.2
Doxycycline	0.075	0.300	99.3	7.8
Iso-chlortetracycline	0.010	0.050	89.6	5.6
Oxytetracycline	0.050	0.150	97.7	9.8
Tetracycline	0.010	0.100	96.5	6.7

Values refer to mg per kg fresh weight; due to missing standard compound, values cannot be provided for the metabolite 4-hydroxy-sulfadiazine

10 $\mu\text{L}/\text{min}$ for tuning the mass spectrometer and optimizing capillary temperature, sheath gas, and auxiliary gas flow rates. The polarity source was set positive for all compounds; the spray needle voltage was 5 kV. Drying gas was nitrogen generated from pressurized air in an Ecoinert 2 ESP nitrogen generator (DWT-GmbH, Gelsenkirchen, Germany). The optimized conditions were as follows: sheath gas flow was set at 100 units, the auxiliary gas was turned off, and the capillary temperature was 150 °C. LOD, LOQ, recovery, and RSD are summarized in Table 1. MS(3) is a technique that can be exclusively performed on ion trap mass spectrometers. The HPLC system employed was a gradient system consisting of a Thermoquest P4000 pump, an AS3000 autosampler (San Jose, CA) and a Puresil C18 column (5 μm , 4.6 \times 150 mm, Waters Corp., Milford, MA) operated at 23 °C. The flow of 1 mL/min was split 1:10 before entrance into the mass spectrometer. The mobile phase consisted of 0.5% formic acid in water with 1 mM ammonium acetate (solvent A, pH 2.5) and acetonitrile (solvent B). The gradient run was 0-50% B in 9 min and then held at 50% B for 1 min. After each run, the column was rinsed for

3 min with 99% acetonitrile and re-equilibrated for 12 min with solvent A. The injection volume was 1-2 μL . After each injection, the autosampler was rinsed with 3 mL of 90% methanol/10% 10 mM oxalic acid in water. Calibration curves constructed ranged from 0.1 to 10 ng per injection and were linear, with $r^2 > 0.99$ for the MS-MS procedure. The quantification procedure was

obtained by comparing the peak areas with external calibration curves, and all data were corrected for recovery.

Results and discussion

Antibiotic use in the animal husbandry in Albania and Kosovo

A report from the World Organization for Animal Health (OIE) concluded that this practice of using antibiotics as growth promoters is banned in many parts of the world. However, it has found that 45 countries out of 155 are still supplying them as a growth promoter (OIE 2019). According to the literature review, there is no published information on statistics for antibiotic use in livestock farming in Albania and Kosovo. An estimation over the VAs use was calculated through extrapolation on each country's meat production data and the average Veterinary Pharmaceutical Consumption Factor (VPCFa) calculated by Kools et al. (2008) based on the reported European animal meat production. Differences that exist between the livestock economies in the EU countries and two countries in our study might not present the exact values of the VAs application in animal husbandry in two states, referring to the VPCFa approach. A combination of the VPCFa (135 mg antibiotics kg^{-1} meat) and the country's annual meat production for 2017 is said to be 161,200 t; eventually, an amount of 21.8 t VAs was estimated to have been used in Albania, while for Kosovo, this value was 15.1 t.

VA occurrence in livestock manure in countries of the study

This study presents the occurrence of VAs in the livestock manures in Albania and Kosovo, two countries situated in Southeastern Europe. The employed analytical method screened the presence of 14 VAs and one metabolite belonging to the sulfonamides and tetracyclines classes (Table 2). One or more antibiotics were simultaneously detected in 11 out of the 38 manure samples or resulting in 28.9% positive manure samples. The concentration levels varied from 0.04 to 10.1 mg kg⁻¹. The VAs levels for both countries are presented in Table 2. The VAs belonging to the tetracyclines class were the most common in ten out of 11 positive manure samples investigated with VAs presence. Referring to the total manure samples, the incidence rate of tetracyclines was 26.3%. The highest value belonged to poultry manure, with 3 out of 5 samples, and co-occurrence of 3 or more tetracyclines. The second group with tetracyclines co-occurrence belonged to the cattle manure samples with residual levels variation of 0.10-3.91 mg kg⁻¹. From 4 tetracyclines and one metabolite, the tetracycline (TC) was the most common antibiotic, even though chlortetracycline (CTC) was detected in the highest concentration (3.91 mg kg⁻¹), in the range of approximately 10-15 times higher compared with TC or OTC. Tetracyclines' presence rates were analyzed in the following sequence order: TC > OTC = CTC = Iso-CTC > DOXY.

The sulfonamides co-occurrence in livestock manure was found less frequent compared with tetracyclines. It is in line with the use of tetracyclines in animal feeding operation,

which is not always familiar with sulfonamides for operators. Meanwhile, the concentration levels showed a variation factor of 250. Regarding three sulfonamides and one metabolite, their presence rates were less common in four out of 11 positive manure samples investigated with VA presence, which means 10.5% to overall analyzed manure samples. However, the highest residue levels of the VA belonged to sulfonamides. The detectable levels to sulfonamides ranged 0.04-10.1 mg kg⁻¹ in the poultry manure. Sulfadiazine was the principal antibiotic both in residue level and incidence rate. Their presence rate was similar among different types of animal manure.

Antibiotics in manure samples from Kosovo

In the analyzed manure samples from Kosovo, in total, six VAs and one metabolite were detected in 6 out of 22 manure samples, marking a presence rate of 27.3%. VAs present in manure samples belonged to tetracyclines, which include oxytetracycline (OTC), tetracycline (TC), chlortetracycline (CTC), and doxycycline (DOXY). Besides, iso-chlortetracycline (iso-CTC) was detected as the primary CTC metabolite, and the second group of antibiotics, sulfonamides, includes sulfadiazine (SDZ) and sulfathiazole (STZ). The highest concentration was found in a poultry manure sample (3.91 mg CTC kg⁻¹ fresh weight). In the same manure sample, two other antibiotics were detected (0.31 mg OTC kg⁻¹ wet weight, 0.25 mg TC kg⁻¹ wet weight) accompanied by the metabolite iso-CTC. In three poultry manure samples, all VAs detected were below the LOQ. Low levels, together

Table 2 Co-occurrence and detected concentrations of seven VAs and two metabolites in animal manure from both countries

VA	Occurrence (%)			Range (mg kg ⁻¹)		
	Cattle (n = 9)	Pig (n = 22)	Poultry (n = 6)	Cattle (n = 9)	Pig (n = 22)	Poultry (n = 6)
SDZ	11.1	4.54	16.6	<LOQ	0.04-0.04	10.1-10.1
SDZ-OH	-	-	16.6	-	-	(1.4)*
STZ	-	4.54	-	-	0.11-0.11	-
SMZ	11.1	-	-	<LOQ	-	-
OTC	11.1	9.09	33.3	1.17-1.17	<LOQ-0.47	<LOQ-0.31
TC	11.1	4.54	83.3	<LOQ	<LOQ	<LOQ-0.25
CTC	11.1	4.54	50.0	0.10-0.10	0.68	0.12-3.91
Iso-CTC	11.1	4.54	50.0	<LOQ	0.25-0.25	<LOQ-0.73
DOXY	22.2	4.54	16.6	<LOQ-0.49	0.30-0.30	<LOQ
ΣSuls ^a				n.d-<LOQ	n.d - 0.15	n.d-11.5
ΣTCs ^a				n.d-1.76	n.d - 1.40	<LOQ-5.10
ΣAntibiotics ^a				<LOQ-1.76	0.15-1.40	<LOQ-13.44

SDZ sulfadiazine, SDZ-OH 4-hydroxy sulfadiazine, STZ sulfathiazole, SMZ sulfamethazine, iso-CTC iso-chlortetracycline, CTC chlortetracycline, DOXY doxycycline, OTC oxytetracycline, TC tetracycline. *SDZ-OH: semi-quantitative as no standard substance available; LOQ limit of quantification; n.d none detected. All values are given in mg kg⁻¹ fresh weight, ΣSuls sum of sulfonamides, ΣTCs sum of tetracyclines, Σantibiotics sum of antibiotics, ^a per positive samples

with low detection frequencies, were found for manure samples belonging to cattle and pig. All in all, the detection rates for at least one VA were 100% in poultry manures, 8.3% in pig manures, and 16.7% for cattle manure samples.

The VAs distribution pattern in poultry manure samples from two countries shows a non-significant difference regarding the antibiotic's occurrence in Kosovo poultry manure samples with three VAs and one metabolite, compared with three VAs and two metabolites found in poultry manure from Albania. The maximum value in poultry manure from Kosovo belonged to CTC (3.91 mg CTC kg⁻¹ fresh weight) compared with SDZ (10.1 mg kg⁻¹ fresh weight) in poultry manure from Albania.

In poultry manure, four tetracyclines and one metabolite analyzed, the antibiotics levels were generally lower compared with VAs concentrations in samples from China, except the CTC (Zhao et al. 2010; Ji et al. 2012). The CTC levels in samples from Kosovo were found in much higher manure concentration (3.91 mg kg⁻¹) compared with data from two studies in poultry manure from China. This pattern was different when compared with CTC (1.09 mg kg⁻¹) in poultry manure from Albania. The OTC presence in one poultry manure sample from Kosovo (0.31 mg kg⁻¹) was considerably lower compared with data published in both studies, respectively,

1.6 mg kg⁻¹ (Zhao et al. 2010) and 21.96 mg kg⁻¹ (Ji et al. 2012). Meanwhile, OTC was not present in samples from Albania. Two other tetracyclines, TC and DOXY, were found in significant differences compared with poultry manure samples from China. So, TC (10.31 mg kg⁻¹) according to (Ji et al. 2012), and DOXY (3.4 mg kg⁻¹) according to (Zhao et al. 2010) occurred in much higher values compared to TC concentration (0.25 mg kg⁻¹) and (0.12 mg kg⁻¹) in samples from Kosovo and Albania, respectively, while the DOXY presence was not observed in this samples. The VAs concentrations in poultry manure from both countries in the study were found in higher levels when compared with data from Austria (Martínez-Carballo et al. 2007). Sulfonamide analysis covered 10 different antibiotics for their presence in poultry manure samples in both countries. Only SDZ was detected in one sample from Albania (10.1 mg kg⁻¹). The SDZ concentration was much lower compared with SDZ levels (51.0 mg kg⁻¹) in poultry manure samples from Austria (Martínez-Carballo et al. 2007) while similar to data from China (8.03 mg kg⁻¹) according to Ji et al. (2012).

Antibiotic in manure samples from Albania

In the manure samples, six different VAs and two metabolites were found in five out of sixteen samples or 31.2% (Table 2). Two VAs belonged to the class of sulfonamides (SDZ and SMZ) while the other four VAs to tetracyclines (OTC, TC,

CTC, and DOXY). Also, two metabolites—4-hydroxy sulfadiazine and iso-chlortetracycline—were as well detected. The maximum concentration of studied VAs was detected in poultry manure, the sulfonamide SDZ (10.1 mg kg⁻¹). Comparison of manure samples concerning their animal species origin reveals that cattle manures are a source of five different VAs, OTC (1.17 mg kg⁻¹), CTC (0.1 mg kg⁻¹), and DOXY (0.49 mg kg⁻¹), together with SMZ and TC under LOQ levels. Three VAs and one metabolite were detected in pig manure, at very low concentrations. Altogether, 31.2% of investigated manure samples contained at least one VA.

The antibiotics spectrum included seven VAs, which belonged to the sulfonamides and tetracyclines classes. Referring to VAs co-occurrence and concentration levels, these data are in good accordance with investigations from different countries, such as Austria, Belgium, and the Netherlands (Table 3) (Martínez-Carballo et al. 2007; Berendsen et al. 2015; Van den Meersche et al. 2016; Widyasari-Mehta et al. 2016), but much lower compared with VAs in manure samples from Germany and China (Zhao et al. 2010; Ji et al. 2012; Spielmeyer et al. 2014).

The antibiotics' concentrations in pig manure, from both countries in the study, were generally similar with those found in pig manure in the Netherlands (Berendsen et al. 2015) but much lower compared with the data published in studies from Germany (Widyasari-Mehta et al. 2016), Belgium (Van den Meersche et al. 2016), and China (Ji et al. 2012). The CTC (0.68 mg kg⁻¹) was detected in samples from Albania and Germany (Widyasari-Mehta et al. 2016), but not in samples from Kosovo, the Netherlands (Berendsen et al. 2015), and China (Ji et al. 2012). The second antibiotic, from the tetracycline class, studied for its presence, DOXY, was not detected in pig manure samples in both countries, similar with data published for swine manure from China (Ji et al. 2012), while found in high levels in samples from Germany (Widyasari-Mehta et al. 2016) and Belgium (Van den Meersche et al. 2016), 27.4 mg kg⁻¹ and 22.8 mg kg⁻¹, respectively. The same pattern is present in the case of OTC, where concentrations found in samples from Albania and Kosovo resulted much lower compared with data from Germany (Widyasari-Mehta et al. 2016), Belgium (Van den Meersche et al. 2016), and China (Ji et al. 2012), while TC was not detected in our study, presenting a similar situation with the Netherlands (Berendsen et al. 2015) and Belgium (Van den Meersche et al. 2016). The concentrations of sulfonamides resulted much lower compared with data from Germany (Widyasari-Mehta et al. 2016), Belgium (Van den Meersche et al. 2016), and China (Ji et al. 2012) while similar with those presenting results from the Netherlands (Berendsen et al. 2015).

Evaluation of VAs concentrations in cattle manure samples revealed a better situation compared with data from recent studies worldwide (Ji et al. 2012; Spielmeyer et al. 2014). The co-occurrence of the analyzed antibiotics in samples from

Table 3 Comparison of antibiotic concentrations present in various manure samples worldwide with our results (mg kg⁻¹)

Country	Matrix	CTC	DOXY	OTC	TC	Iso-CTC	SDZ	SMZ	STZ	Reference
Netherlands	Swine feces	n.d	1.9	0.16	n.d	-	0.13	n.d	-	Berendsen et al. (2015)
Belgium	Swine manure ^a	n.d	22.8	2.0	n.d	-	3.0	n.d	-	Van den Meersche et al. (2016)
Germany	Pig manure ^b	37.4	27.4	13.6	152	-	7.3	2.2	-	Widyasari-Mehta et al. (2016)
China	Swine manure	-	-	18.7	12.27	-	4.87	7.56	-	Ji et al. (2012)
Albania	Pig manure	0.68	n.d	0.47	<LOQ	n.d	n.d	n.d	-	
Kosovo	Pig manure	n.d	n.d	<LOQ	n.d	0.25	0.04	-	0.11	
Netherlands	Cattle feces ^a	n.d	0.04	8.0	0.04	n.d	0.02	0.02	-	Berendsen et al. (2015)
China	Cattle manure	-	-	21.36	12.01	-	4.57	9.36	-	Ji et al. (2012)
Albania	Cattle manure	0.10	0.49	1.17	<LOQ	<LOQ	n.d	<LOQ	-	
Kosovo	Cattle manure	n.d	n.d	n.d	n.d	n.d	<LOQ	n.d	n.d	
Germany	Manure ^b	36.5	n.d	n.d	6.6	-	1.1	201	-	Spielmeier et al. (2014)
Austria	Chicken dung ^b	n.d	n.d	n.d	n.d	-	51	n.d	-	Martínez-Carballo et al. (2007)
China	Chicken dung ^b	1.1	3.4	1.6	n.d	-	0.15	0.43	-	Zhao et al. (2010)
	Chicken dung	-	-	21.96	10.31	-	8.03	8.62	-	Ji et al. (2012)
Albania	Chicken dung	1.09	n.d	n.d	0.12	0.73	10.1	n.d	-	
Kosovo	Chicken dung	3.91	n.d	0.31	0.25	0.63	n.d	n.d	n.d	

CTC chlortetracycline, DC doxycycline, OTC oxytetracycline, TC tetracycline, SDZ sulfadiazine, SMX sulfamethoxazole, SMZ sulfamethazine; ^a mg kg⁻¹ dryweight; ^b mg kg⁻¹ fresh weight

Albania was higher compared with cattle manure samples from Kosovo, 5 VAs and one metabolite, compared with one antibiotic. Overall, VAs from the tetracyclines class were present in the cattle manure samples from Albania, but not in samples from Kosovo. Also, antibiotics belonging to sulfonamides class were found below LOQ for SMZ in one sample from Albania and in the case of SDZ for one sample from Kosovo. VAs concentrations were much higher in studies from Germany (Spielmeier et al. 2014), the Netherlands (Berendsen et al. 2015), and China (Ji et al. 2012). The antibiotics' concentration in manure samples from Germany was CTC (36.5 mg kg⁻¹) and SMZ (201.0 mg kg⁻¹) (Spielmeier et al. 2014). In China, the highest concentrations belonged to OTC (21.36 mg kg⁻¹) and TC (12.01 mg kg⁻¹). VAs concentrations and their co-occurrence in cattle manure found in positive samples from Albania show similar patterns with data presented in positive samples from the Netherlands (Berendsen et al. 2015).

The co-occurrence of VAs in positive samples was evaluated as the sum of tetracyclines (ΣTCs), the sum of sulfonamides (ΣSulfs), and the sum of antibiotics. In the cattle manure samples, the ΣSulfs was detected below LOQ and the ΣTCs with the maximum value of 1.76 mg kg⁻¹ wet weight, which means that tetracyclines were the only contributors to the contaminated cattle manure samples. In pig manure, the ΣSulfs reached the maximum of 0.15 mg kg⁻¹ wet weight, the ΣTCs reached the peak (1.40 mg kg⁻¹ wet weight), and calculated as the sum of antibiotics was found in the interval 0.15

to 1.40 mg kg⁻¹ wet weight. Finally, the VAs concentration in poultry manure samples was calculated as the ΣSulfs, ΣTCs, and the sum of antibiotics: 11.5 mg kg⁻¹, 5.10 mg kg⁻¹, and 13.44 mg kg⁻¹ wet weight, respectively. These values are considerably lower compared with the data on VAs occurrence in livestock manure in China (Ji et al. 2012). Ji et al. (2012) found ΣTCs (30.97 mg kg⁻¹ wet weight) in pig manure, 33.37 mg kg⁻¹ in cattle manure, and 32.27 mg kg⁻¹ in poultry manure. The sum of sulfonamides in pig manure was 18.59 mg kg⁻¹ wet weight, in cattle manure 20.31 mg kg⁻¹, and in poultry manure 24.66 mg kg⁻¹, while the sum of antibiotics in pig manure was 60.57 mg kg⁻¹ wet weight, in cattle manure 66.58 mg kg⁻¹, and poultry manure 74.78 mg kg⁻¹.

Kim et al. (2011) concluded that proper composting practices application in livestock manures will eliminate the excreted VAs and their metabolites on a scale of more than 90%. In the case of Albania, the livestock manure composting practices consist mainly of the composting process. To the best of our knowledge, there is no one intensive farm that has implemented the anaerobic treatment of animal manure for both countries. However, referring to the efficacy, such treatment practice should not overestimate (Hamscher and Bachour 2018). The environmental issue arising from the application of contaminated animal manure with antibiotics and other veterinary drugs, both to arable fields, as well as dumping to surface waters, or wastewater systems, in both of these countries, urges for significant intervention to the treatment practices of livestock manure.

Conclusions

Seven VAs and two metabolites were detected in the livestock manure of three different animal species. Antibiotics belonged to sulfonamides and tetracyclines classes. Based on this study, we conclude that the assessed VAs in manure samples belong to the two most used antibiotic groups. Tetracyclines were most present antibiotics, especially in poultry manure. TC, OTC, CTC, and DOXY were the most typical antibiotics found in animal manure. Six VAs accompanied by two metabolites were found in manure samples from Albania while six VAs and one metabolite in Kosovo manure samples. VAs present in manure samples from Albania resulted in higher levels compared with manure samples from Kosovo. The maximum content was found in poultry manures for both countries, SDZ (10.1 mg kg⁻¹) from Albania and CTC (3.91 mg kg⁻¹) from Kosovo, respectively. Low levels of VAs indicate that the environmental impact originating from livestock farming in both countries is considered not an emergent situation. Lack of national programs to address the issue of manure disposal and implementation of anaerobic treatment procedures to livestock farms indicates that the actual situation in both countries needs to address this issue. Further studying the presence of antibiotics and other veterinary drugs in manure samples, arable land, and surface waters in both countries, including other neighboring countries, will give information on ecological risks accompanying the livestock manure applications for the entire region of Southeastern Europe.

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SEROPREVALENCE OF BLUETONGUE DISEASE VIRUS (BTV) AMONG DOMESTIC RUMINANTS IN KOSOVO AND FIRST RECORD OF BTV SEROTYPE 4 IN SHEEP

N. MARKU¹, K. BËRXHOLI¹, J. SPAHIU², K. SHERIFI³ & A. REXHEPI³

¹Agricultural University of Tirana, Faculty of Veterinary Medicine, Tirana, Albania; ²Food and Veterinary Agency, Kosovo; ³University of Prishtina Hasan Prishtina, Faculty of Agriculture and Veterinary Medicine, Kosovo

Summary

Marku, N., K. Bërxfholi, J. Spahiu, K. Sherifi & A. Rexhepi, 2019. Seroprevalence of bluetongue disease virus (BTV) among domestic ruminants in Kosovo and first record of BTV serotype 4 in sheep. *Bulg. J. Vet. Med.*, **22**, No 1, 50–56.

The objective of the study was to estimate the seroprevalence and serotype of bluetongue virus (BTV) in domestic ruminants in different regions in Kosovo, in years 2014 and 2015. A total of 905 blood sera were analysed: 633 from sheep, 204 from cattle and 68 from goats, collected in 170 farms, 88 villages in 18 municipalities. All samples were analysed with c-ELISA for detection of BTV seroprevalence. From sheep with clinical signs samples were collected and were analysed with specific RT-PCR. Out of all 905 samples analysed with c-ELISA, 105 samples (11.6%) were seropositive (53 ovine, 39 bovine and 13 caprine). The 43 samples from sheep with clinical signs for bluetongue disease were confirmed by RT-PCR, and BTV-4 serotype was identified. The results indicated high seroprevalence of BTV in domestic ruminants, evidence of BTV-4 serotype in sheep, suggesting a need to strengthen national and regional scientific efforts and control strategy to meet the global challenge of this infectious disease.

Key words: bluetongue, BTV-4, domestic ruminants, Kosovo, seroprevalence

INTRODUCTION

Bluetongue is an insect-transmitted viral disease of domestic and wild ruminants (Mertens *et al.*, 2004). Bluetongue virus (BTV) is a RNA virus, belonging to the non-contagious vectorborne *Orbivirus*, family *Reoviridae*. Twenty-six BTV serotypes of the virus are reported (Maan *et al.*, 2012). Multiple BTV serotypes (serotypes 1, 2, 4, 6, 8, 9, 11 and 16) have in-

vaded Europe since 1998 (Mellor *et al.*, 2009; Rodriguez-Sanchez *et al.*, 2008). Bluetongue disease with clinical signs was reported in Kosovo in 2001, caused by BTV-9 serotype (Osmani *et al.*, 2006).

The BTV is transmitted between its ruminant hosts by certain species of biting midges of the genus *Culicoides* (Darpel *et al.*, 2007). There are approximately 30

Culicoides species that play a role in the transmission of BTV across the world (Meiswinkel *et al.*, 2004; 2007). Presence of *Culicoides* containing *C. obsoletus* and *C. pulicaris* complexes but not *C. imiciola* is reported in Kosovo (Berisha *et al.*, 2010).

The global distribution of individual BTV serotypes is attributed to climate change and human influence in environment (Randolph, 2008; Maclachlan, 2010), involving different mechanisms in introduction process including the movement of infected livestock, the passive movement of infected *Culicoides* by the wind (Wilson & Mellor, 2009).

The virus can infect most species of domestic and wild ruminants, but in domestic sheep clinical signs are usually most severe. Cattle are the main reservoir for the BTV, although the cattle and goats disease usually assumes a subclinical course without severe symptoms (Darpel *et al.*, 2007). Serotype-specific vaccines for BTV, inactivated or live-attenuated are currently available (Savini *et al.*, 2008; Zientara *et al.*, 2010).

The seroprevalence of BTV using c-ELISA was reported in many studies, and it is probably the most widely used and validated method with high sensitivity and specificity (Hamblin, 2004). Sensitive and specific real-time RT-PCR assays have been developed to detect BTV types, targeting different genome-segment as reviewed by Hoffmann *et al.* (2009). Real-time RT-PCR assay for BTV-4 targeting genome segment 2, is reported to be a sensitive and reliable method for the identification and differentiation of the twenty-six BTV serotypes (Maan *et al.*, 2012).

The purpose of this research is to assess the distribution, seroprevalence and BTV serotype in domestic ruminants (sheep, cattle and goats) in Republic of Kosovo.

MATERIALS AND METHODS

Samples were collected in 88 villages in 18 municipalities (Klina, Gjilan, Gjakova, Shtërpçë, Kaçanik, Drenas, Dragash, Viti, Deçan, Mitrovica, Skenderaj, Shtime, Hani i Elezit, Lipjan, Vushtrri, Peja, Novobërda and Prizren) from a total of 170 farms. The total number of serum samples collected was 905, including 633 from sheep, 68 from goats, and 204 from cattle. Samples were taken during May-September in 2014 and 2015.

Serum samples were separated from sheep blood and were kept frozen at -20°C until used for detection of BTV-specific IgG. The testing of sera was performed by the competitive-enzyme-linked immunosorbent assay (c-ELISA) for detection of BT antibodies in ruminant sera using a commercial test (IDEXX[®], Westbrook, USA). Tests were conducted according to manufacturer instructions, with the known positive and negative controls tested on each plate.

The 59 blood samples collected from sheep with clinical signs for bluetongue were also tested using type-specific real-time RT-PCR assay for BTV-4 targeting genome segment 2, for the presence of this serotype in the samples tested. Molecular analyses were done at the Pirbright Institute, UK.

Composition of reaction mixes for one-step real-time RT-PCR in total volume of 15 μL contained: EXPRESS SuperScript[®] qPCR SuperMix Universal (10 μL), Forward primer (0.4 μL), Reverse primer (0.4 μL), Probe (0.8 μL), RNase free water (1 μL), ROX (1/10 dilution)(0.4 μL), and EXPRESS SuperScript[®] mix (2 μL).

The primers for RT-PCR and thermal profile are presented in Tables 1 and 2.

Table 1. Primers and probes used for one-step RT-PCR of bluetongue virus (Hofmann *et al.*, 2008)

Primer/ probe	Oligo name	Sequence (5'-3')	Working concentra- tion (µM)
For- ward	Hofm_BTV_IVI_F2	TGGAYAAAGCRATGTCAA	20
Reverse	Hofm_BTV_IVI_R2	ACRTCATCACGAAACGCTTC	20
Probe	Hofm_BTV_IVI_P	FAM-ARGCTGCATTCGCATCGTACGC- Tamra	5

Table 2. Real time RT-PCR thermal profile

Stage of real-time RT-PCR	Temperature	Duration	Number of cycles
Reverse transcription	50 °C	15 min	1 cycle
RT inactivation/ Taq activation	95 °C	20 s	1 cycle
	95 °C	3 s	
PCR	56 °C	30 s	45 cycles
	72 °C*	30 s*	

*Read fluorescence at the end of this stage.

RESULTS

Clinical observations

In summer 2014, clinical symptoms indicative of bluetongue were observed in sheep herds in most of regions in Kosovo. The clinical findings were typical for the disease: abundant salivation, oedema of the tongue, buccal and intermaxillary regions, erosive stomatitis, elevated body temperature, coronitis with resulting lameness. Clinical symptoms of the bluetongue disease were observed in infected cattle as well.

Serological analysis

All samples were analysed by c-ELISA test. The BTV antibodies were detected in 105 from 905 samples (11.6%). Seropositive samples for BTV antibodies are detected in all domestic ruminants: sheep (8.3%), goats (19.1%) and cattle (19.1%) (Table 3).

RT-PCR analysis

The 43 seropositive samples were tested positive by RT-PCR. The RNA samples were also tested using type-specific real-time RT-PCR targeting genome Seg-2 for BTV-4. Results confirmed the BTV-4 serotype.

Table 3. Results from samples analysed with c-ELISA for BTV taken in sheep, cattle and goats in different municipalities in Kosovo in 2014 and 2015

Municipality	Animal species	Number of samples	Positive cases	%
Skenderaj	Sheep	28	0	0.0%
	Sheep	24	3	12.5%
Gjilan	Goat	9	5	55.6%
	Cattle	14	8	57.1%
Gjakova	Sheep	16	1	6.3%
Shtërpc	Cattle	20	4	20.0%
Kaçanik	Sheep	43	4	9.3%
	Goat	2	0	0.0%
Drenas	Sheep	24	1	4.2%
Dragash	Sheep	28	0	0.0%
Viti	Sheep	118	21	17.8%
	Goat	12	3	25.0%
	Cattle	6	3	50.0%
Deçan	Sheep	32	0	0.0%
	Goat	3	0	0.0%
Mitrovicë	Goat	9	0	0.0%
Shtime	Cattle	12	0	0.0%
Hani i Elezit	Sheep	16	2	12.5%
Lipjan	Sheep	9	0	0.0%
	Goat	23	1	4.3%
Vushtrri	Cattle	86	8	9.3%
Prizren	Sheep	28	4	14.3%
	Cattle	20	7	35.0%
Ferizaj	Sheep	141	1	0.7%
	Cattle	46	9	19.6%
Novobërda	Sheep	55	13	23.6%
	Goat	10	4	40.0%
Peja	Sheep	71	3	4.2%
Total		905	105	11.6%

The distribution of BTV seropositive (red dots) and BTV seronegative samples (blue dots) in farms and villages of Kosovo is shown on Fig. 1.

DISCUSSION

This report describes the first evidence of BTV-4 in sheep in Kosovo as well as BTV seroprevalence in domestic rumi-

nants in years 2014 and 2015. After the first BT disease outbreak reported in 2001 caused by BTV serotype 9 (Osmani *et al.*, 2006), in disease outbreak in 2014 in Kosovo the results of this study confirmed high seroprevalence in domestic ruminants of 11.6%: 8.3% in sheep, 19.1% in goats and 19.1% in cattle. The RT-PCR analysis in samples from sheep with clinical signs confirmed presence of BTV se-



Fig. 1. The BTV seropositive (red dots) and seronegative (blue dots) samples in domestic ruminants in different municipalities in Kosovo in 2014 and 2015.

rotype 4 involved in disease outbreak in year 2014. Both BTV-4 and BTV-9 serotypes are reported to be present in European countries (Mellor *et al.*, 2009; Rodriguez-Sanchez *et al.*, 2008).

The high seroprevalence of specific antibodies in domestic ruminants, the presence of disease vectors, presence of two serotypes BTV-9 and BTV-4 in domestic ruminants in Kosovo, suggest that this disease should already considered as endemic disease in Kosovo. Detection and distribution of BTV positive samples showed that the virus was widespread in different areas within the country.

The bluetongue disease is considered a major problem for veterinary medicine

due to the rapid spread, mortality and incurred economic losses (Baylis & Mellor, 2001; Saegerman *et al.*, 2008).

There is still no contingency plan for responding to an outbreak of bluetongue disease in Kosovo. The high rate of seroprevalence and identification of current two BTV serotypes (BTV-4 and BTV-9) suggests the need for the application of control measures for BTV in Kosovo and strengthening regional scientific efforts and control strategy for infectious diseases in animals. Further research studies are necessary to analyse different mechanisms that have been involved in the introduction process, spread of the virus, specific vector of BTV in our country, factors as-

sociated with animals and environment, factors influencing in disease outbreak, and its impact on animal production.

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Correspondence:

Agim Rexhepi
Faculty of Agriculture and
Veterinary Medicine,
10000 Bill Clinton str.,
Prishtina, Kosovo,
phone: +37744171780
email: agim.rexhepi@uni-pr.edu

Kaltrina Zenuni. Ndërlidhja në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë në kohë të pandemisë në QKUK. Revista Farmaceutike. 2021, Korrik; 1(1): 66-80.

Ndërlidhja në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë në kohë të pandemisë në QKUK

Abstrakti

Ky studim me një mostër prej 150 punëtorë shëndetësorë, nga QKUK shqyrtoi ndërlidhjen mes inteligjencës emocionale (aftësitë përkatëse të emocioneve) dhe stresit (ndjenjat e paafësisë për të kontrolluar ngjarjet e jetës) tek punëtorët shëndetësorë në kohë të pandemisë. duke marrë parasysh personalitetin (vetë-perceptimin e tipareve të qartësisë, intensitetit dhe vëmendjes së meta-emocioneve) si një ndryshore moderuese. Gjetjet e këtij studimi sugjerojnë se ka ndërlidhje të rëndësishme në mes inteligjencës emocionale dhe stresit në punë, tek disa individ me rritjen e inteligjencës emocionale bie niveli i stresit në punë. Sa i përket dallimeve gjinore rrezultatet tregojnë femrat arrijnë mesatare të inteligjencës emocionale $M=3.20$, kurse meshkujt kanë mesatare të inteligjencës emocionale $M=3.28$, kurse tek variabla e stresit femrat arrijnë mesatare të stresit në punë $M=2.79$, kurse meshkujt kanë mesatare të stresit në punë $M=2.81$. nga rrezultatet sugjerrojmë se si femrat ashtu edhe meshkujt duhet të përdorin metoda të ndryshme që ndikojnë në rritjen e inteligjencës emocionale.

Fjalët kyçe: *inteligjencë emocionale, stres, pandemi, punëtor shëndetësor*

Falenderimi

Një falenderim dhe mirënjohje për familjen time, për mbështetje dhe kurajo gjatë kohës së realizimit të këtij hulumtimi shkencor.

Një falenderim i veçantë për punëtorët shëndetësorë të repartit të infektivës dhe repartit të pulmologjisë pranë ShSKUK.

Falenderoj Hekuran Sabedini (Fakulteti i Psikologjisë) për këshillat dhe konsultimet e tij gjatë finalizimit të këtij punimi.

1. Hyrje

Në këtë studim flitet rreth inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë në kohë pandemie, gjatë këtij studimi do të kuptohet se sa të rëndësishme janë këto dy variabla për punëtorët shëndetësorë në kohë të pandemisë.

Benet (1905), ka dhënë një ndër përkufizimet më complete për inteligjencën emocionale, sipas të cilit ajo është aftësi e përgjithshme e përbërë prej katër karakteristikave themelore: të kuptuarit; invencioni; direksioni; censura (vërtetimi i zgjidhjeve) (Benet, 1905).

Termi *emocion* shfaq nocionin rreth të cilit kuptim të saktë filozofët dhe psikologët për shumë vite me rrallë kanë kundërtënë në mes vete. Në kuptimin më tekstual, (Fjalori i Oksfordit i Gjuhës Angleze , 2001) emocionin e konceptualizon si “çdo agjitacion apo ngacim i psiqikes, epshit, ndjenjës, çdo gjendje mendore e shpejtë apo joqetësisë”. Me termin *emocion* u atribuohen mendimeve dhe ndjenjave të veçanta, gjendjet biologjike apo psikologjike dhe zinxhirin e prirjeve për veprim. Ekzistojnë shumë emocione, bashkë me hibridet e tyre, mutacionet, variacionet dhe nuancat. Dihet botërisht se emocionet janë shumë më perfide sesa ekzistojnë fjalët me të cilat mund të përshkruhen (Rose, 1976).

Ndryshe prej këtyre fakteve, një pjesë e mjekëve janë skeptikë apo kanë dyshime kur bëhet fjalë për rëndësinë klinike që kanë emocionet. Kjo për faktin se edhe pse është argumentuar se dhe emocionet negative dhe stresi dobësojnë efikasitetin e imuno-qelizave të ndryshme, nuk është gjithmonë e qartë se hallka e këtyre ndryshimeve është mjaft i numërt për të qenë në mënyrë medicinale i ndryshueshëm (Rose, 1976).

Stresi në punë diskutohet si çështja tek të cilat e ndeshmi më së shumti si në jetën e përditshme, ashtu edhe në vendet e punës, pasi që në botën aktuale moderne ka të bëjë me individë të vendeve dhe kulturave të ndryshme të cilët punojnë së bashku në vendin e punës. Puna që çdo punëtor e bën, formon trysni dhe stres, e posaçërisht kur ka të bëjë me vende të mëdha pune siç janë organizatat apo kompanitë e ndryshme, shkollat apo spitalet në rastin tonë konkret (Caplan, 1983).

Pandemia aktuale, e ashtuquajtura pandemia e COVID-19 është një sëmundje akute e frymëmarrjes që mund të jetë e rëndë dhe shkaktohet nga një koronavirus i sapo identifikuar i quajtur zyrtarisht SARS-CoV2. COVID-19. Për ne, ky virus është i panjohur, andaj edhe ka lindur motivimi që të studiohet nga afër problematika e këtij virusi tek punëtorët shëndetësorë (Sher, 2020)..

Nga sa mund të shihet, epidemia aktual e COVID-19 ka shpërfaqur një mungesë të theksuar të përvojës në përballje me problemet e shëndetit mendor të këtyre përmasave. Në anën tjetër, numri i madh i hulumtimeve të kryera është dëshmia më e mirë se përvoja aktuale ka nxitur një seriozitet më të madh tek profesionistët e fushës së shëndetit mendor për t’u përgatitur dhe përballur me situata të tilla në të ardhmen. Fatkeqësisht, në Kosovë nuk është gjetur as edhe një artikull apo

hulumtim të botuar i cili trajton në mënyrë të hollësishme e me të dhëna statistikore inteligjencën emocionale dhe stresin tek punëtorët shëndetësorë. Prandaj, është shumë i rëndësishëm të studiohet ky problem (Sher, 2020).

Qëllimi kryesor i këtij studimi është që të shihet ndërlidhja në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë në kohë të pandemisë, qëllim tjetër është edhe të shihet se a ekziston ndërlidhje në mes inteligjencës emocionale dhe stresit, me të dhënat demografike: moshë, gjinia, vendbanimi dhe niveli i arsimit tek punëtorët shëndetësorë.

2. Literatura

2.1. Koncepte mbi inteligjencën emocionale

Termi *emocion* shfaq nocionin rreth të cilit kuptim të saktë filozofët dhe psikologët për shumë vite me rrallë kanë kundërhënë në mes vete. Në kuptimin më tekstual, (Fjalori i Oksfordit i Gjuhës Angleze, 2001) fjalën *emocion* e konceptualizon si çdo agjitacion apo ngacmim i psikikes, epshit, ndjenjës, çdo gjendje mendore e shpejtë apo joqetësisë”. Me termin *emocion* u atribuohen mendimeve dhe ndjenjave të veçanta, gjendjet biologjike apo psikologjike, dhe zinxhirin e prirjeve për veprim. Ekzistojnë shumë emocione, bashkë me hibridet e tyre, mutacionet, variacionet dhe nuancat. Dihet botërisht se emocionet janë shumë më perfide sesa ekzistojnë fjalët me të cilat mund të përshkruhen (Rose, 1976).

Hulumtuesit vazhdojnë debatin për atë se cilat nga emocionet duhet të jenë primare – të kuqen, të kaltrën, apo të verdhën emocion nga e cila burojnë të gjitha nuancat e emocioneve – krejt deri te ajo se a ekzistojnë fare emocionet themelore (Goleman, 1995). Teoricienët Jacobsen, (1965) dhe Becan, (1996) propozojnë grupet kryesore, edhe pse nuk janë të pajtimit të gjithë rreth të njëjtës. Pretendentët kryesorë, apo disa prej anëtarëve të familjes janë:

2.2. Inteligjenca emocionale në raport me moshën.

Studiuesit e fushës së inteligjencës emocionale kanë bërë po ashtu shumë kërkime për të parë funksionin në mes relacionin e Inteligjencës Emocionale dhe moshës. Kjo fushë po ashtu vë në dukje gjetje kontradiktore në literaturën relevante. Disa studime, pohojnë përmes gjetjeve të tyre

se njerëzit më të moshuar e kanë më të zhvilluar inteligjencën emocionale sesa njerëzit me moshë më të re, përderisa studimet e tjera kanë zbuluar dallime shumë të vogla, ose aspak dallime ndërmjet inteligjencës emocionale dhe moshës. Sidoqoftë, studimet e (Moriarty, 2007) fokusohen në teoritë më relevante të inteligjencës emocionale, supozojnë se një gamë e gjerë e relacionit të inteligjencës emocionale dhe moshës, mund të sqarohet prej atribuimit të faktorëve të të mësuarit përgjatë jetës. Studiuesit mendojnë se një gamë e gjerë e modaliteteve të inteligjencës, mund të përmirësohen dhe avancohen përmes ushtrimeve dhe përgjatë kohës. (Moriarty, 2007), duke theksuar kështu edhe inteligjencën emocionale, si një aset mbi të cili përvoja dhe mosha mund të lënë gjurmë.

2.3. Inteligjenca emocionale në raport me gjinin

Sipas Garcia & Lopez (2009) shqyrtimi i literaturës që trajton dhe adreson çështjet e konstruktit të inteligjencës emocionale, vë në dukje po ashtu ndikimin që gjinia ka (dallimeve gjinore) në të kuptuarit e dallimeve individuale rreth kapacitetit të tyre për të zhvilluar në mënyrë sa më të saktë dhe më efikase informacionin emocional. Në përgjithësi, thuhet se fusha e studimit të inteligjencës emocionale gjatë këtyre 20 viteve ka evoluar jashtëzakonisht shumë, duke na sjellë kështu rezultate të dobishme lidhur me forcën parashikuese të këtij konstrukti në aspektet e ndryshme të jetës tek të dy gjinitë, meshkujve dhe femrave. Sidoqoftë, në përgjithësi, edhe pse shpeshherë gjetjet e studimeve lidhur me ndikimin e konstruktit të inteligjencës emocionale dhe dallimeve gjinore duket të jenë kontradiktore kur merret për bazë i gjithë rezultati i inteligjencës emocionale, megjithatë thuhet se dallimet gjinore në këtë aspekt duket të jenë një ndryshore e rëndësishme për studiuesit e kësaj fushe. Në këtë aspekt, duhet pasur parasysh që këto rezultate të cilat janë kontradiktore dhe sqarimet pasuese për dallimet e tilla gjinore në raport me inteligjencën emocionale, mbeten të kuptohen tutje, kjo sidomos në raport me matjet vetëraportuese të konstruktit të inteligjencës emocionale nga qasjet e ndryshme teorike (Garcia & Lopez, 2009).

2.4. Koncepte mbi stresin në punë

Stresi është një problem në jetën e secilit individ, ai ndikon negativisht në jetën e secilit nga ne. Stresi është një ndjenjë e cila perceptohet e rrezikshme dhe e pakëndshme emocionale si dhe fiziologjike. Nocioni "stres" do të thotë gjëra të ndryshme për njerëz të ndryshëm. Psikologë të ndryshëm botërorë e kanë përkufizuar "stresin" si problem serioz i cili shpesh mund të jetë edhe kronik. Ekzistojnë faktorë të ndryshëm të cilët ndikojnë në formimin e stresit në jetën e një individi. Sipas hulumtimeve të ndryshme psikologjike shkaktarët të cilat shkaktojnë stresin janë quajtur stresorë. Stresi në jetë në përgjithësi, dhe në punë është një faktor që mund të shkaktojë sëmundje mendore në jetën e njerëzve (Orhani, 2011).

Një individ i cili është në gjendje stresuese, zakonisht ka vështirësi në përqendrimin e vëmendjes, sjellje të jashtme të ndryshuar dhe mendime ankthi. Kështu, shtrëngimi i duarve dhe i dhëmbëve, boshllëku në stomak, apo frymëmarrja e vështirë, duart dhe këmbët e ftohta, rritja e rrahjeve të zemrës, goja e tharë, tronditja, ankthi dhe frika, ankesat psiko-somatike, çrregullimi i gjumit e të tjera, janë disa nga shenjat më të zakonshme të stresit që mund të shkaktohen edhe me disa shenjave fiziologjike të emocioneve të ankthit (Caplan, 1983).

Si gjendje unike psikologjike stresi është në relacion me çdo gjë që ushtron kërkesa ndaj të cilave duhet të përshtatemi. Ai në të vërtetë nënkupton të gjitha ndryshimet me natyrë sociale, somatike e psikologjike që bëjnë të nevojshme ngjashmërinë. Pra, çdo rrethanë që shkakton tension të brendshëm teknikisht quhet stresuese. Stresi është i pashmangshëm në pjesën më të madhe të jetës të secilit person. Duke pasur parasysh shkaqet dhe faktorët e tij (Orhani, 2011).

Sipas psikologut Mytahir Haskuka, stresi në përgjithësi është një nocion i ri i cili në aspektin terminologjik do të thotë tendosje, njerëzit ndihen të tendosur dhe reagojnë shpejt. Haskuka ka thënë se stresi paraqitet në mënyra të ndryshme te njerëz të ndryshëm (Orhani, 2011).

2.5. Koncepte mbi pandemin Covid 19

Sipas Organizatës Botërore të Shëndetit (OBSh), sëmundjet infektive siç janë koronavirusët hynë në familjen e sëmundjeve infektive apo viruseve që shkaktojnë sëmundje duke filluar nga më të lehtat siç është ftohja e zakonshme e deri te sëmundjet më të rënda siç janë Sindroma e frymëmarrjes në Lindjen e Mesme (MERS) dhe koronavirusi i rëndë i lidhur me sindromën e frymëmarrjes (SARS) (Sher, 2020).

Kemi parë siç ndodhi me virusin e fundit (koronavirusi) i cili fillimisht u transmetua përmes kafshëve dhe pastaj njerëzve. Koronavirusi, besohet se fillimisht është transferuar nga macet, ndërsa virusi MERS udhëtonte nga një lloj deve tek njerëzit. Ekzistojnë edhe disa koronavirusë të njohura që po qarkullojnë te kafshët që nuk kanë infektuar ende njerëzit. Emri koronavirus vjen nga fjala latine *corona*, që do të thotë *kurorë* ose *halo*. Nën një mikroskop elektronik, imazhi i virusit i përngjan një korone diellore. Një koronavirus i ri, i identifikuar nga autoritetet kineze më 7 janar dhe aktualisht i quajtur 2019-nCoV, është një lloj i ri që nuk ishte identifikuar më herët tek njerëzit. Dihet pak për këtë, megjithëse transmetimi njeri-tek-njeri është konfirmuar (Sher, 2020).

Nga sa mund të shihet, epidemia aktuale e COVID-19 ka shpërfaqur një mungesë të theksuar të përvojës në përballje me problemet e shëndetit mendor të këtyre përmasave. Në anën tjetër, numri i madh i hulumtimeve të kryera është dëshmia më e mirë se përvoja aktuale ka nxitur një seriozitet më të madh tek profesionistët e fushës së shëndetit mendor për t'u përgatitur dhe përballur me situata të tilla në të ardhmen. Fatkeqësisht, në Kosovë nuk kam gjetur as edhe një artikull apo hulumtim të botuar i cili trajton në mënyrë të hollësishme e me të dhëna statistikore temën të cilën është hulumtuar e lëre më ndonjë bazë të të dhënave. Një bazë e tillë elektronike do të ndihmonte në grumbullimin e të dhënave, analizimin dhe kuptimin e tyre dhe do të ishte një mjet i dobishëm

në luftimin, trajtimin dhe sanimin e pasojave psikologjike tek popullata në kohë pandemie. Mbase gjërat do të ndryshojnë për të mirë në muajt apo vitet e ardhshme (Sher, 2020).

Karantimi si rezultat i pandemisë, ka rezultuar me kufizime në socializim, argëtim dhe kontakte fizike si dhe me shumë pacient që trajtohen në shtëpi shëndetësore, kjo ka qenë gjendje e rëndë fizike dhe psikike për punëtorët shëndetësor, ata janë përballur çdo ditë me stresin e punës dhe ka qenë shumë e nevojshme për ta zhvillonin intelegjiencen emocionale të tyre në atë mënyrë që të arrinin ta menaxhonin stresin.

Për ta parë se a ka pasur ndërlidhje në mes inteligjences emocionale dhe stresit në punë ka qenë i nevojshëm të realizohet një studim i tillë, përmes metodes kuantitative apo sasiore ky studim do të na ipte rrezultate të qarta do të na ndihmohnte edhe të krahasojmë rrezultatet në peridhua të ndryshme kohore.

Qëllimi kryesor i këtij studimi është që të shihet ndërlidhja në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë në kohë të pandemisë, qëllim tjetër është edhe të shihet se a ekziston ndërlidhje në mes inteligjencës emocionale dhe stresit, me të dhënat demografike: moshë, gjinia, vendbanimi dhe niveli i arsimit tek punëtorët shëndetësorë

3. Metodologjia

3.1. Metoda kërkimore

Për realizimin e këtij studimi është përdorur metoda kuantitative (sasiore) e studimit. Për këtë studim do të përdoret dizajni i kërkimit korrelacional. Sipas Burns & Grove (2010), kërkimi korrelacional ka për qëllim të përshkruajë marrëdhëniet midis dy ose më shumë variablave, të parashikojë rrezultatet e një variabli nga rrezultatet e pjesëmarrësve në variablin tjetër, ose të testojë marrëdhëniet e supozuara nga propozimet teorike (Bruns & Grove, 2010). Në këtë studim janë bërë ndërlidhje në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë.

3.2. Mostra e hulumtimit

Mostër e këtij hulumtimi janë: punëtorët shëndetësorë të repartit të infektives dhe repartit të pulmologjisë. Popullata e studimit në këto reparte është 168 por që popullata mbetet relative pasi punëtorët shëndetësorë rekrutohen shpesh nga një repart në tjetrin sipas nevojës që kanë ata. Por për mostër të këtij studimi janë marrë 150 punëtorë shëndetësorë. Mostra është përzgjedhur në mënyrë rastësore duke i anketuar të gjithë punëtorët shëndetësorë që ishin prezentë prej të dy ndërrimeve të punëtorëve. Subjektet që janë marrë për mostër janë ndarë në 63 meshkuj dhe 87 femra dhe sillen nga moshë 22 deri në 55 vjet. Arsyeja se përse kemi të limituar deri tek moshë 55 vjetë, është sepse mostra ishte në mënyrë rastësore dhe në ditën e anketimit nuk kishte respondentë

më moshë më të lartë. Të dhënat janë mbledhur në repartin e infektivës dhe repartin e Pulmologjisë në qytetin e Mitrovicës - Kosovë. Profili i respondentëve kanë qenë mjek dhe infermiar që janë grupuar si punëtorë shëndetësor. Subjektet të cilat janë marrë për mostër gërshetojnë mes vete karakteristikat për një mostër adekuate, ata posedojnë të gjitha karakteristikat e popullatës ku do të zhvillohet hulumtimi, subjektet kanë mundësi të barabartë në përfshirje të mostrës.

3.3. Përpunimi i të dhënave

Pas mbledhjes së fakteve në terren, vjen puna në tavolinë, që ka të bëjë me analizën dhe përpunimin e statistikave. Deklaratat e ndryshme kërkojnë analizë specifike për të gjetur treguesit që na interesojnë. Përpunimin i rezultateve është bërë me anë të programin SPSS25, i cili mundëson që të dhënat e futura në databazë të përpunohen më shpejt. Paraprakisht është përpiluar një skemë koduese të dhënash, në shprehje kuantifikuese. Kodimi i informatave është procesi i shndërrimit në një format të kuantifikuar, zakonisht numerik, që të mund të bëhet më lehtë analiza sistematike e tyre. Procesi i kodimit filloi me kodimin e përgjigjeve paraprakisht, duke mundësuar që numrat të rumbullakohen ose të shënuar të futen direkt në bazë të të dhënave. Vlera e të dhënave si numra ose si emërtime varet nga analizat që është bërë. Analizat që janë bërë në këtë studim janë analizat deskriptive pastaj është përdorur analiza e korrelacionit të Pearsonit, Anoven dhe T-testin.

4. Rezultatet

4.1. Rrezultatet lidhur me të dhënat demografike

Tabela 1. Paraqitja e të dhënave demografike të punëtorëve shëndetësorë

		Frekuencat	Përqindja
Gjinia	Femër	87	58.0%
	Mashkull	63	42.0%
	Total	150	100.0%
Moshë	25-30 vjeç	45	30.0%
	31-35 vjeç	31	20.7%
	36-40 vjeç	23	15.3%
	41-45 vjeç	21	14.0%
	46-50 vjeç	15	10.0%
	51-55 vjeç	7	4.7%
	56-60 vjeç	8	5.3%
	Total	150	100.0%
Vendëbanimi	Fshat	54	36.0%
	Qytet	96	64.0%
mostra	Total	150	100.0%

Niveli i arsimit	Arsimim i mesëm	43	28.7%
	Arsimim universitar	85	56.7%
	Arsimim pasuniversitar	22	14.7%
	Total	150	100.0%
Përvoja në punë	0-5 vite	69	46.0%
	6-10 vite	53	35.3%
	11-15 vite	13	8.7%
	16-20 vite	11	7.3%
	Mbi 20 vite	4	2.7%
	Total	150	100.0%

Në studim morën pjesë 150 punëtorë shëndetësorë, ku 42% ishin të gjinisë mashkullore dhe 58% të gjinisë femërore. Moshë minimale e punëtorëve shëndetësorë është 25 vjeç, kurse ajo maksimale 60 vjeç. Numri më i madh i punëtorëve shëndetësorë 30% i përkasin moshës 25-30 vjeç, kurse numri më i vogël 4.7% punëtorë shëndetësorë i përkasin moshës 51-55 vjeç. Gjithsej në studim morën pjesë 150 punëtorë shëndetësorë, ku 36% jetonin në fshat, kurse 64% jetonin në qytet. Pra, këtu shihet se është një dominim të vendbanimit në qytet krahasuar me vendbanimin në fshat. 28.7% e punëtorëve shëndetësorë e kanë të përfunduar arsimimin e mesëm, 56.7% e kanë të përfunduar arsimimin universitar dhe 14.7% kanë të përfunduar arsimimin pasuniversitar. 46% e punëtorëve shëndetësorë kanë përvojë pune 0-5 vite, 35.3% kanë përvojë pune 6-10 vite, 8.7% e punëtorëve shëndetësorë kanë përvojë pune 11-15 vite, 7.3% kanë përvojë pune 16-20 vite dhe 2.7% e punëtorëve shëndetësorë kanë përvojë pune mbi 20 vite.

4.1. Ndërlidhja në mes inteligjencës emocionale dhe stresit

Më poshtë, në tabelën nr. 1 është paraqitur analiza e korrelacionit në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë në kohë të pandemisë.

Tabela1. Korrelacioni në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë

		Inteligjenca emocionale	Stresi
Inteligjenca emocionale	Pearson Correlation	1	.076
	Sig. (2-tailed)		.001
	N	150	150
Stresi	Pearson Correlation	.076	1
	Sig. (2-tailed)	.001	
	N	150	150

Nga analiza korrelative nxirren këto përfundime: inteligjenca emocionale ka marrëdhënie lineare pozitive me stresin në punë gjatë pandemisë $r=.076$. Signifikanca është 0.001. $p<0.05$ në nivelin e rëndësisë 0.05. Nga kjo mund të thuhet se ka lidhje signifikante në mes dy ndryshoreve.

Si përfundim mund të thuhet se ka ndërlidhje signifikante në mes inteligjencës emocionale dhe stresit në punë gjatë pandemisë tek punëtorët shëndetësorë.

4.2. Ndërlidhja në mes inteligjencës emocionale dhe të dhënave demografike

Tabela 2. ANOVA e inteligjencës emocionale lidhur me moshën e punëtorëve shëndetësorë

I. Emocionale

	Shuma e katrorëve	Df (Shkallët e lirisë)	Mesatarja e katrorëve	F	Sig.(p)
Ndërmjet grupeve	5.866	6	.978	1.890	.086
Brenda grupeve	73.985	143	.517		
Total	79.851	149			

Rezultatet nga analiza njëkahëshe e variancës (ANOVA) tregojnë se nuk ka dallime signifikante ($p>05$) në mes inteligjencës emocionale në punë dhe moshës së punëtorëve shëndetësorë për të gjitha nivelet e moshës, ku $F(143)=1.89$, $p>0.05$. Moshë 25 deri në 30 vjet ka ($M=3.40$ dhe $Ds=0.52$), moshë 31 deri 35 vjet ka ($M=3.14$ dhe $Ds=0.68$), moshë 36 deri 40 vjet ka ($M=3.30$ dhe $Ds=0.94$), moshë 41 deri 45 vjet ka ($M=3.15$ dhe $Ds=0.80$), moshë 46-50 vjet ka ($M=3.21$ dhe $Ds=0.82$), moshë 51-55 vjet ka ($M=2.48$ dhe $Ds=0.57$) dhe moshë 56-60 vjet ka ($M=3.32$ dhe $Ds=0.76$). Pra, nga rezultatet del se nuk ka dallime signifikante në mes në mes inteligjencës emocionale dhe moshës së punëtorëve shëndetësorë për të gjitha nivelet e moshës.

Tabela 3. Paraqitja e dallimeve gjinore në kontekst të inteligjencës emocionale përmes T-testit

	Testi i Leventit për barazinë e variancës		t-test për barazimin e mesatareve					95% Diferenca e intervalit të besueshmërisë	
	F	Sig.(p)	t	Df (Shkallët e lirisë)	Sig. (2-tailed)	Diferenca e mesatareve	Diferenca e gabimit standard	E ulët	E lartë
Inteligjenca supozuara emocionale	.348	.556	-.695	148	.488	-.08426	.12132	-.32399	.15548
Inteligjenca supozuara emocionale			-.701	137.739	.485	-.08426	.12027	-.32207	.15356

Duke i vështruar mesataret që arrijnë femrat dhe meshkujt në tabelën 9 dhe 10 shihet se femrat arrijnë mesatare të inteligjencës emocionale $M=3.20$, kurse meshkujt kanë mesatare të inteligjencës emocionale $M=3.28$. Devijimi standard për femrat është $Ds=0.74$, ndërsa për meshkujt $Ds=0.78$ dhe mesatarja e gabimit standard për femrat është $Gs=0.080$ dhe për meshkujt $Gs=0.089$. Signifikanca është 0.556 . $p>0.05$ në nivelin e rëndësisë 0.05 . Nga kjo mund të thuhet se nuk ka ndonjë lidhje signifikante në mes inteligjencës emocionale dhe gjinisë.

4.3. Ndërlidhja në mes stresit në punë dhe të dhënave demografike

Tabela 4. ANOVA e stresit në punë lidhur me moshën e punëtorëve shëndetësorë
Stresi

	Shuma e katrorëve	Df (Shkallët e lirisë)	Mesatarja e katrorëve	F	Sig.
Ndërmjet grupeve	3.218	6	.536	1.772	.109
Brenda grupeve	43.274	143	.303		
Total	46.492	149			

Rezultatet nga analiza njëkahëshe e variancës (ANOVA) tregojnë se nuk ka dallime signifikante ($p>0.05$) në mes stresit në punë dhe moshës së punëtorëve shëndetësorë për të gjitha nivelet e moshës, ku $F(149)=1.77$, $p>0.05$. Moshja 25 deri në 30 vjet ka ($M=2.70$ dhe $Ds=0.56$), moshja 31 deri 35 vjet ka ($M=2.61$ dhe $Ds=0.53$), moshja 36 deri 40 vjet ka ($M=2.88$ dhe $Ds=0.49$), moshja 41 deri 45 vjet ka ($M=2.98$ dhe $Ds=0.61$), moshja 46-50 vjet ka ($M=2.89$ dhe $Ds=0.50$), moshja 51-55 vjet ka ($M=3.10$ dhe $Ds=0.65$) dhe moshja 56-60 vjet ka ($M=2.90$ dhe $Ds=0.42$). Pra, nga rezultatet del se nuk ka dallime signifikante në mes në mes stresit në punë dhe moshës së punëtorëve shëndetësorë për të gjitha nivelet e moshës.

Tabela 5. Paraqitja e dallimeve gjinore në kontekst të stresit në punë përmes T-testit

	Testi i Leventit për barazinë e variancës		t-test për barazimin e mesatareve					95% Diferenca e intervalit të besueshmërisë	
	F	Sig.	t	Df (Shkallët e lirisë)	Sig. (2-tailed)	Diferenca e mesatareve	Diferenca e gabimit standard	E ulët	E lartë
Stresi Varianca të barabarta të supozuara	.791	.375	-.289	148	.773	-.02680	.09269	-.20997	.15638
Varianca të barabarta jo të supozuara			-.285	126.677	.776	-.02680	.09399	-.21279	.15919

Duke i vështruar mesataret që arrijnë femrat dhe meshkujt në tabelën 19 dhe 20 shihet se femrat arrijnë mesatare të stresit në punë $M=2.79$, kurse meshkujt kanë mesatare të stresit në punë $M=2.81$. Devijimi standard për femrat është $Ds=0.53$, ndërsa për meshkujt $Ds=0.58$ dhe mesatarja e gabimit standard për femrat është $Gs=0.05$ dhe për meshkujt $Gs=0.07$. Signifikanca është 0.375 . $p>0.05$ në nivelin e rëndësisë 0.05 . Nga kjo mund të thuhet se nuk ka ndonjë lidhje signifikante në mes stresit në punë dhe gjinisë tek punëtorët shëndetësorë.

Përfundimi

Në këtë studim është shqyrtuar literatura teorike ekzistuese duke përzgjedhur artikujt që lidhen me studimin tonë të cilat shërbejnë për të kuptuar më thellë problemin dhe për të parë lidhjen midis studimit tonë dhe studimeve të tjera të realizuara.

Nga rezultatet e studimit kemi kuptuar se nuk ka dallime signifikante të inteligjencës emocionale për të gjitha nivelet e moshës së punëtorëve shëndetësorë, femrat arrijnë mesatare të inteligjencës emocionale $M=3.20$, ndërsa meshkujt $M=3.28$ por që këto dallime nuk kanë rëndësi signifikante, po ashtu shihet se respondentët me vendbanim në fshat arrijnë mesatare të inteligjencës emocionale $M=3.19$ më të vogël se sa respondentët me vendbanim në qytet, të cilët kanë mesatare të inteligjencës emocionale $M=3.25$ por që këto dallime nuk kanë rëndësi statistikore. Po ashtu nga rezultatet mund të shihet se nuk ka dallime signifikante në mes inteligjencës emocionale në punë

dhe nivelit të arsimit të punëtorëve shëndetësorë për të gjitha nivelet e arsimit po ashtu rezultatet tregojnë se nuk ka dallime signifikante në mes inteligjencës emocionale në punë dhe përvojës në punë për të gjitha nivelet e përvojës së punës.

Nga rezultatet lidhur me stresin në punë dhe të dhënave demografike ka dalë se nuk ka dallime signifikante në mes stresit në punë dhe moshës së punëtorëve shëndetësorë për të gjitha nivelet e moshës, po ashtu nga rezultatet mund të shihet se femrat arrijnë mesatare të stresit në punë $M=2.79$ më të ulët se sa meshkujt, të kanë mesatare të stresit në punë $M=2.81$ por që ky dallim nuk është signifikant, respondentët me vendbanim në fshat arrijnë mesatare të stresit në punë $M=2.70$ më të vogël se sa respondentët me vendbanim në qytet, të kanë mesatare të stresit në punë $M=2.85$ por që as ky dallim nuk është signifikant. Nga rezultatet gjithashtu mund të shihet se nuk ka dallime signifikante në mes stresit në punë dhe nivelit të arsimit të punëtorëve shëndetësorë për të gjitha nivelet e arsimit, po ashtu rezultatet tregojnë se nuk ka dallime signifikante në mes stresit në punë dhe përvojës në punë për të gjitha nivelet e përvojës së punës.

Diskutimi i rrezultateve

Në këtë kapitull janë përmbledhur gjetjet kryesore të studimit duke i diskutuar ato në lidhje me literaturën përkatëse. Këto gjetje janë krahasuar me studime të tjera nga autorë të ndryshëm dhe është paraqitur këndvështrimi lidhur me to ku është kryer edhe ky studim. Fokusi i këtij studimi është të paraqesë rezultatet lidhur me stresin dhe inteligjencën emocionale në punë tek punëtorët shëndetësorë gjatë pandemisë Covid-19 në QKUK të lidhura këto dhe me të dhëna demografike (gjinia, mosha, vendbanimi, niveli i arsimit dhe përvoja e punës).

Diskutime për hipotezën H1: ekziston ndërlidhje në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë në kohë të pandemisë.

Për ta parë ndërlidhjen në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë në kohë të pandemisë është përdorur analiza e korrelacionit. Nga analiza korrelative në studim janë nxjerrë këto përfundime: inteligjenca emocionale ka marrëdhënie lineare pozitive me stresin në punë gjatë pandemisë si dhe ndërlidhja mes këtyre variablave është signifikante. Nga këto rezultate vijmë në përfundim se aprovohet hipoteza 1.

Arsyeja pse ka dalë një rezultat i tillë është sepse inteligjenca emocionale tek punëtorët shëndetësorë ka mbetur pothuajse ajo siç ka qenë para pandemisë edhe tani gjatë pandemisë, kurse stresi tek punëtorët shëndetësorë mendohet të ketë qenë më i ulët, kurse tani gjatë pandemisë kemi ngritje të lirë të stresit në punë tek punëtorët shëndetësorë.

Lidhjet midis dimensioneve të këtij niveli janë të ngjashme me ato që raportohen nga studimet ndërkombëtare (Kulkarni, Fareill, Erasi, & Koschar, 1998) edhe pse koeficienti i korrelacionit të inteligjencës emocionale stresit në punë nuk është signifikant. Për shembull, në një studim ndërkombëtar dimensionet e lidhur më fort me njëri-tjetrin midis stresit në punë dhe inteligjencës emocionale (Spearman's $\rho=0.63$) (Kulkarni, Fareill, Erasi, & Koschar, 1998), kurse në këtë

studim ndërlidhja mes stresit në punë dhe inteligjencës emocionale ishte (Pearson's -0.76 dhe sginikanca 353).

Diskutime për hipotezën H2: ekziston ndërlidhje në mes inteligjencës emocionale dhe të dhënave demografike: moshë, gjinia, vendbanimi, niveli i arsimit dhe përvoja në punë tek punëtorët shëndetësorë në kohë të pandemisë.

Për të parë nëse ekziston ndërlidhje në mes inteligjencës emocionale dhe të dhënave demografike: moshë, gjinia, vendbanimi, niveli i arsimit dhe përvoja në punë tek punëtorët shëndetësorë në kohë të pandemisë janë mbledhur të dhëna përmes *Instrumentit për matjen e inteligjencës emocionale* i standardizuar nga (Schutte, 1990) i cili iu ka përshtatur jashtëzakonisht kërkesave, kurse për mbledhjen e të dhënave është përdorur metoda kuantitative e studimit e cila mendojmë se është më adekuate për një studim të tillë.

Për të parë nëse ekziston ndërlidhje në mes inteligjencës emocionale dhe të dhënave demografike: moshë, gjinia, vendbanimi, niveli i arsimit dhe përvoja në punë tek punëtorët shëndetësorë në kohë të pandemisë është përdorur analizat e ANOVA dhe T-testi, nga këto analiza del se nuk ka dallime signifikante për të gjitha nivelet e moshës së punëtorëve. Duke i vështruar mesataret që arrijnë femrat dhe meshkujt nga rezultatet e t-testit del se femrat arrijnë mesatare të inteligjencës emocionale $M=3.20$ se sa meshkujt $M=3.28$ por që këto dallime nuk kanë rëndësi signifikante. Nga analiza t-testi po ashtu shihet se respondentët me vendbanim në fshat arrijnë mesatare të inteligjencës emocionale $M=3.19$ më të vogël se sa respondentët me vendbanim në qytet, të cilët kanë mesatare të inteligjencës emocionale $M=3.25$ por që këto dallime nuk kanë rëndësi statistikore. Rezultatet nga analiza njëkahëshe e variancës (ANOVA) tregojnë se nuk ka dallime signifikante në mes inteligjencës emocionale në punë dhe nivelit të arsimit të punëtorëve shëndetësorë për të gjitha nivelet e arsimit, po ashtu rezultatet nga analiza njëkahëshe e variancës (ANOVA) tregojnë se nuk ka dallime signifikante në mes inteligjencës emocionale në punë dhe përvojës në punë për të gjitha nivelet e përvojës së punës. Si përfundim mund të thuhet se nga analizat statistikore shihet se nuk ka ndërlidhje signifikante në mes inteligjencës emocionale me moshën, gjininë, vendbanimin, nivelin të arsimit dhe përvojës në punë tek punëtorët shëndetësorë. Nga këto rezultate mund të përfundohet se nuk aprovohet hipoteza 2.

Arsyeja se pse nuk është ndërlidhje signifikante në mes inteligjencës emocionale me moshën, gjininë, vendbanimin, nivelin e arsimit dhe përvojës në punë tek punëtorët shëndetësorë është sepse shumica e punëtorëve shëndetësorë kanë nivel të njëjtë të arsimit, prandaj kjo i bën të padallueshëm si tek gjinia ashtu edhe tek moshë dhe vendbanimi i tyre.

Po të krahasohen rezultatet e këtij studimi me studiuesit e fushës së inteligjencës emocionale, disa studime, pohojnë përmes gjetjeve të tyre se njerëzit më të moshuar e kanë më të zhvilluar inteligjencën emocionale sesa njerëzit me moshë më të re, përderisa studimet e tjera kanë zbuluar dallime shumë të vogla, ose aspak dallime ndërmjet inteligjencës emocionale dhe moshës. Sidoqoftë, studimet e (Moriarty, 2007) fokusohen në teorinë më relevante të inteligjencës emocionale, supozojnë se një gamë e gjerë e relacionit të inteligjencës emocionale dhe moshës, mund të sqarohet prej atribuitit të faktorëve të të mësuarit përgjatë jetës. Studiuesit mendojnë se një gamë e gjerë e modaliteteve të inteligjencës, mund të përmirësohen dhe avancohen përmes

ushtrimeve dhe përgjatë kohës. (Moriarty, 2007), duke theksuar kështu edhe inteligjencën emocionale, si një aset mbi të cilin përvoja dhe mosha mund të lënë gjurmë.

Sipas Garcia & Lopez (2009) shqyrtimi i literaturës që trajton dhe adreson çështjet e konstruktit të inteligjencës emocionale, vë në dukje po ashtu ndikimin që gjinia ka (dallimeve gjinore) në të kuptuarit e dallimeve individuale rreth kapacitetit të tyre për të zhvilluar në mënyrë sa më të saktë dhe më efikase informacionin emocional. Në përgjithësi, thuhet se fusha e studimit të inteligjencës emocionale gjatë këtyre 20 viteve ka evoluar jashtëzakonisht shumë, duke na sjellë kështu rezultate të dobishme lidhur me forcën parashikuese të këtij konstrukti në aspektet e ndryshme të jetës tek të dy gjinitë, meshkujve dhe femrave. Sidoqoftë, në përgjithësi, edhe pse shpeshherë gjetjet e studimeve lidhur me ndikimin e konstruktit të inteligjencës emocionale dhe dallimeve gjinore duket të jenë kontradiktore kur merret për bazë i gjithë rezultati i inteligjencës emocionale, megjithatë thuhet se dallimet gjinore në këtë aspekt duket të jenë një ndryshore e rëndësishme për studiuesit e kësaj fushe. Në këtë aspekt, duhet pasur parasysh që këto rezultate të cilat janë kontradiktore dhe sqarimet pasuese për dallimet e tilla gjinore në raport me inteligjencën emocionale, mbeten të kuptohen tutje, kjo sidomos në raport me matjet vetëraportuese të konstruktit të inteligjencës emocionale nga qasjet e ndryshme teorike (Garcia & Lopez, 2009).

Diskutime për hipotezën H3: ekziston ndërlidhje në mes stresit në punë dhe të dhënave demografike: mosha, gjinia, vendbanimi, niveli i arsimit dhe përvoja në punë tek punëtorët shëndetësorë në kohë të pandemisë.

Për të parë nëse ekziston ndërlidhje në mes stresit në punë dhe të dhënave demografike: mosha, gjinia, vendbanimi, niveli i arsimit dhe përvoja në punë tek punëtorët shëndetësorë në kohë të pandemisë janë mbledhur të dhëna përmes *Instrumentit për matjen e stresit në punë*. Instrumenti i cili u përdorë për matjen e nivelit të stresit të punëtorëve shëndetësorë në ballafaqim me pandeminë Covid-19 është instrumenti i standardizuar nga Kristensen & Borg (2003) i cili iu ka përshtatur jashtëzakonisht kërkesave tona, kurse për mbledhjen e të dhënave është përdorur metoda kuantitative e studimit e cila mendohet se është më adekuate për një studim të tillë.

Për të parë nëse ekziston ndërlidhje në mes stresit në punë dhe të dhënave demografike: mosha, gjinia, vendbanimi, niveli i arsimit dhe përvoja në punë tek punëtorët shëndetësorë në kohë të pandemisë, është përdorur analizat e ANOVA dhe T-testi, nga këto analiza del se nuk ka dallime signifikante në mes stresit në punë dhe moshës së punëtorëve shëndetësorë për të gjitha nivelet e moshës. Nga analiza e t-testit shihet se femrat arrijnë mesatare të stresit në punë $M=2.79$ më të ulët sesa meshkujt, të cilët kanë mesatare të stresit në punë $M=2.81$ por që ky dallim nuk është signifikant, po ashtu nga analiza e t-testit del se respondentët me vendbanim në fshat arrijnë mesatare të stresit në punë $M=2.70$ më të vogël se sa respondentët me vendbanim në qytet, të cilët kanë mesatare të stresit në punë $M=2.85$ por që as ky dallim nuk është signifikant. Rezultatet nga analiza njëkahëshe e variancës (ANOVA) tregojnë se nuk ka dallime signifikante në mes stresit në punë dhe nivelit të arsimit të punëtorëve shëndetësorë për të gjitha nivelet e arsimit, po ashtu rezultatet nga analiza njëkahëshe e variancës (ANOVA) tregojnë se nuk ka dallime signifikante në mes stresit në punë dhe përvojës në punë për të gjitha nivelet e përvojës së punës. Si përfundim mund të thuhet se nga analizat statistikore shihet se nuk ka ndërlidhje signifikante në mes stresit

në punë me moshën, gjininë, vendbanimin, nivelin e arsimit dhe përvojës në punë tek punëtorët shëndetësorë. Nga këto rezultate mund të përfundohet se nuk aprovohet hipoteza 3.

Arsyeja se pse nuk është ndërlidhje signifikante në mes stresit në punë me moshën, gjininë, vendbanimin, nivelin e arsimit dhe përvojës në punë tek punëtorët shëndetësorë mund të jetë si rezultat i ndonjë problemi statistikor në analizën e të dhënave.

Hulumtuesit kanë gjetur se të dhënat demografike janë faktorë shumë të rëndësishëm në shqyrtimin e burimeve dhe strategjive të përballimit të stresit midis punëtorëve shëndetësorë (Lofquist & Dawis, 1969). Rolet gjinore dhe sjellja e përshtatshme gjinore përbëjnë dy burime kryesore të stresit për ta. Literatura e mëparshme zbuloi se punëtorët shëndetësorë femra kishin gjasa të ndesheshin me nivele më të larta të ngacmimit, armiqësisë së hapur dhe ndërveprimeve të tjera negative në punë (Tenant, 2001). Theksojmë se po të krahasohen gjetjet e këtij studimi me një studim të kryer në pesë institucione të shëndetësisë së Hamburgut me 155 pjesëmarrës të gjinisë femërore dhe mashkullore të gjithë spektrit të gradave, moshës, funksioneve dhe kohëzgjatjes në shërbim rezultoi se 19% e tyre u diagnostikuan me stres të lartë në vendin e punës, 56% me nivel të mesëm të stresit dhe 25% e punëtorëve shëndetësorë kishin nivel të ulët të stresit.

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Ndërlidhja në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë në kohë të pandemisë në QKUK

Abstrakti

Ky studim me një mostër prej 150 punëtorë shëndetësorë, nga QKUK shqyrtoi ndërlidhjen mes inteligjencës emocionale (aftësitë përkatëse të emocioneve) dhe stresit (ndjenjat e paafësisë për të kontrolluar ngjarjet e jetës) tek punëtorët shëndetësorë në kohë të pandemisë. duke marrë parasysh personalitetin (vetë-perceptimin e tipareve të qartësisë, intensitetit dhe vëmendjes së meta-emocioneve) si një ndryshore moderuese. Gjetjet e këtij studimi sugjerojnë se ka ndërlidhje të rëndësishme në mes inteligjencës emocionale dhe stresit në punë, tek disa individ me rritjen e inteligjencës emocionale bie niveli i stresit në punë. Sa i përket dallimeve gjinore rrezultatet tregojnë femrat arrijnë mesatare të inteligjencës emocionale $M=3.20$, kurse meshkujt kanë mesatare të inteligjencës emocionale $M=3.28$, kurse tek variabla e stresit femrat arrijnë mesatare të stresit në punë $M=2.79$, kurse meshkujt kanë mesatare të stresit në punë $M=2.81$. nga rrezultatet sugjerrojmë se si femrat ashtu edhe meshkujt duhet të përdorin metoda të ndryshme që ndikojnë në rritjen e inteligjencës emocionale.

Fjalët kyçe: *inteligjencë emocionale, stres, pandemi, punëtor shëndetësor*

Falenderimi

Një falenderim dhe mirënjohje për familjen time, për mbështetje dhe kurajo gjatë kohës së realizimit të këtij hulumtimi shkencor.

Një falenderim i veçantë për punëtorët shëndetësorë të repartit të infektivës dhe repartit të pulmologjisë pranë ShSKUK.

Falenderoj Hekuran Sabedini (Fakulteti i Psikologjisë) për këshillat dhe konsultimet e tij gjatë finalizimit të këtij punimi.

1. Hyrje

Në këtë studim flitet rreth inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë në kohë pandemie, gjatë këtij studimi do të kuptohet se sa të rëndësishme janë këto dy variabla për punëtorët shëndetësorë në kohë të pandemisë.

Benet (1905), ka dhënë një ndër përkufizimet më complete për inteligjencën emocionale, sipas të cilit ajo është aftësi e përgjithshme e përbërë prej katër karakteristikave themelore: të kuptuarit; invencioni; direksioni; censura (vërtetimi i zgjidhjeve) (Benet, 1905).

Termi *emocion* shfaq nocionin rreth të cilit kuptim të saktë filozofët dhe psikologët për shumë vite me rrallë kanë kundërthënë në mes vete. Në kuptimin më tekstual, (Fjalori i Oksfordit i Gjuhës Angleze , 2001) emocionin e konceptualizon si “çdo agjitacion apo ngacim i psiqikes, epshit, ndjenjës, çdo gjendje mendore e shpejtë apo joqetësisë”. Me termin *emocion* u atribuohen mendimeve dhe ndjenjave të veçanta, gjendjet biologjike apo psikologjike dhe zinxhirin e prirjeve për veprim. Ekzistojnë shumë emocione, bashkë me hibridet e tyre, mutacionet, variacionet dhe nuancat. Dihet botërisht se emocionet janë shumë më perfide sesa ekzistojnë fjalët me të cilat mund të përshkruhen (Rose, 1976).

Ndryshe prej këtyre fakteve, një pjesë e mjekëve janë skeptikë apo kanë dyshime kur bëhet fjalë për rëndësinë klinike që kanë emocionet. Kjo për faktin se edhe pse është argumentuar se dhe emocionet negative dhe stresi dobësojnë efikasitetin e imuno-qelizave të ndryshme, nuk është gjithmonë e qartë se hallka e këtyre ndryshimeve është mjaft i numërt për të qenë në mënyrë medicinale i ndryshueshëm (Rose, 1976).

Stresi në punë diskutohet si çështja tek të cilat e ndeshmi më së shumti si në jetën e përditshme, ashtu edhe në vendet e punës, pasi që në botën aktuale moderne ka të bëjë me individë të vendeve dhe kulturave të ndryshme të cilët punojnë së bashku në vendin e punës. Puna që çdo punëtor e bën, formon trysni dhe stres, e posaçërisht kur ka të bëjë me vende të mëdha pune siç janë organizatat apo kompanitë e ndryshme, shkollat apo spitalet në rastin tonë konkret (Caplan, 1983).

Pandemia aktuale, e ashtuquajtura pandemia e COVID-19 është një sëmundje akute e frymëmarrjes që mund të jetë e rëndë dhe shkaktohet nga një koronavirus i sapo identifikuar i quajtur zyrtarisht SARS-CoV2. COVID-19. Për ne, ky virus është i panjohur, andaj edhe ka lindur motivimi që të studiohet nga afër problematika e këtij virusi tek punëtorët shëndetësorë (Sher, 2020)..

Nga sa mund të shihet, epidemia aktual e COVID-19 ka shpërfaqur një mungesë të theksuar të përvojës në përballje me problemet e shëndetit mendor të këtyre përmasave. Në anën tjetër, numri i madh i hulumtimeve të kryera është dëshmia më e mirë se përvoja aktuale ka nxitur një seriozitet më të madh tek profesionistët e fushës së shëndetit mendor për t’u përgatitur dhe përballur me situata të tilla në të ardhmen. Fatkeqësisht, në Kosovë nuk është gjetur as edhe një artikull apo

hulumtim të botuar i cili trajton në mënyrë të hollësishme e me të dhëna statistikore inteligjencën emocionale dhe stresin tek punëtorët shëndetësorë. Prandaj, është shumë i rëndësishëm të studiohet ky problem (Sher, 2020).

Qëllimi kryesor i këtij studimi është që të shihet ndërlidhja në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë në kohë të pandemisë, qëllim tjetër është edhe të shihet se a ekziston ndërlidhje në mes inteligjencës emocionale dhe stresit, me të dhënat demografike: moshë, gjinia, vendbanimi dhe niveli i arsimit tek punëtorët shëndetësorë.

2. Literatura

2.1. Koncepte mbi inteligjencën emocionale

Termi *emocion* shfaq nocionin rreth të cilit kuptim të saktë filozofët dhe psikologët për shumë vite me rrallë kanë kundërthënë në mes vete. Në kuptimin më tekstual, (Fjalori i Oksfordit i Gjuhës Angleze, 2001) fjalën *emocion* e konceptualizon si çdo agjitacion apo ngacmim i psikikes, epshit, ndjenjës, çdo gjendje mendore e shpejtë apo joqetësisë”. Me termin *emocion* u atribuohen mendimeve dhe ndjenjave të veçanta, gjendjet biologjike apo psikologjike, dhe zinxhirin e prirjeve për veprim. Ekzistojnë shumë emocione, bashkë me hibridet e tyre, mutacionet, variacionet dhe nuancat. Dihet botërisht se emocionet janë shumë më perfide sesa ekzistojnë fjalët me të cilat mund të përshkruhen (Rose, 1976).

Hulumtuesit vazhdojnë debatin për atë se cilat nga emocionet duhet të jenë primare – të kuqen, të kaltrën, apo të verdhën emocion nga e cila burojnë të gjitha nuancat e emocioneve – krejt deri te ajo se a ekzistojnë fare emocionet themelore (Goleman, 1995). Teoricienët Jacobsen, (1965) dhe Becan, (1996) propozojnë grupet kryesore, edhe pse nuk janë të pajtimit të gjithë rreth të njëjtës. Pretendentët kryesorë, apo disa prej anëtarëve të familjes janë:

2.2. Inteligjenca emocionale në raport me moshën.

Studiuesit e fushës së inteligjencës emocionale kanë bërë po ashtu shumë kërkime për të parë funksionin në mes relacionin e Inteligjencës Emocionale dhe moshës. Kjo fushë po ashtu vë në dukje gjetje kontradiktore në literaturën relevante. Disa studime, pohojnë përmes gjetjeve të tyre

se njerëzit më të moshuar e kanë më të zhvilluar inteligjencën emocionale sesa njerëzit me moshë më të re, përderisa studimet e tjera kanë zbuluar dallime shumë të vogla, ose aspak dallime ndërmjet inteligjencës emocionale dhe moshës. Sidoqoftë, studimet e (Moriarty, 2007) fokusohen në teoritë më relevante të inteligjencës emocionale, supozojnë se një gamë e gjerë e relacionit të inteligjencës emocionale dhe moshës, mund të sqarohet prej atribuimit të faktorëve të të mësuarit përgjatë jetës. Studiuesit mendojnë se një gamë e gjerë e modaliteteve të inteligjencës, mund të përmirësohen dhe avancohen përmes ushtrimeve dhe përgjatë kohës. (Moriarty, 2007), duke theksuar kështu edhe inteligjencën emocionale, si një aset mbi të cili përvoja dhe mosha mund të lënë gjurmë.

2.3. Inteligjenca emocionale në raport me gjinin

Sipas Garcia & Lopez (2009) shqyrtimi i literaturës që trajton dhe adreson çështjet e konstruktit të inteligjencës emocionale, vë në dukje po ashtu ndikimin që gjinia ka (dallimeve gjinore) në të kuptuarit e dallimeve individuale rreth kapacitetit të tyre për të zhvilluar në mënyrë sa më të saktë dhe më efikase informacionin emocional. Në përgjithësi, thuhet se fusha e studimit të inteligjencës emocionale gjatë këtyre 20 viteve ka evoluar jashtëzakonisht shumë, duke na sjellë kështu rezultate të dobishme lidhur me forcën parashikuese të këtij konstrukti në aspektet e ndryshme të jetës tek të dy gjinitë, meshkujve dhe femrave. Sidoqoftë, në përgjithësi, edhe pse shpeshherë gjetjet e studimeve lidhur me ndikimin e konstruktit të inteligjencës emocionale dhe dallimeve gjinore duket të jenë kontradiktore kur merret për bazë i gjithë rezultati i inteligjencës emocionale, megjithatë thuhet se dallimet gjinore në këtë aspekt duket të jenë një ndryshore e rëndësishme për studiuesit e kësaj fushe. Në këtë aspekt, duhet pasur parasysh që këto rezultate të cilat janë kontradiktore dhe sqarimet pasuese për dallimet e tilla gjinore në raport me inteligjencën emocionale, mbeten të kuptohen tutje, kjo sidomos në raport me matjet vetëraportuese të konstruktit të inteligjencës emocionale nga qasjet e ndryshme teorike (Garcia & Lopez, 2009).

2.4. Koncepte mbi stresin në punë

Stresi është një problem në jetën e secilit individ, ai ndikon negativisht në jetën e secilit nga ne. Stresi është një ndjenjë e cila perceptohet e rrezikshme dhe e pakëndshme emocionale si dhe fiziologjike. Nocioni "stres" do të thotë gjëra të ndryshme për njerëz të ndryshëm. Psikologë të ndryshëm botërorë e kanë përkufizuar "stresin" si problem serioz i cili shpesh mund të jetë edhe kronik. Ekzistojnë faktorë të ndryshëm të cilët ndikojnë në formimin e stresit në jetën e një individi. Sipas hulumtimeve të ndryshme psikologjike shkaktarët të cilat shkaktojnë stresin janë quajtur stresorë. Stresi në jetë në përgjithësi, dhe në punë është një faktor që mund të shkaktojë sëmundje mendore në jetën e njerëzve (Orhani, 2011).

Një individ i cili është në gjendje stresuese, zakonisht ka vështirësi në përqendrimin e vëmendjes, sjellje të jashtme të ndryshuar dhe mendime ankthi. Kështu, shtrëngimi i duarve dhe i dhëmbëve, boshllëku në stomak, apo frymëmarrja e vështirë, duart dhe këmbët e ftohta, rritja e rrahjeve të zemrës, goja e tharë, tronditja, ankthi dhe frika, ankesat psiko-somatike, çrregullimi i gjumit e të tjera, janë disa nga shenjat më të zakonshme të stresit që mund të shkaktohen edhe me disa shenjave fiziologjike të emocioneve të ankthit (Caplan, 1983).

Si gjendje unike psikologjike stresi është në relacion me çdo gjë që ushtron kërkesa ndaj të cilave duhet të përshtatemi. Ai në të vërtetë nënkupton të gjitha ndryshimet me natyrë sociale, somatike e psikologjike që bëjnë të nevojshme ngjashmërinë. Pra, çdo rrethanë që shkakton tension të brendshëm teknikisht quhet stresuese. Stresi është i pashmangshëm në pjesën më të madhe të jetës të secilit person. Duke pasur parasysh shkaqet dhe faktorët e tij (Orhani, 2011).

Sipas psikologut Mytahir Haskuka, stresi në përgjithësi është një nocion i ri i cili në aspektin terminologjik do të thotë tendosje, njerëzit ndihen të tendosur dhe reagojnë shpejt. Haskuka ka thënë se stresi paraqitet në mënyra të ndryshme te njerëz të ndryshëm (Orhani, 2011).

2.5. Koncepte mbi pandemin Covid 19

Sipas Organizatës Botërore të Shëndetit (OBSh), sëmundjet infektive siç janë koronavirusët hynë në familjen e sëmundjeve infektive apo viruseve që shkaktojnë sëmundje duke filluar nga më të lehtat siç është ftohja e zakonshme e deri te sëmundjet më të rënda siç janë Sindroma e frymëmarrjes në Lindjen e Mesme (MERS) dhe koronavirusi i rëndë i lidhur me sindromën e frymëmarrjes (SARS) (Sher, 2020).

Kemi parë siç ndodhi me virusin e fundit (koronavirusi) i cili fillimisht u transmetua përmes kafshëve dhe pastaj njerëzve. Koronavirusi, besohet se fillimisht është transferuar nga macet, ndërsa virusi MERS udhëtonte nga një lloj deve tek njerëzit. Ekzistojnë edhe disa koronavirusë të njohura që po qarkullojnë te kafshët që nuk kanë infektuar ende njerëzit. Emri koronavirus vjen nga fjala latine *corona*, që do të thotë *kurorë* ose *halo*. Nën një mikroskop elektronik, imazhi i virusit i përngjan një korone diellore. Një koronavirus i ri, i identifikuar nga autoritetet kineze më 7 janar dhe aktualisht i quajtur 2019-nCoV, është një lloj i ri që nuk ishte identifikuar më herët tek njerëzit. Dihet pak për këtë, megjithëse transmetimi njeri-tek-njeri është konfirmuar (Sher, 2020).

Nga sa mund të shihet, epidemia aktuale e COVID-19 ka shpërfaqur një mungesë të theksuar të përvojës në përballje me problemet e shëndetit mendor të këtyre përmasave. Në anën tjetër, numri i madh i hulumtimeve të kryera është dëshmia më e mirë se përvoja aktuale ka nxitur një seriozitet më të madh tek profesionistët e fushës së shëndetit mendor për t'u përgatitur dhe përballur me situata të tilla në të ardhmen. Fatkeqësisht, në Kosovë nuk kam gjetur as edhe një artikull apo hulumtim të botuar i cili trajton në mënyrë të hollësishme e me të dhëna statistikore temën të cilën është hulumtuar e lëre më ndonjë bazë të të dhënave. Një bazë e tillë elektronike do të ndihmonte në grumbullimin e të dhënave, analizimin dhe kuptimin e tyre dhe do të ishte një mjet i dobishëm

në luftimin, trajtimin dhe sanimin e pasojave psikologjike tek popullata në kohë pandemie. Mbase gjërat do të ndryshojnë për të mirë në muajt apo vitet e ardhshme (Sher, 2020).

Karantimi si rezultat i pandemisë, ka rezultuar me kufizime në socializim, argëtim dhe kontakte fizike si dhe me shumë pacient që trajtohen në shtëpi shëndetësore, kjo ka qenë gjendje e rëndë fizike dhe psikike për punëtorët shëndetësor, ata janë përballur çdo ditë me stresin e punës dhe ka qenë shumë e nevojshme për ta zhvillonin intelegjiencen emocionale të tyre në atë mënyrë që të arrinin ta menaxhonin stresin.

Për ta parë se a ka pasur ndërlidhje në mes inteligjences emocionale dhe stresit në punë ka qenë i nevojshëm të realizohet një studim i tillë, përmes metodes kuantitative apo sasiore ky studim do të na ipte rrezultate të qarta do të na ndihmohnte edhe të krahasojmë rrezultatet në peridhua të ndryshme kohore.

Qëllimi kryesor i këtij studimi është që të shihet ndërlidhja në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë në kohë të pandemisë, qëllim tjetër është edhe të shihet se a ekziston ndërlidhje në mes inteligjencës emocionale dhe stresit, me të dhënat demografike: moshë, gjinia, vendbanimi dhe niveli i arsimit tek punëtorët shëndetësorë

3. Metodologjia

3.1. Metoda kërkimore

Për realizimin e këtij studimi është përdorur metoda kuantitative (sasiore) e studimit. Për këtë studim do të përdoret dizajni i kërkimit korrelacional. Sipas Burns & Grove (2010), kërkimi korrelacional ka për qëllim të përshkruajë marrëdhëniet midis dy ose më shumë variablave, të parashikojë rrezultatet e një variabli nga rrezultatet e pjesëmarrësve në variablin tjetër, ose të testojë marrëdhëniet e supozuara nga propozimet teorike (Bruns & Grove, 2010). Në këtë studim janë bërë ndërlidhje në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë.

3.2. Mostra e hulumtimit

Mostër e këtij hulumtimi janë: punëtorët shëndetësorë të repartit të infektives dhe repartit të pulmologjisë. Popullata e studimit në këto reparte është 168 por që popullata mbetet relative pasi punëtorët shëndetësorë rekrutohen shpesh nga një repart në tjetrin sipas nevojës që kanë ata. Por për mostër të këtij studimi janë marrë 150 punëtorë shëndetësorë. Mostra është përzgjedhur në mënyrë rastësore duke i anketuar të gjithë punëtorët shëndetësorë që ishin prezentë prej të dy ndërrimeve të punëtorëve. Subjektet që janë marrë për mostër janë ndarë në 63 meshkuj dhe 87 femra dhe sillen nga moshë 22 deri në 55 vjet. Arsyeja se përse kemi të limituar deri tek moshë 55 vjetë, është sepse mostra ishte në mënyrë rastësore dhe në ditën e anketimit nuk kishte respondentë

më moshë më të lartë. Të dhënat janë mbledhur në repartin e infektivës dhe repartin e Pulmologjisë në qytetin e Mitrovicës - Kosovë. Profili i respondentëve kanë qenë mjek dhe infermiar që janë grupuar si punëtorë shëndetësor. Subjektet të cilat janë marrë për mostër gërshetojnë mes vete karakteristikat për një mostër adekuate, ata posedojnë të gjitha karakteristikat e popullatës ku do të zhvillohet hulumtimi, subjektet kanë mundësi të barabartë në përfshirje të mostrës.

3.3. Përpunimi i të dhënave

Pas mbledhjes së fakteve në terren, vjen puna në tavolinë, që ka të bëjë me analizën dhe përpunimin e statistikave. Deklaratat e ndryshme kërkojnë analizë specifike për të gjetur treguesit që na interesojnë. Përpunimin i rezultateve është bërë me anë të programin SPSS25, i cili mundëson që të dhënat e futura në databazë të përpunohen më shpejt. Paraprakisht është përpiluar një skemë koduese të dhënash, në shprehje kuantifikuese. Kodimi i informatave është procesi i shndërrimit në një format të kuantifikuar, zakonisht numerik, që të mund të bëhet më lehtë analiza sistematike e tyre. Proces i kodimit filloi me kodimin e përgjigjeve paraprakisht, duke mundësuar që numrat të rumbullakohen ose të shënuar të futen direkt në bazë të të dhënave. Vlera e të dhënave si numra ose si emërtime varet nga analizat që është bërë. Analizat që janë bërë në këtë studim janë analizat deskriptive pastaj është përdorur analiza e korrelacionit të Pearsonit, Anoven dhe T-testin.

4. Rezultatet

4.1. Rrezultatet lidhur me të dhënat demografike

Tabela 1. Paraqitja e të dhënave demografike të punëtorëve shëndetësorë

		Frekuencat	Përqindja
Gjinia	Femër	87	58.0%
	Mashkull	63	42.0%
	Total	150	100.0%
Moshë	25-30 vjeç	45	30.0%
	31-35 vjeç	31	20.7%
	36-40 vjeç	23	15.3%
	41-45 vjeç	21	14.0%
	46-50 vjeç	15	10.0%
	51-55 vjeç	7	4.7%
	56-60 vjeç	8	5.3%
	Total	150	100.0%
Vendëbanimi	Fshat	54	36.0%
	Qytet	96	64.0%
mostra	Total	150	100.0%

Niveli i arsimit	Arsimim i mesëm	43	28.7%
	Arsimim universitar	85	56.7%
	Arsimim pasuniversitar	22	14.7%
	Total	150	100.0%
Përvoja në punë	0-5 vite	69	46.0%
	6-10 vite	53	35.3%
	11-15 vite	13	8.7%
	16-20 vite	11	7.3%
	Mbi 20 vite	4	2.7%
	Total	150	100.0%

Në studim morën pjesë 150 punëtorë shëndetësorë, ku 42% ishin të gjinisë mashkullore dhe 58% të gjinisë femërore. Moshë minimale e punëtorëve shëndetësorë është 25 vjeç, kurse ajo maksimale 60 vjeç. Numri më i madh i punëtorëve shëndetësorë 30% i përkasin moshës 25-30 vjeç, kurse numri më i vogël 4.7% punëtorë shëndetësorë i përkasin moshës 51-55 vjeç. Gjithsej në studim morën pjesë 150 punëtorë shëndetësorë, ku 36% jetonin në fshat, kurse 64% jetonin në qytet. Pra, këtu shihet se është një dominim të vendbanimit në qytet krahasuar me vendbanimin në fshat. 28.7% e punëtorëve shëndetësorë e kanë të përfunduar arsimimin e mesëm, 56.7% e kanë të përfunduar arsimimin universitar dhe 14.7% kanë të përfunduar arsimimin pasuniversitar. 46% e punëtorëve shëndetësorë kanë përvojë pune 0-5 vite, 35.3% kanë përvojë pune 6-10 vite, 8.7% e punëtorëve shëndetësorë kanë përvojë pune 11-15 vite, 7.3% kanë përvojë pune 16-20 vite dhe 2.7% e punëtorëve shëndetësorë kanë përvojë pune mbi 20 vite.

4.1. Ndërlidhja në mes inteligjencës emocionale dhe stresit

Më poshtë, në tabelën nr. 1 është paraqitur analiza e korrelacionit në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë në kohë të pandemisë.

Tabela1. Korrelacioni në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë

		Inteligjenca emocionale	Stresi
Inteligjenca emocionale	Pearson Correlation	1	.076
	Sig. (2-tailed)		.001
	N	150	150
Stresi	Pearson Correlation	.076	1
	Sig. (2-tailed)	.001	
	N	150	150

Nga analiza korrelative nxirren këto përfundime: inteligjenca emocionale ka marrëdhënie lineare pozitive me stresin në punë gjatë pandemisë $r=.076$. Signifikanca është 0.001. $p<0.05$ në nivelin e rëndësisë 0.05. Nga kjo mund të thuhet se ka lidhje signifikante në mes dy ndryshoreve.

Si përfundim mund të thuhet se ka ndërlidhje signifikante në mes inteligjencës emocionale dhe stresit në punë gjatë pandemisë tek punëtorët shëndetësorë.

4.2. Ndërlidhja në mes inteligjencës emocionale dhe të dhënave demografike

Tabela 2. ANOVA e inteligjencës emocionale lidhur me moshën e punëtorëve shëndetësorë

I. Emocionale

	Shuma e katrorëve	Df (Shkallët e lirisë)	Mesatarja e katrorëve	F	Sig.(p)
Ndërmjet grupeve	5.866	6	.978	1.890	.086
Brenda grupeve	73.985	143	.517		
Total	79.851	149			

Rezultatet nga analiza njëkahëshe e variancës (ANOVA) tregojnë se nuk ka dallime signifikante ($p>05$) në mes inteligjencës emocionale në punë dhe moshës së punëtorëve shëndetësorë për të gjitha nivelet e moshës, ku $F(143)=1.89$, $p>0.05$. Moshë 25 deri në 30 vjet ka ($M=3.40$ dhe $Ds=0.52$), moshë 31 deri 35 vjet ka ($M=3.14$ dhe $Ds=0.68$), moshë 36 deri 40 vjet ka ($M=3.30$ dhe $Ds=0.94$), moshë 41 deri 45 vjet ka ($M=3.15$ dhe $Ds=0.80$), moshë 46-50 vjet ka ($M=3.21$ dhe $Ds=0.82$), moshë 51-55 vjet ka ($M=2.48$ dhe $Ds=0.57$) dhe moshë 56-60 vjet ka ($M=3.32$ dhe $Ds=0.76$). Pra, nga rezultatet del se nuk ka dallime signifikante në mes në mes inteligjencës emocionale dhe moshës së punëtorëve shëndetësorë për të gjitha nivelet e moshës.

Tabela 3. Paraqitja e dallimeve gjinore në kontekst të inteligjencës emocionale përmes T-testit

	Testi i Leventit për barazinë e variancës		t-test për barazimin e mesatareve					95% Diferenca e intervalit të besueshmërisë	
	F	Sig.(p)	t	Df (Shkallët e lirisë)	Sig. (2-tailed)	Diferenca e mesatareve	Diferenca e gabimit standard	E ulët	E lartë
Inteligjenca supozuara emocionale	.348	.556	-.695	148	.488	-.08426	.12132	-.32399	.15548
Varianca të barabarta të supozuara			-.701	137.739	.485	-.08426	.12027	-.32207	.15356
Varianca të barabarta jo të supozuara									

Duke i vështruar mesataret që arrijnë femrat dhe meshkujt në tabelën 9 dhe 10 shihet se femrat arrijnë mesatare të inteligjencës emocionale $M=3.20$, kurse meshkujt kanë mesatare të inteligjencës emocionale $M=3.28$. Devijimi standard për femrat është $Ds=0.74$, ndërsa për meshkujt $Ds=0.78$ dhe mesatarja e gabimit standard për femrat është $Gs=0.080$ dhe për meshkujt $Gs=0.089$. Signifikanca është 0.556 . $p>0.05$ në nivelin e rëndësisë 0.05 . Nga kjo mund të thuhet se nuk ka ndonjë lidhje signifikante në mes inteligjencës emocionale dhe gjinisë.

4.3. Ndërlidhja në mes stresit në punë dhe të dhënave demografike

Tabela 4. ANOVA e stresit në punë lidhur me moshën e punëtorëve shëndetësorë
Stresi

	Shuma e katrorëve	Df (Shkallët e lirisë)	Mesatarja e katrorëve	F	Sig.
Ndërmjet grupeve	3.218	6	.536	1.772	.109
Brenda grupeve	43.274	143	.303		
Total	46.492	149			

Rezultatet nga analiza njëkahëshe e variancës (ANOVA) tregojnë se nuk ka dallime signifikante ($p>0.05$) në mes stresit në punë dhe moshës së punëtorëve shëndetësorë për të gjitha nivelet e moshës, ku $F(149)=1.77$, $p>0.05$. Moshë 25 deri në 30 vjet ka ($M=2.70$ dhe $Ds=0.56$), moshë 31 deri në 35 vjet ka ($M=2.61$ dhe $Ds=0.53$), moshë 36 deri në 40 vjet ka ($M=2.88$ dhe $Ds=0.49$), moshë 41 deri në 45 vjet ka ($M=2.98$ dhe $Ds=0.61$), moshë 46-50 vjet ka ($M=2.89$ dhe $Ds=0.50$), moshë 51-55 vjet ka ($M=3.10$ dhe $Ds=0.65$) dhe moshë 56-60 vjet ka ($M=2.90$ dhe $Ds=0.42$). Pra, nga rezultatet del se nuk ka dallime signifikante në mes në mes stresit në punë dhe moshës së punëtorëve shëndetësorë për të gjitha nivelet e moshës.

Tabela 5. Paraqitja e dallimeve gjinore në kontekst të stresit në punë përmes T-testit

		Testi i Leventit për barazinë e variancës		t-test për barazimin e mesatareve					95% Diferenca e intervalit të besueshmërisë	
		F	Sig.	t	Df (Shkallët e lirisë)	Sig. (2-tailed)	Diferenca e mesatareve	Diferenca e gabimit standard	E ulët	E lartë
Stresi	Varianca të barabarta të supozuara	.791	.375	-.289	148	.773	-.02680	.09269	-.20997	.15638
	Varianca të barabarta jo të supozuara			-.285	126.677	.776	-.02680	.09399	-.21279	.15919

Duke i vështruar mesataret që arrijnë femrat dhe meshkujt në tabelën 19 dhe 20 shihet se femrat arrijnë mesatare të stresit në punë $M=2.79$, kurse meshkujt kanë mesatare të stresit në punë $M=2.81$. Devijimi standard për femrat është $Ds=0.53$, ndërsa për meshkujt $Ds=0.58$ dhe mesatarja e gabimit standard për femrat është $Gs=0.05$ dhe për meshkujt $Gs=0.07$. Signifikanca është 0.375 . $p>0.05$ në nivelin e rëndësisë 0.05 . Nga kjo mund të thuhet se nuk ka ndonjë lidhje signifikante në mes stresit në punë dhe gjinisë tek punëtorët shëndetësorë.

Përfundimi

Në këtë studim është shqyrtuar literatura teorike ekzistuese duke përzgjedhur artikujt që lidhen me studimin tonë të cilat shërbejnë për të kuptuar më thellë problemin dhe për të parë lidhjen midis studimit tonë dhe studimeve të tjera të realizuara.

Nga rezultatet e studimit kemi kuptuar se nuk ka dallime signifikante të inteligjencës emocionale për të gjitha nivelet e moshës së punëtorëve shëndetësorë, femrat arrijnë mesatare të inteligjencës emocionale $M=3.20$, ndërsa meshkujt $M=3.28$ por që këto dallime nuk kanë rëndësi signifikante, po ashtu shihet se respondentët me vendbanim në fshat arrijnë mesatare të inteligjencës emocionale $M=3.19$ më të vogël se sa respondentët me vendbanim në qytet, të cilët kanë mesatare të inteligjencës emocionale $M=3.25$ por që këto dallime nuk kanë rëndësi statistikore. Po ashtu nga rezultatet mund të shihet se nuk ka dallime signifikante në mes inteligjencës emocionale në punë

dhe nivelit të arsimit të punëtorëve shëndetësorë për të gjitha nivelet e arsimit po ashtu rezultatet tregojnë se nuk ka dallime signifikante në mes inteligjencës emocionale në punë dhe përvojës në punë për të gjitha nivelet e përvojës së punës.

Nga rezultatet lidhur me stresin në punë dhe të dhënave demografike ka dalë se nuk ka dallime signifikante në mes stresit në punë dhe moshës së punëtorëve shëndetësorë për të gjitha nivelet e moshës, po ashtu nga rezultatet mund të shihet se femrat arrijnë mesatare të stresit në punë $M=2.79$ më të ulët se sa meshkujt, të kanë mesatare të stresit në punë $M=2.81$ por që ky dallim nuk është signifikant, respondentët me vendbanim në fshat arrijnë mesatare të stresit në punë $M=2.70$ më të vogël se sa respondentët me vendbanim në qytet, të kanë mesatare të stresit në punë $M=2.85$ por që as ky dallim nuk është signifikant. Nga rezultatet gjithashtu mund të shihet se nuk ka dallime signifikante në mes stresit në punë dhe nivelit të arsimit të punëtorëve shëndetësorë për të gjitha nivelet e arsimit, po ashtu rezultatet tregojnë se nuk ka dallime signifikante në mes stresit në punë dhe përvojës në punë për të gjitha nivelet e përvojës së punës.

Diskutimi i rrezultateve

Në këtë kapitull janë përmbledhur gjetjet kryesore të studimit duke i diskutuar ato në lidhje me literaturën përkatëse. Këto gjetje janë krahasuar me studime të tjera nga autorë të ndryshëm dhe është paraqitur këndvështrimi lidhur me to ku është kryer edhe ky studim. Fokusi i këtij studimi është të paraqesë rezultatet lidhur me stresin dhe inteligjencën emocionale në punë tek punëtorët shëndetësorë gjatë pandemisë Covid-19 në QKUK të lidhura këto dhe me të dhëna demografike (gjinia, mosha, vendbanimi, niveli i arsimit dhe përvoja e punës).

Diskutime për hipotezën H1: ekziston ndërlidhje në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë në kohë të pandemisë.

Për ta parë ndërlidhjen në mes inteligjencës emocionale dhe stresit në punë tek punëtorët shëndetësorë në kohë të pandemisë është përdorur analiza e korrelacionit. Nga analiza korrelative në studim janë nxjerrë këto përfundime: inteligjenca emocionale ka marrëdhënie lineare pozitive me stresin në punë gjatë pandemisë si dhe ndërlidhja mes këtyre variablave është signifikante. Nga këto rezultate vijmë në përfundim se aprovohet hipoteza 1.

Arsyeja pse ka dalë një rezultat i tillë është sepse inteligjenca emocionale tek punëtorët shëndetësorë ka mbetur pothuajse ajo siç ka qenë para pandemisë edhe tani gjatë pandemisë, kurse stresi tek punëtorët shëndetësorë mendohet të ketë qenë më i ulët, kurse tani gjatë pandemisë kemi ngritje të lirë të stresit në punë tek punëtorët shëndetësorë.

Lidhjet midis dimensioneve të këtij niveli janë të ngjashme me ato që raportohen nga studimet ndërkombëtare (Kulkarni, Fareill, Erasi, & Koschar, 1998) edhe pse koeficienti i korrelacionit të inteligjencës emocionale stresit në punë nuk është signifikant. Për shembull, në një studim ndërkombëtar dimensionet e lidhur më fort me njëri-tjetrin midis stresit në punë dhe inteligjencës emocionale (Spearman's $\rho=0.63$) (Kulkarni, Fareill, Erasi, & Koschar, 1998), kurse në këtë

studim ndërlidhja mes stresit në punë dhe inteligjencës emocionale ishte (Pearson's -0.76 dhe sginikanca 353).

Diskutime për hipotezën H2: ekziston ndërlidhje në mes inteligjencës emocionale dhe të dhënave demografike: moshë, gjinia, vendbanimi, niveli i arsimit dhe përvoja në punë tek punëtorët shëndetësorë në kohë të pandemisë.

Për të parë nëse ekziston ndërlidhje në mes inteligjencës emocionale dhe të dhënave demografike: moshë, gjinia, vendbanimi, niveli i arsimit dhe përvoja në punë tek punëtorët shëndetësorë në kohë të pandemisë janë mbledhur të dhëna përmes *Instrumentit për matjen e inteligjencës emocionale* i standardizuar nga (Schutte, 1990) i cili iu ka përshtatur jashtëzakonisht kërkesave, kurse për mbledhjen e të dhënave është përdorur metoda kuantitative e studimit e cila mendojmë se është më adekuate për një studim të tillë.

Për të parë nëse ekziston ndërlidhje në mes inteligjencës emocionale dhe të dhënave demografike: moshë, gjinia, vendbanimi, niveli i arsimit dhe përvoja në punë tek punëtorët shëndetësorë në kohë të pandemisë është përdorur analizat e ANOVA dhe T-testi, nga këto analiza del se nuk ka dallime signifikante për të gjitha nivelet e moshës së punëtorëve. Duke i vështruar mesataret që arrijnë femrat dhe meshkujt nga rezultatet e t-testit del se femrat arrijnë mesatare të inteligjencës emocionale $M=3.20$ se sa meshkujt $M=3.28$ por që këto dallime nuk kanë rëndësi signifikante. Nga analiza t-testi po ashtu shihet se respondentët me vendbanim në fshat arrijnë mesatare të inteligjencës emocionale $M=3.19$ më të vogël se sa respondentët me vendbanim në qytet, të cilët kanë mesatare të inteligjencës emocionale $M=3.25$ por që këto dallime nuk kanë rëndësi statistikore. Rezultatet nga analiza njëkahëshe e variancës (ANOVA) tregojnë se nuk ka dallime signifikante në mes inteligjencës emocionale në punë dhe nivelit të arsimit të punëtorëve shëndetësorë për të gjitha nivelet e arsimit, po ashtu rezultatet nga analiza njëkahëshe e variancës (ANOVA) tregojnë se nuk ka dallime signifikante në mes inteligjencës emocionale në punë dhe përvojës në punë për të gjitha nivelet e përvojës së punës. Si përfundim mund të thuhet se nga analizat statistikore shihet se nuk ka ndërlidhje signifikante në mes inteligjencës emocionale me moshën, gjininë, vendbanimin, nivelin të arsimit dhe përvojës në punë tek punëtorët shëndetësorë. Nga këto rezultate mund të përfundohet se nuk aprovohet hipoteza 2.

Arsyeja se pse nuk është ndërlidhje signifikante në mes inteligjencës emocionale me moshën, gjininë, vendbanimin, nivelin e arsimit dhe përvojës në punë tek punëtorët shëndetësorë është sepse shumica e punëtorëve shëndetësorë kanë nivel të njëjtë të arsimit, prandaj kjo i bën të padallueshëm si tek gjinia ashtu edhe tek moshë dhe vendbanimi i tyre.

Po të krahasohen rezultatet e këtij studimi me studiuesit e fushës së inteligjencës emocionale, disa studime, pohojnë përmes gjetjeve të tyre se njerëzit më të moshuar e kanë më të zhvilluar inteligjencën emocionale sesa njerëzit me moshë më të re, përderisa studimet e tjera kanë zbuluar dallime shumë të vogla, ose aspak dallime ndërmjet inteligjencës emocionale dhe moshës. Sidoqoftë, studimet e (Moriarty, 2007) fokusohen në teorinë më relevante të inteligjencës emocionale, supozojnë se një gamë e gjerë e relacionit të inteligjencës emocionale dhe moshës, mund të sqarohet prej atribuit të faktorëve të të mësuarit përgjatë jetës. Studiuesit mendojnë se një gamë e gjerë e modaliteteve të inteligjencës, mund të përmirësohen dhe avancohen përmes

ushtrimeve dhe përgjatë kohës. (Moriarty, 2007), duke theksuar kështu edhe inteligjencën emocionale, si një aset mbi të cilin përvoja dhe mosha mund të lënë gjurmë.

Sipas Garcia & Lopez (2009) shqyrtimi i literaturës që trajton dhe adreson çështjet e konstruktit të inteligjencës emocionale, vë në dukje po ashtu ndikimin që gjinia ka (dallimeve gjinore) në të kuptuarit e dallimeve individuale rreth kapacitetit të tyre për të zhvilluar në mënyrë sa më të saktë dhe më efikase informacionin emocional. Në përgjithësi, thuhet se fusha e studimit të inteligjencës emocionale gjatë këtyre 20 viteve ka evoluar jashtëzakonisht shumë, duke na sjellë kështu rezultate të dobishme lidhur me forcën parashikuese të këtij konstrukti në aspektet e ndryshme të jetës tek të dy gjinitë, meshkujve dhe femrave. Sidoqoftë, në përgjithësi, edhe pse shpeshherë gjetjet e studimeve lidhur me ndikimin e konstruktit të inteligjencës emocionale dhe dallimeve gjinore duket të jenë kontradiktore kur merret për bazë i gjithë rezultati i inteligjencës emocionale, megjithatë thuhet se dallimet gjinore në këtë aspekt duket të jenë një ndryshore e rëndësishme për studiuesit e kësaj fushe. Në këtë aspekt, duhet pasur parasysh që këto rezultate të cilat janë kontradiktore dhe sqarimet pasuese për dallimet e tilla gjinore në raport me inteligjencën emocionale, mbeten të kuptohen tutje, kjo sidomos në raport me matjet vetëraportuese të konstruktit të inteligjencës emocionale nga qasjet e ndryshme teorike (Garcia & Lopez, 2009).

Diskutime për hipotezën H3: ekziston ndërlidhje në mes stresit në punë dhe të dhënave demografike: mosha, gjinia, vendbanimi, niveli i arsimit dhe përvoja në punë tek punëtorët shëndetësorë në kohë të pandemisë.

Për të parë nëse ekziston ndërlidhje në mes stresit në punë dhe të dhënave demografike: mosha, gjinia, vendbanimi, niveli i arsimit dhe përvoja në punë tek punëtorët shëndetësorë në kohë të pandemisë janë mbledhur të dhëna përmes *Instrumentit për matjen e stresit në punë*. Instrumenti i cili u përdorë për matjen e nivelit të stresit të punëtorëve shëndetësorë në ballafaqim me pandeminë Covid-19 është instrumenti i standardizuar nga Kristensen & Borg (2003) i cili iu ka përshtatur jashtëzakonisht kërkesave tona, kurse për mbledhjen e të dhënave është përdorur metoda kuantitative e studimit e cila mendohet se është më adekuate për një studim të tillë.

Për të parë nëse ekziston ndërlidhje në mes stresit në punë dhe të dhënave demografike: mosha, gjinia, vendbanimi, niveli i arsimit dhe përvoja në punë tek punëtorët shëndetësorë në kohë të pandemisë, është përdorur analizat e ANOVA dhe T-testi, nga këto analiza del se nuk ka dallime signifikante në mes stresit në punë dhe moshës së punëtorëve shëndetësorë për të gjitha nivelet e moshës. Nga analiza e t-testit shihet se femrat arrijnë mesatare të stresit në punë $M=2.79$ më të ulët sesa meshkujt, të cilët kanë mesatare të stresit në punë $M=2.81$ por që ky dallim nuk është signifikant, po ashtu nga analiza e t-testit del se respondentët me vendbanim në fshat arrijnë mesatare të stresit në punë $M=2.70$ më të vogël se sa respondentët me vendbanim në qytet, të cilët kanë mesatare të stresit në punë $M=2.85$ por që as ky dallim nuk është signifikant. Rezultatet nga analiza njëkahëshe e variancës (ANOVA) tregojnë se nuk ka dallime signifikante në mes stresit në punë dhe nivelit të arsimit të punëtorëve shëndetësorë për të gjitha nivelet e arsimit, po ashtu rezultatet nga analiza njëkahëshe e variancës (ANOVA) tregojnë se nuk ka dallime signifikante në mes stresit në punë dhe përvojës në punë për të gjitha nivelet e përvojës së punës. Si përfundim mund të thuhet se nga analizat statistikore shihet se nuk ka ndërlidhje signifikante në mes stresit

në punë me moshën, gjininë, vendbanimin, nivelin e arsimit dhe përvojës në punë tek punëtorët shëndetësorë. Nga këto rezultate mund të përfundohet se nuk aprovohet hipoteza 3.

Arsyeja se pse nuk është ndërlidhje signifikante në mes stresit në punë me moshën, gjininë, vendbanimin, nivelin e arsimit dhe përvojës në punë tek punëtorët shëndetësorë mund të jetë si rezultat i ndonjë problemi statistikor në analizën e të dhënave.

Hulumtuesit kanë gjetur se të dhënat demografike janë faktorë shumë të rëndësishëm në shqyrtimin e burimeve dhe strategjive të përballimit të stresit midis punëtorëve shëndetësorë (Lofquist & Dawis, 1969). Rolet gjinore dhe sjellja e përshtatshme gjinore përbëjnë dy burime kryesore të stresit për ta. Literatura e mëparshme zbuloi se punëtorët shëndetësorë femra kishin gjasa të ndesheshin me nivele më të larta të ngacmimit, armiqësisë së hapur dhe ndërveprimeve të tjera negative në punë (Tenant, 2001). Theksojmë se po të krahasohen gjetjet e këtij studimi me një studim të kryer në pesë institucione të shëndetësisë së Hamburgut me 155 pjesëmarrës të gjinisë femërore dhe mashkullore të gjithë spektrit të gradave, moshës, funksioneve dhe kohëzgjatjes në shërbim rezultoi se 19% e tyre u diagnostikuan me stres të lartë në vendin e punës, 56% me nivel të mesëm të stresit dhe 25% e punëtorëve shëndetësorë kishin nivel të ulët të stresit.

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Comparison Study between Angio CT and USG Doppler for Early Detection of Arterial Stenosis of Lower Extremities in University Clinical Center of Kosovo

Lavdim H. Ymeri¹, Vjosa A.Zejnullahu^{1,3}, Serbeze Kabashi Muqaj¹,
Muharrem Sadiku¹, Valon A.Zejnullahu²

1. Department of Radiology, University Clinical Centre of Kosova, 10000 Prishtina, Kosovo.

2. Department of General Surgery University Clinical Centre of Kosova, 10000 Prishtina, Kosovo.

3. Department of Gynecology University Clinical Center of Kosovo.

Abstract

Peripheral artery disease (PAD), also known as atherosclerotic disease leading to peripheral artery obstruction, with a variety of symptoms and signs revealing extremity ischemia. The signs and symptoms of arterial insufficiency are due to low blood flow to the musculature. Low blood circulation leads to the changes in the metabolism. These changes are manifested with the pain in the precious muscle relating to the anatomical structure in which the obstruction occurs. Undoubtedly the peripheral arterial disease (PAD) is a common disease that can affect different groups of population with higher prevalence in different groups with risk factors such as: age (older age), hypertension, smoking, diabetes mellitus, obesity, inflammation, stress, live style, family history of cardio vascular (CV) disease, gender, heredity and dyslipidemia.

The main purpose of this study is to determine the role of USG Doppler and CT angiography examination in patient with PAD, furthermore to estimate the incidence rate of PAD in different group of population based on the age, profession and to identify the potential risk factors.

Material and method: This study was implemented at the University Clinical Center of Kosovo in the department of Radiology for patient with clinical signs of PAD in the lower extremities admitted from the emergency unit and patients who were referred by different unit over a period of one year. All patient were subjected to the duplex ultrasound examination and Angio-CT.

In this study included 150 patients from which 73.3 % were male and 26.7 female. Among the total number of patients 17.3% were urban residence and 82.7 % rural residence. 59.3% of patient were active smokers. During our study we found that most of the patient with peripheral disease were previously diagnosed with diabetes mellitus(DM). In our study we found that just 9.3 % of patient were without chronic disease.36.0% of patients were in the age-group from 60-70 years old. Our study showed that 39.4% of patients were with intensive claudication, 49.3% with moderate and 11.3% with mild claudication. The sensitivity of Duplex Doppler was 88.36%, specify is 50.0 % and accuracy 87.33%.

Angio CT together with duplex ultrasound has higher predictive value that determines the level of occlusion. According to our results the accurate occlusion/sub occlusion is better defined if we use Angio CT and Duplex Doppler together to improve the diagnostic accuracy in PAD. More than half of patients participating in this study were heavy smoker.

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Introduction

Atherosclerosis is consequence from gathering of lipid and fibrosis in the layers of the

***Corresponding author:**

Valon A. Zejnullahu PhD
Department of General Surgery,
University Clinical Centre of Kosova, 10000 Prishtina
E-mail: valonzejnullahu14@gmail.com

arterial wall. With time these changes lead to the narrowing of coronary, cerebral and peripheral arteries. Due to the atherosclerosis the narrowing of the peripheral vessel will cause disease known as lower extremity peripheral artery disease.

Results from recent research, report that prevalence of peripheral arterial disease in the lower extremity occurs approximately 10 percent of the population before the age of 55.¹ The data from the United States research suggest that this prevalence of peripheral arterial disease has higher rate among women than in men.² There are different risk factors that can contribute in the peripheral arterial disease such as smoking, older age, hypertension diabetes mellitus and dyslipidemia.^{3,4}

Occlusion due to atherosclerosis may be asymptomatic or with clinical manifestation with symptoms of peripheral disease. The clinical manifestation includes unilaterally or bilaterally claudication in one part of the extremities or in combination. The manifestation of this disease can be with acute or chronic symptoms. Vascular system includes each organ system in our body. The manifestations of vascular disease are different. The insufficient of blood supply to specific organs typically is followed with pain; such as calf pain with lower extremity claudication, postprandial abdominal pain from mesenteric ischemia, and arm pain with axillosubclavian arterial occlusion.⁵ Symptoms of arterial disease typically are separated into acute and chronic types. The beginning of pain usually indicates whole occlusion of vessel clinically manifested with severe pain and ischemia in the specific part of the body. Low blood supply in the crucial artery finally causes limb gangrene or intestinal infarction. The diagnosis of peripheral arterial disease is proven by physical examination, measurement of ankle - brachial index, duplex ultrasonography, computed tomography, angiography and magnetic resonance angiography.⁶

The classification of peripheral artery disease (PAD) in the lower extremities are based on sorting out symptoms and anatomic lesions as follows:

Claudication is confirmed by initial and absolute walking distance (Rutherford scale graded from 1 to 3).

Classification based on the recommendation from Trans-Atlantic InterSociety Consensus (TASC-II) for the atherosclerosis disease in the lower extremity.⁷

The Global Vascular Guidelines (GVG) recommends classification system based on the clinical manifestation (wound, Ischemia and foot infection- WIfI).⁸

Referring to the GLASS classification system peripheral arterial disease are distinguished based on the anatomical distribution such is femoropopliteal segment, infrapopliteal segment, infrainguinal stage (I to III).⁸ Diagnosis of PAD in the initial phase of disease prevents further complication in different group of

population. The examination using the color Doppler imagery are noninvasive procedure and inexpensive can help in early diagnosis and early treatment in comparison to Computed tomography angiography and magnetic resonance imagery that are expensive and difficult to reach in the developing countries. There is no data for PAD in our country regarding the incidence rate, risk factors and age of patient which are mostly affected. **Aim of the Study**

The purpose of this study is to:

1. Determine the role of USG Doppler and CT angiography examination in patient with PAD
2. Estimating the incidence rate of PAD in different group of populations based on the age, profession and as well to identify the potential risk factors.

Materials and methods

Study Design

This study was conducted at the University Clinical Center of Prishtina in the Department of Radiology in patients admitted with clinical signs of the peripheral arterial disease (PAD) during the period of time from October 2018 up to October 2019.

Study samples

This study included 150 patients in each of them USG Doppler and Angio CT was performed in order to determine the level of occlusion, scale of occlusion comparing with patients ages, residency and co morbidities. Using duplex Doppler and CT angiography we performed examination in every patient to determine level and scale of occlusion in peripheral arterial system. This study has been approved by ethical committee at the University Clinical Center of Prishtina and informed consent was provided by all subjects participating in this study.

Inclusion criteria

All patients referred upon clinically suspicious for peripheral occlusion in the department of emergency.

Patients with chronic disease referred by other units (neurology, internal medicine, surgery)

1. Pregnant women were excluded from this study
2. Patient with high level of BUN
3. Allergic patient to iodinated contrast.
4. Patient who refused to be part of this protocol.

In all these cases with exclusion criteria we have performed only USG Doppler scan.

Duplex Doppler evaluation was performed by Philips ultrasound system. Patients were positioned in supine position while we performed examination of the iliac artery, tibial artery together with dorsalis pedis, while for popliteal artery the examination was done in prone position. The evaluation of patient suspicious for peripheral arterial disease was performed by using the linear probe (from 6 up to 12 MHz) together with color flow.

In all patients, except for patient with exclusion criteria after the Duplex Doppler in our unit we performed the CT Angiography. The evaluation was done using the Siemens 64 slice CT multidetector. All images obtained by examination, have been interpreted by radiologist of University Clinical Center of Pristina.

Stenotic findings were classified according to scale of occlusion/sub occlusion that was identified in Duplex Doppler and CT angiography as follows:

- Normal or mild stenosis (0-19%)

- Moderate arterial stenosis (20-50 %)
- Significant stenosis (> 50-79%) - Sever or “critical” stenosis (80-99%)
- occlusion (100%)

STATISTICAL ANALYSIS

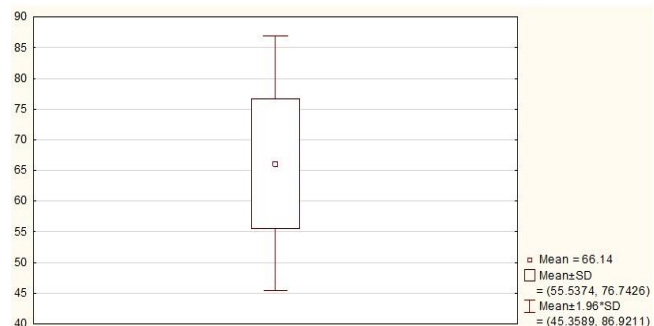
Statistical analyses are performed using statistical package SPSS 17.0 version software. All categorical variables are presented as proportion in percentages with their 95% confidence interval (95%CI). The mean values of all continuous variables will also present with their 95% CI. X2-analysis or Fisher exact test is performed to test the difference in proportions of qualitative variables between groups. The level $P < 0.05$ will be considered as the cut-off value for significance.

Results

A total of 150 cases that met inclusion criteria were included and analyzed in the current study using two diagnostic methods Duplex Doppler and CT Angiography. The mean age of the patients was 66.1 ± 10.6 years, with a range from 47 to 87 years (Table 1 and Graph 1).

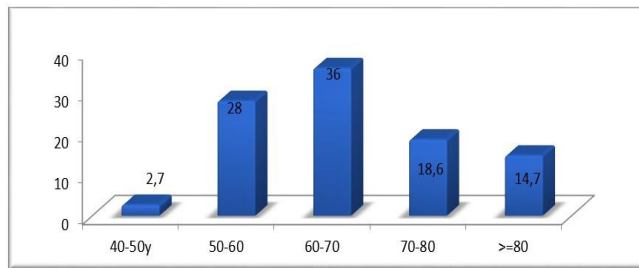
	Valid N	Mean	Minimum	Maximum	Std.Dev.
Age	150	66.14	47.0	87.0	10.62037

Table 1. Mean age of the patients.



Graph 1. Mean age of the patients.

More than half, 54 patients (36.0%) were the age-group 60-70 years (Graph 2). We discovered statistically significant association between the age-group equal to or above 60 years and claudication (11.648, $p = .000642$ using chi-square test). Age equal or above 60 years were associated with 2-fold increased risk of arterial stenosis of the lower extremities (OR=2.2609; 95%; 1.4101-3.6250)

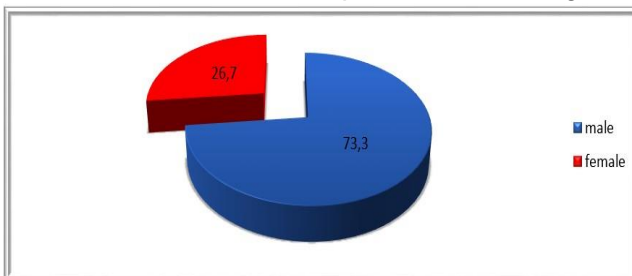


Graph 2. Distribution of the patients according to the age-groups.

Of the total number 150 patients (73.3%) were male and 40 patients (26.7%) were female (Table 2 and Graph 3). Statistically significant association was found between the arterial stenosis of the lower extremities and gender (31.74, $p=.0001$ using chi-square test). Male gender increases risk for arterial stenosis of lower extremities by nearly 3 times (OR=2.7500; 95%; 1.6964-4.4581)

gender	Count	Percent
M	110	73.3
F	40	26.7
total	150	100.0

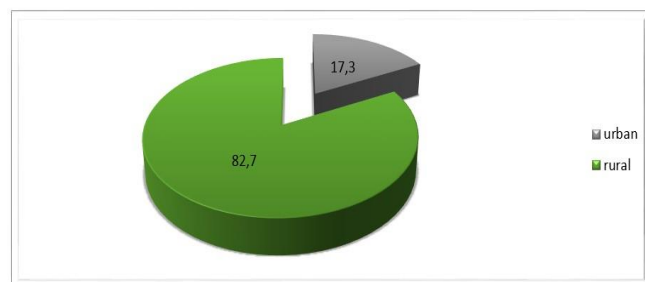
Table 2. Distribution of the patients according to the gender.



according to the gender.

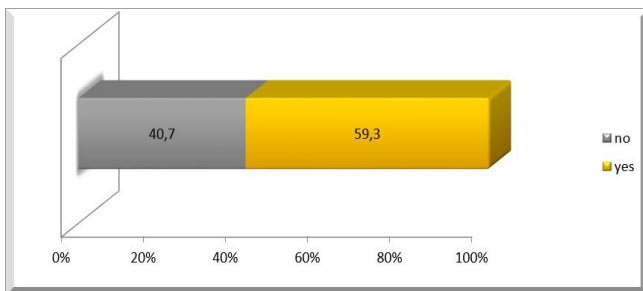
Graph 3. Distribution of the patients

124 patients (82.7%) are rural residence and only 26 patients (17.3%) are urban residence (Graph4). Association between the arterial stenosis of lower extremities and patient residence was considered statistically significant (35.837, $p=.0000$ using chi-square). Patients with rural residence were 4.7 times more likely to develop arterial stenosis of the lower extremities compared to those with urban residence (OR=4.7692; 95%; 2.8065-8.1047).



Graph 4. Distribution of the patients according to the residence.

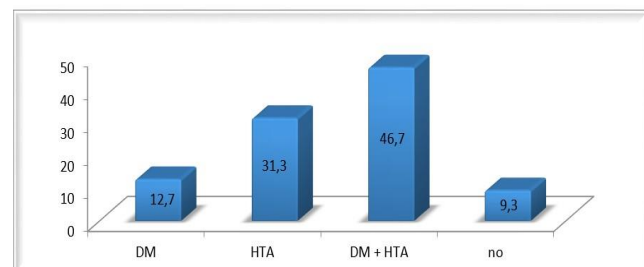
89 patients (59.3%) were active smokers and 61 patients (40.7 %) were nonsmokers (Graph 5). There was not statistically significant association between the smoking habit and arterial stenosis of the lower extremities (Pearson Chi-square 2.6363, $p=.104447$) in the current study.



Graph 5. Distribution of the patients according to the smoking habit.

Out of the total number of participants in this study, 70 patients (46.7%) had two comorbidities namely, diabetes mellitus and arterial hypertension, only hypertension 47 patients (31.3%), diabetes mellitus 19 patients (12.7%) and 14 patients (9.3 %) were without chronic diseases.

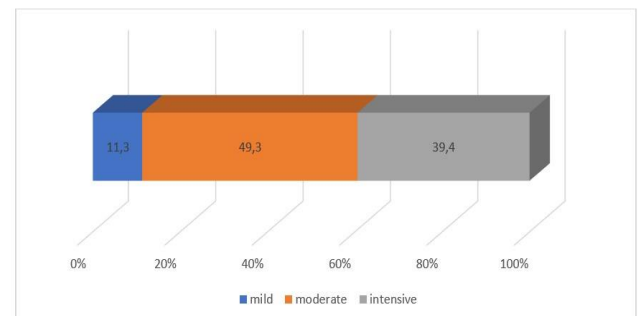
(Graph 6). Association between the arterial stenosis of the lower extremities and chronic hypertension was statistically significant (Pearson Chi- 21.7503, $p=.00001$).



Graph 6. Distribution of the patients with chronic disease/comorbidities.

Hypertension was associated with threefold increased risk for arterial stenosis of the lower extremities (OR=3.1667; 95%;1.93465.1835). No significant association was found between the diabetes mellitus and arterial stenosis of the lower extremities (Pearson Chisquare 2.6363, $p=.104447$).

Severity of the claudication was categorized as mild, moderate and intensive. In our study 59 patients (39.4%) had intensive claudication, 74 patients (49.3%) moderate and 17 patients (11.3%) mild claudication. Differences between intensive vs mild and moderate vs mild claudication were statistically significant ($p<0.05$) (Graph 7).



Graph 7. Distribution of the patients according to the severity of pain (claudication).

We found statistically significant association between the intensive claudication and age > 60 years, hypertension, diabetes mellitus and smoking ($p=.0058$, $p=.0000$, $p=.0005$ and $p=.0433$ respectively) (data not shown).

Ankle-brachial index (ABI) is highly reproducible and noninvasive test and normal range for the ABI is between 0.90 and 1.30. ABI lower than 0.9 indicates PAD with high sensitivity and specificity. ABI values from 0.50 to 0.89 indicate mild to moderate peripheral artery disease and values 0.50 or lower values indicate severe disease.

In the current study, 122 patients (81.3%) had severe arterial disease while 24 patients (16.0%) moderate arterial disease, according to the ankle-brachial index (Graph 8).

Graph 9. CT Angiography and USG Doppler and the degree of stenosis.

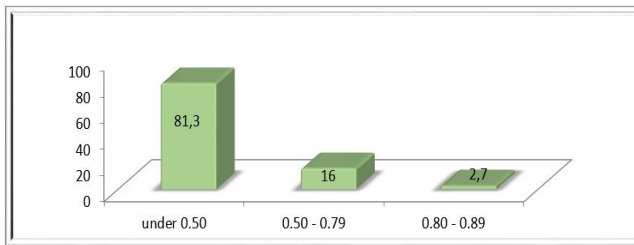
In 150 subjects participating in the study Duplex Doppler and CT Angiography was performed to categorize the degree of the stenosis. Degree of stenosis is classified as low, moderate and high. Low degree stenosis was found in 19 patients(12.7%) with USG Doppler and in 4 patients (2.7%) with CT Angiography, therefore the difference was statistically significant for

Moderate occlusion was found in 35 patients (23.3 %) with USG Doppler and in 13 patients (8.7%) with CT Angiography. The difference was statistically significant for $p<0.05$. CT Angiography revealed high occlusion in 133 patients (88.6%) while these findings were revealed in 96 patients (64.0%) with USG Doppler and the difference was statistically significant for $p<0.05$ (Difference test, $p=.0000$) (Graph 9).

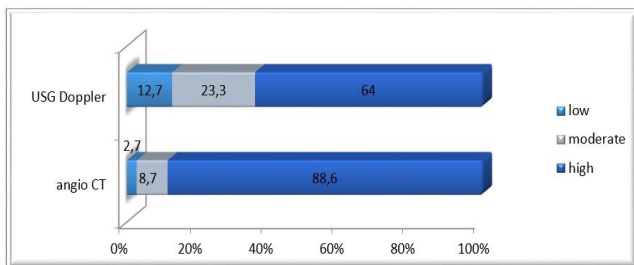
In this study application of two methods, CT Angiography and USG Doppler detected the highest percentage of the arterial stenosis of the lower extremities for a.femoralis and

a.infrapoplitea in 47 patients(31.3%). In 19 patients (12.7%) arterial stenosis was detected in a.infrapoplitea alone and the same percentage was found for the a.femoralis, poplitea and infrapoplitea together (Table 10).

The difference between the a.femoralis vs a.iliaca and a.poplitea as well as the difference between the a.infrapoplitea vs. a.poplitea and a.iliaca was statistically significant for $p < 0.05$



Graph 8. Distribution of the patients according to the ankle -brachial index (ABI).



(Difference test, $p = .0000$) (data not shown).

Angio CT and USG Doppler	Count	Percent
a.iliaca	4	2.7
a.femoralis	17	11.3
a.poplitea	1	0.7
a.infrapoplitea	19	12.7
a.femoralis ,a. infrapoplitea	47	31.3
a.poplitea, a.infrapoplitea	5	3.3
a.femoralis, a.poplitea, a.infrapoplitea	19	12.7
a.femoralis, a.poplitea	12	8.0
a.iliaca, a.femoralis	13	8.7
a.iliaca, a.femoralis, a.infrapoplitea	5	3.3
a.iliaca, a.femoralis,a.poplitea	3	2.0
a.iliaca, a.femoralis, a.poplitea,a.infrapoplitea	5	3.3
Total	150	100.0

Table 10. Distribution of the arterial stenosis of the lower extremities using CT Angiography and USG Doppler.

Discussion

The incidence of peripheral arterial disease is different, but according to the different studies occurrence is around 3 up to 12 percentage^{7,9}. Delay diagnosis and complication are important outcome measures during the clinical evaluation. Peripheral artery disease (PAD) of the upper extremities can be asymptomatic disease with years and also can be symptomatic, causing pains because of ischemic changes and finally leading to gangrenous and ulcerative alterations. Dealing with patients which potentially are suffering from PAD is a clinical challenge and require multidisciplinary approach between clinicians and radiologist.

This cross-sectional study was conducted with intent to find the best diagnostic approach in patient related to PAD. During this study our intention was to determined the role of CT angiography and Doppler ultrasound in early detection of PAD. We accomplished to find the relation between the age of patient, gender, residency, smoking habits, chronic disease together with clinical appearance such is claudication and arterial stenosis in lower extremities. The data from our study were compared with corresponding studies.

In our study we analyzed 150 patients with symptoms of PAD in which after clinical examination, Duplex doppler and CT angiography were performed. From overall patients participated in this study 36.0% were in age group from 60 up to 70 years old.

According to this data the percentage difference was statistically significance with $p < 0.05$. Based on our study the age over 60-year-old and equal have increased risk for arterial stenosis for two-fold in comparison to other age groups. According to the recommendations from the American College of Cardiology (ACC/AHA) the occurrence of PAD are in group of age over 70 years^{10,12}.

Result in our study show that the percentage of patients with peripheral arterial disease are raising in the group of age from 60 up to 80-year-old which is roughly with recommendations from ACC and AHA. In the present study of 150 patients, 73.3% were male and 26.7% were female. There is a statistically significance comparing this two groups with $p < 0.05$. All though the prevalence of PAD between male and female are not well evaluated. The study from Hiramoto JS and al, found that women were considerably more probable to suffer from peripheral arterial disease comparing to the male gender.¹³

A recent study found that peripheral arterial disease has equal prevalence in male gender vs female in high-income countries, but this incidence is higher in women than men in low-income countries.^{14,16}

In this study we have analyzed the difference between the place of residence for all patient participating in this study. Almost all of patient were from rural residence (82.7%) while just 17.3% were from urban residence. This proportion are statistically significant for $p < 0.05$. Recently several studies have investigated ethnic-associated differences in preponderance rates of peripheral arterial disease and found that PAD is more frequently in the African Americans than non-Hispanic.^{15,16} While we surf in the different database for peripheral arterial disease we did not find any paper related to comparison the prevalence of this disease between rural and urban population.

During this study we also analyzed the relation between the smoking habitis and PAD. From the total number of patient 59.3 were heavy smokers while 40.7 were non smokers. Based on different studies smoking is related with cardiovascular disease.¹⁷ The evidence from different studies suggest that vascular disease are more frequent in smokers patient in comparison with non-smokers in the sense of having symptomatic disease earlier in patient with smoking habits.¹⁸

In the present study we found that chronic disease can increase the incidence of PAD. Based on the result of our study the patient with diabetes mellitus and arterial hypertension are

more likely to have peripheral arterial disease than patient without DM and HTA. Related to the different studies from USA and Rotterdam there is a strong relation between the arterial hypertension and risk for symptomatic PAD.^{19,21}

We have analyzed the strength of claudication and we found that out the total number 11.3.% of patient that were in the group with mild claudication, 49.3% moderate and 39.4 intensive claudication's this percent difference were statistically significant($p < 0.05$). Analyzing the Ankle-brachial index in our patients we revealed that 81.3% of patient were with severe arterial disease while 16.0% with moderate arterial disease. Referring to the earlier studies the sensitivity of ankle-brachial index in patient with PAD is around 80% while the specificity is more than 95 %.²⁵

In this cross-sectional study we managed to categorize the degree of stenosis in lower extremities using two methods CT angiography and USG Doppler. Low degree stenosis was found in 12.7% of patient while using the USG Doppler and 2.7% of patient with CT angiography. Moderate stenosis was found in 23.3.% of patient with Doppler and 8.7% with CT angiography, while high occlusion was identified in 88.6% with CT angiography and 64.0% using USG Doppler. These results are statistically significant with $p < 0.05$. The results from our study are more or less in line with other previous studies.^{20,24}

Conclusions

Duplex ultrasound and Angio-CT examination are both effective to accomplish the diagnosis of peripheral arterial disease. The result from our study show that combining of these two methods will raise the accuracy of diagnosis and can contribute in higher diagnostic accuracy and better outcomes .

Ethics Approval and Consent to Participate

The study protocol was approved by the ethical Review Committee, University Clinical Center of Kosovo- Prishtine

Availability of data and material Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Declaration of Interest

The authors report no conflict of interest.

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A rare case of thoracic-abdominal aortic aneurism in conjunction with bilateral superficial femoral artery occlusion

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Lavdim Ymeri MD, PhD cand.¹; Flaka Pasha MD, PhD cand.^{1,2}; Valon Zejnullahu MD, PhD cand.³; Edona Leci- Desku MD⁴; Dardan Dreshaj MD, PhD cand.⁵

1. Clinic of Radiology, University Clinical Center of Kosovo
2. Department of Pharmacology and Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Prishtina “ Hasan Prishtina”
3. Department of Abdominal Surgery, University Clinical Center of Kosovo
4. Department of Radiology, Sheikh Zayed Hospital at Vushtri
5. Head and Neck Surgery, University Clinical Center of Kosovo

Corresponding author:

Ass.Dr. Flaka Pasha, MD PhD cand.

Str: Fehmi Agani no. 141, Prishtine, 10000 Kosovo

flaka.pasha@uni-pr.edu; +383 44 584 005

Abstract

Background

Aneurysms represent bulging of the weakened blood vessel area, mostly as a result of cystic medial degeneration, being the 15th leading cause of patients' deaths worldwide.

The incidence of thoracic aorta aneurisms is increasing, explained by aging population and frequent imaging.

Over time, aneurisms are at high risk of rupture or dissection, leading to bleeding and death.

Therefore, patients with thoracic-abdominal aorta aneurisms require frequent monitoring with magnetic resonance angiography and computed tomography angiography, rigorous management of risk factors, regular use of antihypertensive drugs and statins, open repair surgery or endovascular aneurysm repair.

Case presentation

We, herein, present a very uncommon case of ruptured thoracic aorta aneurism in conjunction with bilateral superficial femoral occlusion, as incidental findings in Covid-19 positive patient.

The patient initially presented to emergency room with cough, shortness of breath, fever, muscle aches and leg claudication.

Suspecting thromboembolic event, Doppler ultrasonography of lower extremities was quickly performed, revealing bilateral superficial femoral arteries occlusion. The patient was immediately administered high doses of parenteral anticoagulants.

In addition, hemoptysis followed, leading to chest and abdominal MSCT, showing right pleural effusion, atelectasis, and right active perihilar infiltrates with inter-lobar pleurisy. Due to inflammatory changes on the lung parenchyma, the patient got tested for Sars-Cov-2, and resulted positive.

Contrast-enhanced MSCT also revealed thoracic-abdominal aortic aneurism with its highest diameter measuring 10 centimeters, and massive per-aortal thrombus/hematoma of 5 centimeters, which was further ruptured and patient died untreated in the fourth day of hospitalization.

Discussion

Questions arise if the Covid-19 was the main cause for bilateral superficial femoral artery occlusion, and were the high doses of parenteral anticoagulants the main precipitating factor for thoracic aneurism rupture.

Current studies report that despite high doses of prophylactic parenteral anticoagulation in Covid-19, 30% of the patients developed thromboembolic events. Meanwhile, anticoagulant drugs present increased risk of bleeding in the event of aneurysm rupture.

Thus, a careful balance must be found between the detrimental and protective contributions of anticoagulants in the patients presenting with Covid-19 and thoracic-abdominal aortic aneurysm.

Keywords: ruptured aneurism; SFA occlusion; COVID-19; CTA; MSCT; Doppler ultrasonography

Background

Aneurysms represent bulging of the weakened blood vessel area, mostly as a result of cystic medial degeneration, where smooth muscle cell dropout and elastic fiber degenerate.

Thoracic and abdominal aorta aneurysms are the 15th leading cause of deaths in patients' older than 55 years-old.

Sixty percent of thoracic aorta aneurysms occur in the root of ascending aorta, 40% in the descending aorta, 10% in the arch and 10% in the thoracic-abdominal aorta.

Thoracic aortic aneurysms can be broadly divided into true aneurysms, containing all three layers of the aortic wall, and false aneurysms (pseudo-aneurysms).

Thoracic aortic aneurysms are mostly caused by degenerative diseases, genetic diseases (Marfan and Turner syndrome, or familiar thoracic aortic aneurysm syndrome), bicuspid aortic valve, atherosclerosis, syphilis, aortic arteritis, aortic dissection and trauma.

The incidence of thoracic aorta aneurysms is increasing, explained by aging population and more frequent imaging.

Over time, aneurysms are at high risk of rupture or dissection, leading to bleeding and death.

Therefore, patients with thoracic aorta aneurysms require frequent monitoring with MRA or CTA, rigorous management of risk factors, use of antihypertensive and statins, open repair surgery or endovascular aneurysm repair.

We, herein, present a very uncommon case of ruptured thoracic aorta aneurism in conjunction with bilateral superficial femoral occlusion, as incidental findings in Covid-19 positive patient.

Case report

A 70-years-old female, chronic smoker, hypertensive, presents at emergency room with cough, shortness of breath, fever, muscle aches and leg claudication.

She was conscientious, timely and spatially oriented, her blood pressure measured 100/80 mmHg, had a body temperature of 37,3 C, while the blood saturation measured 93%. Blood biochemistry parameters were all normal, excluding a CRP of 163.7 mg/l. Suspecting thromboembolic event, a Doppler ultrasonography was immediately performed, showing a considerate flow reduction of bilateral superficial femoral arteries before occlusion, and fastened flow after occlusion.

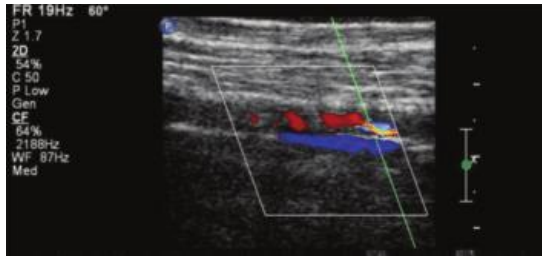


Figure1. Doppler ultrasonography showing superficial femoral artery occlusion

Further, the CTA was performed, confirming the bilateral superficial femoral arteries occlusion.



Figure2. Coronal, sagittal and axial computed tomography angiography showing bilateral superficial femoral arteries occlusion (marked red), followed with arterial mural calcifications

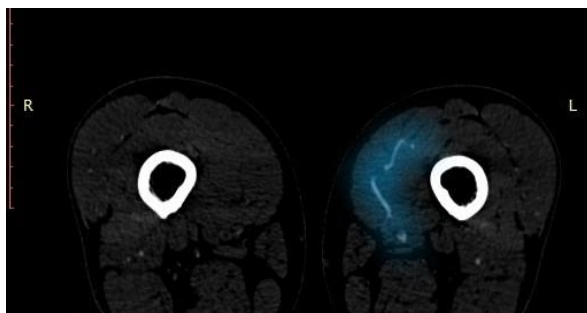


Figure3. Axial CTA showing left collateral circulation (marked with blue).

Accordingly, the patient was immediately administered high doses of parenteral anticoagulants. Yet, patient's clinical condition worsened with hemoptysis, thus clinicians suspected a bleeding peptic ulcer, and gastroscopy was timely performed, with its findings being totally normal. Due to gastroenterologists' suspicion, the bleeding had a respiratory origin; the patient was referred for thoracic and abdominal multi-slice computed tomography-MSCT.

MSCT findings showed right pleural effusion followed with atelectasis, and right active perihilar infiltrates with inter-lobar pleurisy. Due to inflammatory changes on the lung parenchyma, the patient got tested for Sars-Cov-2, and resulted positive.

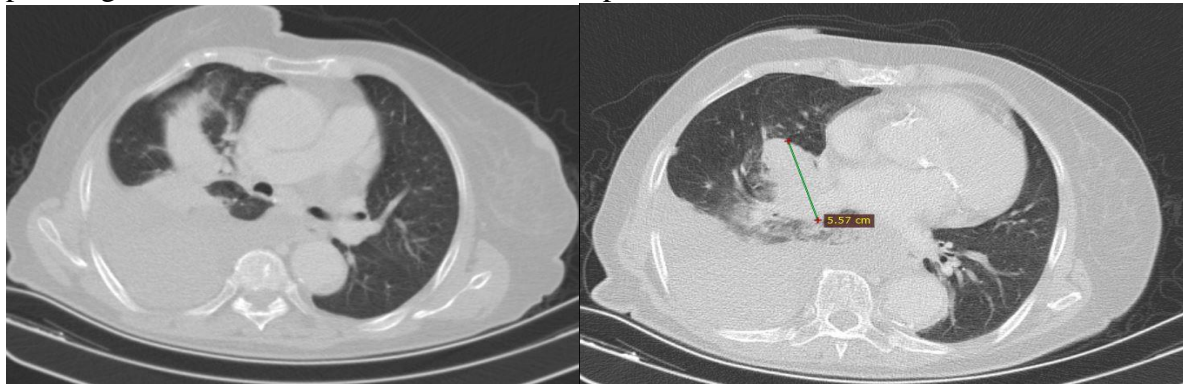


Figure4. MSCT images showing right pleural effusion, atelectasis, right active perihilar infiltrates and inter-lobar pleurisy.

Contrast-enhanced MSCT also revealed thoracic-abdominal aortic aneurism with its highest diameter measuring 10 centimeters, with extravasation and massive per-aortal thrombus/hematoma of 5 centimeters.



Figure5. Axial, coronal and sagittal projections showing thoracic-abdominal aortic aneurism

Patient clinical condition worsened with time, thus the pulmonologist drained 700ml of hemorrhagic exudate from the right lung, under ultrasonography control, which was further sent for histopathology identification.

Event though, patients PT, INR, PTT, TT and D-dimer were normal, high doses of anticoagulants were maintained. Additionally the patient was treated with intravenous saline, antibiotics, analgesics and anxiolytics.

Despite the fact that the patient remained hospitalized for four days and was controlled by six subspecialists, as gastroenterologists, radiologist, pulmonologist, cardiologist, psychologists and

vascular surgeon, she was not treated with open repair surgery or endovascular aneurysm repair, and thus ended up lethally. Autopsy was not performed, due to family members' refusal.

Discussion

Patient's clinical development leads to many open fronts and unanswered questions, such as was the Covid-19 cough the main precipitating factor for thoracic aneurysm rupture, or in contrary, the ruptured thoracic aneurysm lead to respiratory distress.

In addition, there is a further point for discussion if Covid-19 was responsible for the bilateral superficial femoral artery occlusion, and where high doses of parenteral anticoagulants truly needed for patient's leg claudication, thus potentially advancing the thoracic aneurysm to rupture.

So far, studies reveal that Covid-19 triggers thrombosis and disseminated intravascular coagulation, mostly progressing to thromboembolic events at 7,7% of the cases, ischemic strokes at 2.5%, and specifically leading to arterial thrombosis in three documented cases by Griffin et al. Aggravated platelet aggregation, increased blood viscosity, expression of von Willebrand coagulation factors, increased fibrinogen and D-dimer, in addition with ageing, obesity, systematic inflammation, fever and immobility, are considered to be the main precipitating factors of diminished peripheral blood flow, thus leading to arterial thrombosis in Covid-19 patients.

He et al. and Middeldorp et al. reported that despite high doses of parenteral anticoagulation as prophylactic therapy in Covid-19, 30% of the patients developed thromboembolic events. Meanwhile, anticoagulant drugs present increased risk of bleeding in the event of rupture, discouraging their clinical use in patients' with aneurysms.

Given the existent evidence, the decision to initiate anticoagulant therapy in the setting of aortic aneurysm should be patient-specific customized, considering aortic wall injury, stability, and growth rate of intramural thrombus.

Although the thrombus contributes to the size, growth, and proteolytic injury of the arterial wall, it may decrease mechanical stress on the aortic wall and maintain aortic aneurysm stability.

In consequence, even though antithrombotic and anticoagulant therapy could reduce proteolytic injury, it could concurrently reduce mechanical stability of the aneurysm leading to potential rupture and detrimental patients' results.

Thus, a careful balance must be found between the detrimental and protective contributions of the thrombus to aneurysm progression, before starting anticoagulants in the patients presenting with comorbidities as Covid-19, and already diagnosed with thoracic-abdominal aortic aneurysm.

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https://www.researchgate.net/publication/347997511_Colloid_Cyst_of_the_Third_Ventricle_Case_report_and_Literature_Review.

Colloid Cyst of the Third Ventricle: Case report and Literature Review Antigona Kabashi¹, Kreshnike Dedushi^{1,2}, Lavdim Ymeri¹, Ilir Ametxhekaj¹, Mete Shatri² 1 Clinic of Radiology, University Clinical Centre of Kosovo, Prishtina, Kosovo 2 Faculty of Medicine, University of Prishtina "Hasan Prishtina", Kosovo Corresponding author: Kreshnike Dedushi, MD, PHD. Faculty of Medicine, Pristine University, National Institute of Public Health of Kosovo, Pristine, Kosovo. ORCID ID: <http://orcid.org/0000-0003-3639-0891>. E-mail: moc.liamg@ihsudedekinhserk.

ABSTRACT Background: Colloid cysts account for approximately 1% of all intracranial tumors and are the most common type of the neuroepithelial cysts. They are benign, that most commonly occur in the third ventricle near the Monro foramen. Sometimes they have no specific signs and symptoms and may be confused and misdiagnosed with other pathology of nervous systems, but sometimes they may produce acute hydrocephalus, brain herniation, and lead to death. Objective: We present a case of 40 years old male patient with brain colloid cyst. Discussion: The patient was admitted at Clinic of Radiology, for headache and episode of vomit. He was treated for signs and symptoms of depression since 2017. In clinical examination patient had symptoms of brain tumor. The MRI scan demonstrated a cyst in foramen Monroe region with typical signal intensities in the formation that confirm the diagnosis of colloid cyst as dilated of lateral ventricle as signs of early hydrocephalus. After diagnosis the patient was recommended to consult with a Neurosurgeon for intervention. The cyst was removed and similar findings revealed also on histopathological examination. Many of colloid cysts manifest with no specific signs and symptoms that may be confused with depression. Conclusion: On rare occasions, they may completely and irreversibly obstruct the foramen of Monroe, resulting in sudden loss of consciousness and, if patients are not treated, in coma and death. So carefully check the MRI scans is very important, not only in diagnosis but also in planning of treatment. Keywords: Colloid cyst, brain, MRI scans, depression, headache.

1. **INTRODUCTION** Colloid cysts are rare intracranial lesions occurring in approximately three individuals per million per year (1). Some colloid cysts result in acute onset of hydrocephalus and may lead to sudden death. The most common imaging finding is that of a rounded mass in the anterior aspect of the third ventricle. On computer tomography (CT), the lesions are often hyperdense, and their magnetic resonance imaging (MRI) features are variable (2). We present the clinical and imaging findings in a patient with a colloid cyst, and provide a gross anatomic and histopathologic correlation. Correlations between MRI features and cyst content could potentially help surgeons decide on the extent of resection and approach Colloid cysts are among rare benign tumors of the third ventricle. Although the most frequent symptoms are headache and syncope, arrested hydrocephalus or sudden death could appear with colloid cysts. The aim of this pictorial essay was to increase awareness of the clinical presentation

and MRI spectrum, and treatment options of the colloid cysts. Cysts that are especially rich in protein and cholesterol tend to be hyperdense on CT, hypointense on T2-weighted sequences and hyperintense on T1-weighted sequences. These cysts are viscous, and the success of aspiration is significantly low. In the diagnosis and evaluation of small-sized cysts that have an ingredient similar to cerebrospinal fluid, 3-dimensional sequences might be useful. The radiologic appearances of colloid cysts could play an important role in directing these patients to alternative surgical modalities, including resection. In CT typically seen as a well-defined, rounded lesion at the roof of the 3rd ventricle: unilocular, typically hyperdense, isodense and hypodense cysts are uncommon, calcification is uncommon (2). MR signal characteristics include: FLAIR: cysts which are of low signal on T2 will appear similar to attenuated cerebrospinal fluid (CSF) on FLAIR, and are thus difficult to appreciate (4). T1: variable ~50% high signal, the rest are hypointense or isointense to adjacent brain. T1 C+ (Gd): only rarely demonstrates thin rim enhancement, but usually this represents an enhancement of the adjacent and stretched septal veins (3). T2: most are of low T2/T2* signal (short T2), related to thick "motor oil" consistency fluid, some have central low T2 and high peripheral T2 signal (4, 5), some are homogeneously high signal (4, 5).

2. 2. CASE PRESENTATION We present a case of 40-year-old male patient with a colloid cyst in the third ventricle. The patient was admitted at Clinic of Radiology, with symptoms of headache and episodes of vomit. In clinical examination patient had symptoms of brain tumor. He was treated for depression since 2017. A year ago he underwent a MRI head scan, where dilation of lateral ventricle was seen as sign of early hydrocephalus. The MRI scan demonstrated a cyst in foramen of Monro region with typical signal intensities in the cystic formation that confirmed the diagnosis of colloid cyst (Figure 1). In MRI images, they present as homogeneously to heterogeneously hypointense or hyperintense masses in T1 and T2 weighted acquisitions. The outstanding characteristic is a sharply outlined round or ovoid mass in the anterior and superior third ventricle, near or at the foramen of Monro region. Hydrocephalus is observed in most patients (4). After diagnosis the patient was recommended to consult with a Neurosurgeon for intervention. The patient underwent surgical intervention and the cyst was removed. Similar findings were revealed also on histopathological examination (Figure 2, Panels a, b, c, d, e and f). After the intervention the patient did post-operative MRI exam control as recommended where it was evidenced successful tumoral extraction while the histopathologic results described findings compatible with colloid cyst (Figure 2). A colloid cyst can be removed with a craniotomy. A craniotomy is a surgery where an incision is made in the scalp, and part of the skull is removed for the duration of the surgery then the skull is put back in place. On the T1-weighted images, the lesion showed a central signal intensity slightly higher than that of the surrounding brain with a mildly hypointense rim (Figure 2, Panel a). On proton density-weighted images, the colloid cyst was of homogeneous signal intensity, and on T2-weighted images, it showed a central region of hypointensity surrounded by a rim of relative hyperintensity (similar to that of white matter) (Figure 2, Panels b and c). The T2-weighted appearance was a reversal of that seen on the T1-weighted study. The colloid cyst appeared slightly oval on this projection.
3. 3. PATHOLOGY Colloid cysts originate from the abnormal folding of the primitive neuroepithelium (the paraxial elements) (2, 3). They contain mucin, old blood (hemosiderin), cholesterol, and various ions, accounting for a wide range of imaging appearance. These cysts are lined by a

single layer of columnar epithelium which produces mucin, which appears as a thick yellow-green fluid when the cyst is open. We present the imaging-pathologic correlation of a patient with a colloid cyst as well as data supporting the fact that the presence of cholesterol is probably responsible for the MRI features exhibited by some colloid cysts.

. DISCUSSION Colloid cysts of the third ventricle are rare lesions comprising 0.5–1% of primary brain tumors. Most reported cases occur in the third to fifth decades of life (3). Headache occurs in 68–100% of patients and is often the presenting symptom. Headaches are characterized as brief, lasting seconds to minutes, and are initiated, exacerbated, or relieved by a change in position (6). Although colloid cysts are histologically benign, they may obstruct the foramina of Monro and produce acute hydrocephalus. These lesions are a recognized cause of sudden death (7). The cyst's attachment to the third ventricular roof may impart a pendulous character to the lesion, whereby foramina obstruction may be intermittent. Some patients, upon awakening, complain of headache that is relieved by standing. Other symptoms include progressive dementia, drop attacks, and spells of transient loss of consciousness. In children, the most common symptoms are headache, nausea, vomiting, papilledema, and diplopia. Although the great majority of colloid cysts arise in the anterior third ventricle, rare examples in the lateral ventricles, fourth ventricle and outside the ventricular system have been reported (8-11). Histologically, colloid cysts are characterized by a simple to pseudostratified epithelial lining with interspersed mucous goblet cells and scattered ciliated cells (Figure 4). Using MR imaging, colloid cysts have a variable appearance. MRI may occasionally show intracystic fluid levels or central and peripheral components in the lesion. Some colloid cysts are homogeneous in appearance. About 50% of colloid cysts are hyperintense on T1-weighted images, and the rest are either isointense or hypointense with respect to brain (Figure 2, Panel a). On T2-weighted images, most colloid cysts are hypointense to the brain (Figure 2, Panel b). Cysts that are hypointense on T2-weighted sequences may be difficult to visualize using fluid-attenuated inversion recovery images (FLAIR) (Figure2, Panels a and b). Isointense cysts may be difficult to identify on MRI and may be more easily seen on CT scans (8). The T2 features of some colloid cysts are the reversal of their pattern as seen on T1- weighted images (as shown in our patient). Because the central portion of most colloid cysts tends to be of low T2 signal intensity, it has been suggested that paramagnetic effects may be responsible for their MRI characteristics.

4. CONCLUSION Colloid cysts are rare lesions arising in the superior third ventricle and may present in acute hydrocephalus, which may be fatal. The origin of colloid cysts continues to be a matter of debate. Their MRI signal characteristics are variable and mostly dependent on the cholesterol and protein contents and not on the presence of paramagnetic minerals. Despite their variable signal characteristics, their location and shape allow for the correct preoperative diagnosis in most patients. The patient underwent magnetic resonance imaging which clearly showed a colloid cyst of the third ventricle with accompanying obstructive hydrocephalus. Such lesions are potentially life-threatening if undiagnosed. It is suggested that magnetic resonance

imaging has an important role to play in rapidly and clearly demonstrating the location, size and complications of such lesions.

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FREQUENCY OF COMPLETE AND REMOVABLE PARTIAL DENTURE TREATMENT IN THE PRIMARY HEALTH CENTRES IN THREE DIFFERENT REGIONS OF KOSOVO FROM 2002 TO 2013

POGOSTOST OSKRBE S TOTALNO IN DELNO PROTEZO V PRIMARNIH ZDRAVSTVENIH CENTRIH V TREH RAZLIČNIH REGIJAH NA KOSOVU V OBDOBJU 2002-2013

Manushaqe SELMANI BUKLETA^{1,2}, Dashnor BUKLETA³, Mimoza SELMANI⁴, Milan KUCHAR^{5*}

¹University of Ljubljana, Medical Faculty, Doctoral School, Vrazov trg 2, 1000 Ljubljana, Slovenia

²Dental Clinic, Mdent Family Dentistry, Eqrem Qabej 74, 10000 Pristina, Kosovo

³Dental Polyclinic, Department of Oral Surgery, Nene Tereza NN, 30000 Peje, Kosovo

⁴AAB College, Zona Industriale, Fushe Kosove, 10000 Pristina, Kosovo

⁵University of Ljubljana, Medical Faculty, Department for Prosthodontics, Hrvatski trg 6, 1000 Ljubljana, Slovenia

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ABSTRACT

Keywords:

edentulism, prosthodontic treatment, complete dentures, removable partial dentures

Introduction: Edentulism and prosthodontic care are very common, especially in the elderly. The study investigated the treatment with complete dentures (CDs) and acrylic removable partial dentures (ARPDs) among people receiving new prosthodontic treatment in the Primary Health Centres of the three regions in Kosovo from 2002 to 2013.

Methods: The data on ARPDs delivery and CDs delivery was obtained from the archives of primary health centres from three Kosovo regions (Prizren, Peje, Ferizaj) from 2002 to 2013. The data was analysed concerning year of treatment, type of dentures, jaw, age, gender and urban or rural origin of the patients. The trend of treatment was determined, and the binomial logistic regression model was used for predicting odds of ARPD versus CD treatment by year of treatment and patient characteristics.

Results: From 2002 to 2013, 9,478 patients received 11,655 CDs and 4,401 ARPDs. Delivery of CDs significantly increased by 57.45 dentures per year ($R^2=0.609$) and delivery of ARPDs by 30.39 dentures per year ($R^2=0.569$). Each year the odds for ARPD versus CD increased by 4.2% (95% CI: 3.0%-5.4%). Younger patients have higher odds for ARPD rather than CD and odds for ARPD are decreasing as the age of patients rises. The gender, residence, and jaw all had a significant impact on prosthodontic treatment too.

Conclusions: In Primary Health Centres of Kosovo, there is a trend for higher frequencies of both dentures (more obvious for ARPD), and the frequency is highly dependent on the age of patients.

IZVLEČEK

Ključne besede:

brezzobost, protetična oskrba, totalne proteze, delne proteze

Uvod: Brezzobost in protetična oskrba sta zelo pogosti, še zlasti pri starejših osebah. V študiji so preučevali zdravljenje s totalnimi in akrilatnimi delnimi protezami pri osebah, ki so v obdobju od 2002 do 2013 prejele novo protetično oskrbo v primarnih zdravstvenih centrih v treh regijah na Kosovu.

Metode: Iz arhivov primarnih zdravstvenih centrov na Kosovu (Prizren, Peje in Ferizaj) so bili iz obdobja od 2002 do 2013 zbrani podatki o vstavljenih novih totalnih in akrilatnih delnih protezah. Analizirani so bili glede na leto vstavitve, vrste protez, prisotnosti protez v zgornji in/ali spodnji čeljusti, starost, spol in prebivališče (mesto, podeželje) oskrbovancev. Od leta 2002 do 2013 so določili trend pogostosti oskrbe s protezama. Za napovedovanje verjetnosti oskrbe z delno protezo v primerjavi s totalno protezo glede na leto, ko je bila proteza vstavljena, in značilnosti bolnikov so uporabili binomsko logistično regresijo.

Rezultati: V študiji je bilo zajetih 9.478 bolnikov, ki so jim vstavili 11.655 totalnih protez in 4.401 akrilatnih delnih protez. Število vstavitvev totalnih protez se je v letih 2002-2013 pomembno povečevalo za 57,45 na leto ($R^2 = 0,609$), vstavitvev akrilatnih delnih protez pa za 30,39 na leto ($R^2 = 0,569$). Vsako leto se je obet za vstavitvev akrilatne delne proteze glede na totalno protezo povečal za 4,2 % (95 %, CI: 3, 05, 4 %). Mlajši pacienti imajo večje obete za oskrbo z akrilatno delno protezo kot za oskrbo s totalno protezo. Verjetnost zdravljenja z akrilatno delno protezo se s staranjem zmanjšuje. Statistično značilen vpliv na protetično zdravljenje so imeli tudi spol in prebivališče pacientov ter lokacija proteze v ustih.

Sklepi: V primarnih zdravstvenih centrih na Kosovu se povečuje pogostost oskrbe z obema zobnima protezama (intenzivneje pri akrilatni delni protezi) in pogostost je močno odvisna od starosti pacientov.

*Corresponding author: Tel. + 386 41 778 621; E-mail: milan.kuhar@mf.uni-lj.si

1 INTRODUCTION

Thanks to preventive dentistry, which was introduced in the mid-20th century, most people are now aware that natural teeth can be maintained throughout an entire lifetime (1-3). In developed countries, the prevalence and extent of tooth loss has significantly decreased in recent decades (1, 2, 4, 5). The prevalence of edentulism decreased by approximately 4-10% over ten years (1, 5, 6). However, the growing population of elderly, which will continue to grow dramatically in the next few decades, is the main reason for the persistence of high levels of edentulism (1, 7). Consequently, prosthodontic care is prevalent, especially in the elderly, in most European countries (8, 9). Correlations between the prevalence of edentulism and prosthodontic care as well as national prosperity, irregular-regular visits to the dentist, urban-rural residence, age, gender, and education were also demonstrated (1, 10).

The primary purpose of prosthodontic care is to replace missing teeth and to restore function and aesthetics, as well as to preserve the health of the remaining oral tissues of partially or completely edentulous patients. The prosthodontic treatment combines several clinical and laboratory procedures that lead to the insertion of the fixed (crowns and bridges) or removable (partial and complete dentures) prosthodontic device (11). In principle, because treatment with various types of removable dentures is generally less successful than with fixed prosthodontic devices, removable prosthodontic treatment is indicated when clinical or financial factors exclude fixed prosthodontic treatment on teeth or dental implants (12-14). In the last three decades, the removable dentures supported by dental implants have proven to be a very successful form of prosthodontic treatment for edentulous people and treatment with them is continuously increasing. However, due to increased complexity (needs surgery) and higher costs are significantly less common than conventional denture treatment without dental implants (15, 16).

Currently, conventional mucosa-born complete dentures (CDs) are still the most common removable form of prosthodontic treatment for complete edentulous jaws, while conventional mucosa and teeth-born removable partial dentures (RPDs) of all types continue to be a standard treatment modality in the maxillary and mandibular partial edentulous arches (17). The RPDs with a metal framework (MRPDs) and acrylic based RPDs without a metal framework (ARPDs) are the most commonly used removable partial dentures (8). More expensive and technically more demanding MRPDs have some crucial advantages over ARPDS. The main advantages are better dental support, oral tissue release from the extended resin plate coverage, and facilitated maintenance of hygiene, which impacts the higher success rate of MRPDS.

Mobility of abutment teeth and fracture of dentures are observed more often in ARPDS than in MRPDS (18, 19). A study among general dentists in Ireland showed that the average survival rate of an ARPD is 5.7 years, whereas an MRPD has an average survival rate of 10.6 years (20).

Data on prosthodontic care in different countries, environments, and patient groups can be relevant, in particular to all those who are involved in planning and organizing healthcare, as well as in health education (15, 21). Epidemiological studies published from 2004 to 2013 show that, of all RPDs constructed, the acrylic without metal framework ones were used in 3.2-75% of cases in different countries (17, 20, 22-24). A review study of 43 articles, which related to 13 European countries, concluded that there is a trend toward a higher prevalence of fixed prosthodontic treatments, more various types of RPDs and a reduction in CDs in most European countries. The majority of these articles presented the prevalence of various prosthodontic devices at a given time, and only five articles presented the incidence of newly used prosthodontic treatments over a specific period (8). A study performed in Croatia shows that Croatian Health Insurance has been covering the cost of CDs, ARPDS, and MRPDS for the past twenty years. At the same time, an upward trend particular to MRPDS has been detected in the majority of Croatian regions (25). Since 2013, the costs of MRPD treatment have been covered by health insurance in Slovenia as well (26). Subjective clinical estimations show an upward trend in the use of MRPDS since that date but, unfortunately, this has not yet been scientifically confirmed. Ever since the war in 1999, primary health centres in Kosovo only provide low-cost prosthodontic treatment, which means that edentulous persons are treated with either ARPDS or CDs. A considerable number of patients seek medical and dental services in these centres, and most of them are socially deprived, war veterans, relatives of war victims and older adults. Treatment with MRPD as a more advanced form of the removable partial dentures is only available in the state-funded University Clinical Centre in Pristina, Kosovo. In other institutions, the patients need to pay MRPD, like fixed restorations, by themselves.

No epidemiological studies have been made on the use of any prosthodontic treatment in the Republic of Kosovo. The purpose of the study was to investigate the treatment with CDs and ARPDS among people receiving prosthodontic treatment in the Primary Health Centres of the three regions in Kosovo. Therefore, the aims of this study were: 1) to find out what was the trend in the frequency of new treatments with CDs and ARPDS from 2002 to 2013; 2) to analyse the relationship between two prosthodontic treatments (ARPDs versus CDs) and according to different factors (age, sex, jaw, living environment); 3) to determine the proportion of the population receiving new CD and ARPD treatment at the annual level by age.

2 METHODS

This study included all patients who received new prosthodontic treatment with CDs and ARPDs at Primary Health Centres of three Kosovo regions: Prizren, Peje (Peč) and Ferizaj (Uroševac) from 2002 to 2013 (Table 1). 35.2% of the total data comes from Prizren, 37.2% from Peja, and 27.6% from Ferizaj. The data collected from the archives of primary health centres included: the year of delivery of the denture, the type of the denture delivered (CD or ARPD), the location of the denture in the patient's mouth (maxilla, mandible), the patient gender, the urban or rural origin of the patient, and the age of the patient upon denture delivery.

This study includes 9,478 patients who received treatment with CDs and ARPDs. 72.1% of patients live in urban areas and only 27.9% in rural areas. The participants were divided into 6 age groups as follows: younger than 35

years of age (1.9%), 35 to 44 years of age (8.9%), 45 to 54 years of age (21.8%), 55 to 64 years of age (26.0%), 65 to 74 years of age (30.8%) and more than 75 years of age (10.5%).

Table 1. The number of patients according to the number and type of dentures they received in prosthodontic treatment in the Primary Health Centres of the three regions of Kosovo from 2002 to 2013.

Patients	Dentures		
	CD	ARPD	Total
4,483	8,966		8,966
1,647	1,647		1,647
1,042	1,042	1,042	2,084
1,053		2,086	2,106
1,253		1,253	1,253
Σ9,478	Σ11,655	Σ4,401	Σ16,056

The obtained data was statistically analysed using SPSS 22.0 statistical package. The separate linear trends for CD and ARPD treatment were calculated. To draw inference from our data and to lose the outliers, we have normalized the data into a range of between zero and one. The absolute number, as well as the normalized values of the delivered CDs and ARPDs for each year of delivery, are presented in the graphs.

A prediction of ARPD treatment versus CD treatment in people receiving prosthodontic care from 2002 to 2013 was statistically analysed using binomial logistic regression. Sex, age, residence and denture location in the jaw were proposed as categorical covariates and the year of

odds ratios (AORs) and 95% confidence interval (95% CI) are presented. For statistical significance, p-values <0.05 were considered.

To calculate the proportion of the population that is on an annual basis receiving new dentures in Public Health Centres, a four-year (2010-2013) average of patients who received CDs or ARPDs and population data from the last census were used. At the same time, patients and the population in all three investigated regions were arranged into six age groups (<35, 35-44, 45-54, 55-64, 65-74, 75+), in the age group 35+ and to all ages (total). The population for each age group was based on the data from the 2011 population census in the Republic of Kosovo (27). Of the 1,739,825 residents of Kosovo registered in the year 2011, the number living in Prizren, Peje, and Ferizaj was 382,841 (22% of the total number of residents in Kosovo) (Table 2).

Table 2. Population size in the three regions of Kosovo registered in 2011.

Age group	Prizren	Peje/Peč	Ferizaj/Uroševac	Total
<35	111,468	57,811	69,212	238,491
35-44	24,767	13,150	14,750	52,667
45-54	18,452	10,658	10,822	39,932
55-64	11,690	7,337	7,269	26,296
65-74	7,443	4,917	4,570	16,930
75+	3,961	2,577	1,987	8,525
Total	177,781	96,450	108,610	382,841

3 RESULTS

In the Primary Health Centres of all three regions of Kosovo, 9,478 patients received 16,056 removable dentures, of which 6,130 (64.7%) patients received only CDs, 2,306 (24.3%) patients received only ARPDs, and 1,042 (11.0%) patients received both CD and ARPD from denture delivery as a continuous covariate. The adjusted

The absolute number of the new treatments with CDs and ARPDs for each year of denture delivery, as well as the trends of the normalized frequencies of the treatment with CDs and ARPDs from the year 2002 to 2013, are presented (Figures 1 and 2). There is a significant upward trend line in the treatment with CDs: $p=0.003$, $R^2=0.609$. On average, the absolute number of CD deliveries increase by 57.45 CDs per year. Results regarding ARPDs show that there is a significant rising trend in their delivery as well: $p=0.005$, $R^2=0.569$. On average, the absolute number of ARPD deliveries increase by 30.39 per year. Although the average increase of the absolute number of CD deliveries is higher than the average increase of the

absolute number of the ARPD deliveries, the normalized data shows that the average number of ARPD deliveries increases more rapidly than the average number of CD deliveries. To further verify these findings, the odds for receiving ARPDs versus CDs - dependent on the years of delivery - were calculated.

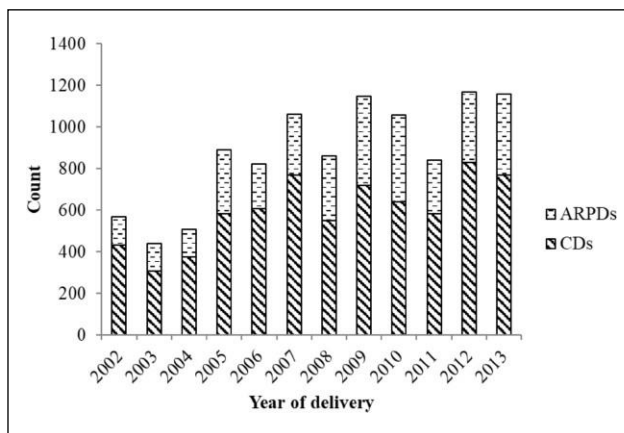


Figure 1. The number of new treatments with CDs and ARPDs for each year of denture delivery in the Primary Health Centres of Kosovo.



Figure 2. The linear trend of CD and ARPD delivery in three regions of Kosovo from 2002 to 2013.

To determine the effects of age, gender, residence and denture location on the likelihood of the type of the denture treatment among the patients, a binomial logistic regression model was conducted (Table 3). The model explained 14.7% (Nagelkerke R²) of the variance in the type of denture predicted and correctly classified 73.9% cases.

The results indicate that the year of delivery was statistically significant: $p < 0.05$. Each year the odds for treatment with ARPD versus CD increased by 4.2% (95% CI: 3.0%-5.4%). Males have 29.1% higher odds for treatment with ARPD rather than CD. In comparison with the reference group (older than 75) patients younger than 34 years have the highest odds for treatment with ARPD (AOR=13.935; 95% CI: 10.296-18.861) rather than CD and odds for ARPD are decreasing as the age of patients rises. Moreover, in comparison with the same reference group (older than 75) all compared age groups have higher odds for treatment regarding ARPD versus CD. Significant results were also found regarding the residence of the patients. Compared with patients from urban residence, patients from rural residence have 10.2% higher odds for treatment with ARPDs over CDs. Results indicate that it is more likely for patients to receive ARPD than CD treatment on the mandibula than maxilla (AOR=2.996; 95% CI: 2.778-3.231).

Table 3. Binominal logistic regression model predicting odds of ARPD versus CD treatment by year of receiving the treatment (2002-2013) and patient characteristics.

Observed category	Reference category	AOR (95% CI) ARPD to CD	p
Year of delivery			
2013	2002	1.042 (1.030-1.054)	0.001*
Sex			
[Male]	[Female]	1.291 (1.197-1.392)	0.001*
Age			
[<34 years]	[75+ years]	13.935 (10.296-18.861)	0.001*
[35-44 years]	[75+ years]	6.201 (5.181-7.422)	0.001*
[45-54 years]	[75+ years]	3.541 (3.031-4.137)	0.001*
[55-64 years]	[75+ years]	2.519 (2.163-2.934)	0.001*
[65-74 years]	[75+ years]	1.512 (1.299-1.759)	0.001*
Residence			
[Rural]	[Urban]	1.102 (1.008-1.205)	0.033*
Jaws			
[Mandibula]	[Maksila]	2.996 (2.778-3.231)	0.001*

*marks statistically significant differences ($p < 0.05$)

From 2010 to 2013, at an annual level, 0.18% of the total population in three Kosovo regions received a new CD and 0.09% a new ARPD in the Public Health Centres. In the 35+ year-old population, the proportion of the population treated with new CDs was 0.48% and 0.23% for new ARPDs. The proportion gradually increased with the increasing age of recipients of dentures up to the 75+ age group, and a decrease was detected in the 75+ age group (Figure 3).

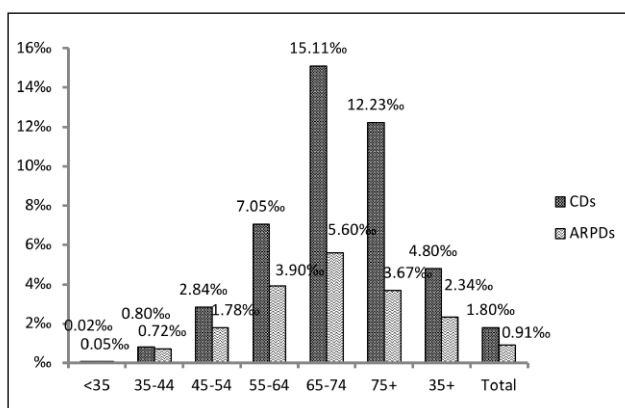


Figure 3. The distribution of the proportion of population treated with CDs and ARPDs from 2010 to 2013, at an annual level, by the age groups >35, 35-44, 45-54, 55-64, 75+, 35+ and in total.

4 DISCUSSION

In the primary health centres in the three Kosovo regions combined, where almost a quarter of Kosovo's inhabitants live, treatment with CDs was more than twice as frequent as treatment with ARPDs over the entire period of 2002-2013. Among those who were seeking prosthodontic care in these centres, most of them are completely edentulous, and the most commonly used prosthodontic devices are CDs. In the same centres, the absolute number of removable prosthodontic treatments with both dentures rose continuously from 2002 to 2013. The increase in treatment with CDs was significantly higher (more than 27 treatments per year on average) than in the case of treatment with ARPDs. In most European countries, however, there has been a clear trend toward reducing removable prosthodontic devices, especially CDs (7-9). In contrast to our study, the frequency of treatment with CDs in Croatia was significantly lower than the frequency of treatment with RPDs from 1996 to 2002 (23). Similarly, the trend toward increasing the frequency of fixed prosthodontics devices and RPDs and the reduction of CDs has been identified in thirteen other European countries (8, 9). Unfortunately, these studies differ significantly concerning age, socioeconomic status, and origin of subjects as well as providers of dental care. Therefore, we can only compare trends, while a direct comparison of data is difficult. The data of our study is obtained from Primary Health Centres, where only primary dentists perform prosthodontic care. While the study in Croatia also includes institutions where specialists perform prosthodontic care. It should also be noted that in these centres dental care is provided to the most vulnerable groups of people in Kosovo. Given that edentulism is the main factor dictating the need for treatment with the CD

(7, 8), we can conclude that there is a high probability that the number of edentulous patients in the Primary Health Centres in Kosovo has also increased from 2002 to 2013.

The normalized frequencies of the treatment with CDs and ARPDs and, in particular, logistic regression predictions of ARPD treatment probability compared to CD by the year of treatment (2002-2013) reveals more interesting data on trends that were previously covered with high absolute values of CD treatment. The probability of ARPD deliveries versus CD significantly increases over the years, and CD deliveries decrease. A more detailed analysis revealed that, in the background of unfavourable absolute values of prosthodontic treatment trends, there is a slight drop in CD delivery in comparison with ARPD delivery, which is also reflected by a slight drop in edentulism. It is also more likely that ARPDs are more commonly used for the prosthodontic treatment of younger patients and, vice versa, that CDs are more often used than ARPDs for the prosthodontic treatment of the elderly. The prevalence of edentulism is most affected by the aging of the population (1, 7, 10). According to the Kosovo Agency of Statistics, the number of people aged 35 years and older increased by 25.0% between 1991 and 2011 (27). Our results also show that the aging of the population is one of the important factors that affects prosthodontic treatment, as the probability of CD treatment increased with the age of the patient. The proportion of ARPDs compared to CDs decreased with the age of patients and at the same time increased over the years. As the number of lost teeth increases with age, it is quite reasonable that, with the increasing age of patients, the CD and ARPD treatment ratio gradually tilts to the side of the CD, which is evident in our and many other studies (8, 28). However, aging is not the only factor affecting the frequency of edentulism and its treatment, since treatment with CDs increased by more than 100% in this same period.

In addition to the year of treatment and age of patients, many additional factors are described that have an impact on the frequency of prosthodontic care and edentulism (8, 9). The additional factors that were available to us in Primary Health Centres - gender, urban versus rural residence and mandibula versus maxilla as denture sites - have had a significant impact on prosthodontic treatment. Treatment with ARPD is more likely to occur in male subjects and people in a rural environment, and CD treatment is more likely in women and people from an urban environment. An appropriate explanation for the more likely deliveries of CDs in an urban environment is currently difficult to justify. Further investigation will be necessary to clarify this.

The results of this study lead us to conclude that CD treatments were significantly more likely in the maxilla than in the mandible and ARPD treatments were more likely in the mandibula. Similarly, studies in Western European countries have found a higher frequency of treatment with a removable partial denture in the mandible (29, 30).

The present study shows that the proportion of the population treated with CDs and ARPDs per year steadily increased from the <35 up to the 75+ age group and decreased in the 75+ age group. Similarly, findings from a study in Croatia indicate that the delivery of prosthodontic appliances in the oldest age group dropped significantly. A less frequent delivery of CDs to persons who are 75 years of age or older can be explained by the fact that older adults rarely visit a dentist, wear prostheses for a more extended period than younger persons, and replace old dentures with new ones less frequently (15, 21, 25, 28).

An important fact is that every prosthodontic treatment also has unwanted side effects and, especially if it is inadequate, harms oral health and tooth loss (29-32). In the contemporary planning of prosthodontic treatment, RPD is indicated when indications for fixed prosthodontic treatment on teeth or implants are ruled out due to biological or socioeconomic factors. It is therefore not surprising that RPD treatment generally has a lower survival rate, more complications and is less comfortable for the patient than fixed prosthodontic treatment (12-14, 32). According to our study, the Primary Health Centres in Kosovo have witnessed a continually growing trend of ARPD delivery in the period from 2002 to 2013. Furthermore, both the absolute number of ARPDs delivered and their proportion in comparison to CDs has increased. In Croatia, Slovenia and many developed countries, the proportion of RPDs in comparison to CDs has also been increasing, but mainly due to an increase in the proportion of MRPDs and a decrease in the proportion of ARPDs (8, 22, 25, 28). MRPDs have significantly better survival and success rate and, above all, improve the survival of the supporting teeth compared to ARPDs (14, 18-20). In the Primary Health Centres of Kosovo, only ARPDs are used for the treatment of partial edentulism. Consequently, although many individuals in these centres met the clinical criteria for a fixed prosthesis or even an MRPD, they were treated with ARPDs, even though these were less appropriate. Except at the University Clinical Centre in Priština, the patients themselves cover the cost of MRPD treatment, which is beyond the reach of most patients looking for dental care in the Primary Health Centres in Kosovo. Treatment with ARPDs can lead to rapid loss of remaining teeth and can increase the overall level of complete edentulism, thereby increasing the need for treatment with a CD (31, 32).

This study undoubtedly has certain limitations, mainly because the prevalence of edentulism was deduced from new dentures treatments. We must be aware that the real prevalence of edentulism in Kosovo is significantly higher than the proportion of new denture treatment. Namely, the data from Primary Health Centres included no information on how long individual patients wore dentures for. According to the population census in Kosovo (25), 61.7% of the population was living in rural areas in 2011. On the other hand, almost three-quarters of patients who received dentures in Primary Health Centres came from the urban environment, which leads us to conclude that only a small proportion of the rural population received dentures in these centres. This may either mean that people in rural areas had better oral health and had less edentulism or, more likely, that they visited a dentist less frequently, were edentulous and without dentures or were using dentures for a long time and rarely changed them. When interpreting the results of this study, it should be borne in mind that our investigated population represents the most vulnerable groups of people in Kosovo.

The number of preserved natural teeth and, indirectly, the presence of prosthodontic restorations is a significant indicator as well as a factor of oral and general health (8, 33). Data on the trend in the frequency of prosthodontic treatment can be important for developing national dental health services and dental health policy, as well as for dental school and research programs in Kosovo and the wider region. Sufficient dental centres that offer adequate care should be made available. In the case of prosthodontic care, this means that partial edentulism should be treated based on the professional indication, including with fixed prostheses and MRPDs, not only ARPDs. Considering the model of the neighbouring countries, the possibility of financing dental treatment, at least with MRPDs, could also be considered in Kosovo.

5 CONCLUSIONS

The trend in the frequency of new CD and ARPD treatment increased linearly from 2002 to 2013 in the Primary Health Centres in three regions of Kosovo. The need for CD treatment in absolute numbers is more than twice as high as the need for treatment with ARPD.

However, the proportion of ARPDs compared to CDs significantly increased over the years. Younger people have higher odds for treatment with ARPD and odds for ARPD are decreasing as the age of patients rises. Males have higher odds for treatment with ARPD. Patients from the rural environment have higher odds for treatment with ARPDs. CD treatments are more likely in the maxilla, and ARPD treatments are more likely in the mandibula.

In the period of 2010-2013, the proportion of the population treated with new CDs and ARPDs per year steadily increased from the <34 to 65-74 age group and decreased in the 75+ age group. 0.48% of the population older than 34 years of age received new CDs, and 0.23% received new ARPDs in these centres.

Professional guidelines for treatment should be taken into consideration as much as possible, to improve oral health in people who need prosthodontic care, while efforts should also be made to reduce the impact of disadvantaged socio-economic factors on treatment decision, especially in older individuals and others seeking dental care in Primary Health Centres in Kosovo.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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There is no financial interest or risk.

ETHICAL APPROVAL

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Bone and soft tissue changes associated with a removable partial denture. A novel method with a fusion of CBCT and optical 3D images

Marko Kuralt ^{a, b}, Manushaqe Selmani Bukleta ^{b, c}, Milan Kuhar ^{d, e}, Aleš Fidler ^{f, g, *}

^a Division of Stomatology, University Medical Centre Ljubljana, Slovenia

^b Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

^c School of Dentistry, Faculty of Medicine, University of Prishtina, Kosovo

^d Department of Prosthetic Dentistry, Faculty of Medicine, University of Ljubljana, Slovenia

^e Department of Prosthodontics, University Medical Centre Ljubljana, Slovenia

^f Department of Endodontics and Operative Dentistry, Faculty of Medicine, University of Ljubljana, Slovenia

^g Department of Restorative Dentistry and Endodontics, University Medical Centre Ljubljana, Slovenia

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ABSTRACT

Background: The purpose of this study was to propose a novel method for 3D evaluation of bone and mucosal changes in removable partial denture (RPD) foundation area using a fusion of CBCT and optical 3D images.

Method: Two CBCT scans and three impressions, taken at insertion and after ten months of wearing the RPD, were acquired from five patients. 3D models of bone and surface were created from CBCT images and gypsum casts, respectively, spatially aligned and saved in STL file format. Visual and numerical analysis of differences between the models allows evaluation of surface, mucosal and bone changes in regions of interest (ROI) defined as narrow ROI (nROI), denture foundation area ROI (dROI) and wide ROI (wROI). Site-specific analysis was performed in mesiodistal and buccolingual direction.

Results: Visual evaluation of 3D color-coded deviation maps showed irregular distribution of bone and surface changes. The differences between mandibles and also between left and right sides were found. Mean volume of bone change in dROI was -135.86 (range = -456.18 to 21.20) mm^3 . The average bone change thickness in dROI was -0.26 (range = -0.96 to 0.07) mm. The mean volume changes in nROI were -38.31 (range = -118.26 to 45.87) mm^3 , -51.96 (range = -182.54 to 5.6) mm^3 and 13.66 (range = -80.62 to 79.46) mm^3 for surface, bone and mucosa, respectively.

Conclusions: The proposed method facilitates separate visual and numerical evaluation of surface, mucosa and bone changes. It opens possibilities for a better understanding of denture-supporting tissues remodeling, objective evaluation and comparison of different treatment options.

1. Introduction

The resorption of the residual ridge following tooth loss is an irreversible physiological process [1], which can be significantly increased with denture wearing [2]. During wearing a denture, occlusal forces are transferred to denture supporting tissues (DST) [3], consisting of the mucosa, (the submucosa and periosteum), and the underlying bone [4]. The shape and thickness of both soft and mineralized DST form a denture foundation area, a contact area between a denture and mucosal surface [4]. It is a well-known fact that wearing a denture is almost invariably accompanied by undesirable and irreversible morpho-

logical changes of DST [4], resulting in poor denture fit with substantial clinical complications [5].

Evaluation of DST morphological changes, associated with wearing a denture, has been studied in the past. The bone changes were evaluated with impressions and casts [6], two-dimensional (2D) radiographic methods including panoramic [7] and cephalometric radiography [8]; and recently with three-dimensional (3D) radiographic methods, using cone beam computed tomography (CBCT) [9–11]. The use of the casts allows evaluation of surface changes only, and consequently, no distinct data for bone and mucosa were available. Radiographic methods are suitable for the evaluation of bone changes only

* Corresponding author. Faculty of Medicine, Department of Endodontics and Operative Dentistry, University of Ljubljana, Vrazov trg 2, 1000, Ljubljana, Slovenia.
Email address: ales.fidler@mf.uni-lj.si (A. Fidler)

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[12]. Use of 2D panoramic and cephalometric radiographic methods was limited by the fact that the 3D structures are shown on the 2D image and inherent projection errors [13]. An introduction of 3D imaging techniques overcomes those limitations. A CBCT imaging opens the possibility of 3D evaluation of bone at a much lower radiation dose in contrast to medical CT [14].

In addition to bone changes, denture also can induce mucosal changes - whether decrease (atrophy) or increase (keratinization, hyperplasia) in its thickness [15,16]. In the present literature, there is only one study evaluating the thickness of mandible mucosa in denture foundation area [17] and several studies evaluating the thickness of maxillary mucosa for mucosal graft harvesting purposes [18-21]. Currently, there are no available studies on mucosal changes with time, associated with wearing a denture.

The fusion of computed tomography data and optical 3D images is a new and promising method [22], facilitating 3D evaluation of both hard and soft tissue changes. Recently it has been introduced in maxillofacial - orthognathic surgery and orthodontics [23], implantology [24] and endodontics [25], but not in other areas of dentistry.

The purpose of this study was to propose a novel method for 3D evaluation of bone and mucosal changes in removable partial denture (RPD) foundation area using a fusion of CBCT and optical 3D images.

2. Materials and methods

2.1. Patients selection

This pilot study is a part of a research project and was conducted to validate and improve the methodology of evaluation of prosthodontic treatment with RPD with and without a metal framework on oral health. Ethical approval was obtained from the Ethics Committee of Hospital and University Clinical Service of Kosovo and University Clinical Centre of Kosovo (555/18.05.2017).

The inclusion criteria of patients for the primary study were patients of both genders, aged 45-65 years, Kennedy class I status - a bilateral edentulous area, located posterior to the remaining natural teeth, no previous RPD, prosthodontically unrestored dentition, no caries lesions, and absence of active periodontal disease.

All patients were informed of the protocol of the study, including the two exposures to CBCT and three impressions taken at two time points, and gave their written consent to participate. Image data from first five patients were used to develop a novel method for 3D evaluation of bone and mucosal changes associated with RPD.

2.2. Clinical protocol - 3D data acquisition

All patients eligible for this study had two CBCT scans taken by one experienced radiology technician. The first CBCT scan was taken at insertion of RPD (T1) and the second one after ten months wearing RPD (T2). All CBCT images were taken with the same device (ORTHOPHOS XG 3D; Dentsply Sirona). The exposure parameters were set at 85 kVp, 7 mA for female and 10 mA for male patients, 5 s effective time, 8 × 8 cm field of view and a voxel size of 0,16 mm. The images were acquired and saved in DICOM format for further processing.

Three impressions with irreversible hydrocolloid impression material (XantALGIN Select Fast Set; Heraeus Kulzer GmbH) and perforated stainless steel impression tray (Hi-Tray Metal; Zhermack SpA) were made by one experienced clinician. The impression material was mixed with automatic mixer (Cavex Alginate Mixer II; Cavex Holland BV) using manufacturers recommended mixing ratios between powder and water and using tap water with consistent temperature between 12 and 15-degree Celsius for reproducibility consistency and viscosity. Pressure

during impression making was controlled in a feedback manner through the clinician sensation. In the cases where the tray was visible or the material thickness was insufficient, the impression was repeated.

The first impression was taken at insertion of RPD (T1) and the second one after ten months wearing RPD (T2). The third impression was taken at T2 together with denture in place to obtain the denture foundation area. Impressions were poured with gypsum immediately after making by a dental technician. Gypsum cast were then digitized with a laboratory scanner (Ceramill Map 400; Amann Gyrbach AG) and exported in Standard Tessellation Language (STL) file format.

2.3. Segmentation of 3D bone models from CBCT scans

Segmentation of teeth and bone was performed using implants planning software (RealGUIDE Software version 5.0; 3DIEMME Srl) with "Tooth/Bone Segmentation" software extension. The segmentation procedure is based on graph cuts segmentation technique [26]. It requires selection of the following parameters: bone and background threshold values, bone and background seeds and a smooth factor. The segmentation parameters and minor manual corrections after segmentation were provided by an experienced operator. Both T1 and T2 CBCT images were segmented as described above and stored in STL file format as BONE1 and BONE2, respectively.

2.4. Registration of 3D models

The rigid spatial registration (RealGUIDE Software version 5.0; 3DIEMME Srl) requires manual definition of multiple reference points for initial alignment, which is then automatically refined using best fit matching based on Iterative closest point (ICP) algorithm (Fig. 1) [27].

First BONE1 and BONE2 registration were performed (Fig. 2). As the objects were from different time points, bone structures that were not affected by denture were selected for initial alignment, i.e., left and right mental foramina and mental spine. For refining initial alignment, best fit matching on expanded regions - spheres around initially selected points were used. In the second step, the SURFACE1 and SURFACE2 objects were registered to corresponding bone objects, BONE1 and BONE2, respectively. The structures present in both models were selected for registration between SURFACE and BONE objects from same time, i.e., teeth. Distinct structures like canine cusps and incisal edges were selected for initial alignment. Spheres around those points were then used for refining initial alignment using best fit matching. All four aligned STL objects were exported for further 3D analysis.

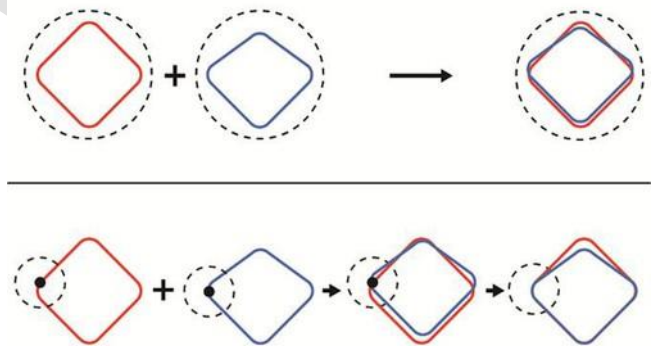


Fig. 1. Comparison of whole object based (upper) and point based (lower) best-fit alignment.

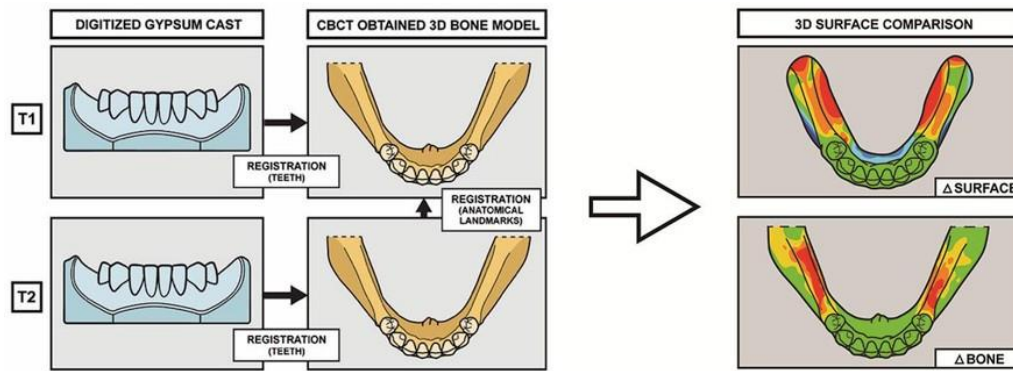


Fig. 2. Surface and bone models registration flowchart. 3D models are obtained with digitizing gypsum casts (surface models) and CBCT segmentation (bone models). Full arrows are showing registration process - SURFACE1 on BONE1 (teeth as registration point) and the same for SURFACE2 on BONE2. So then follows BONE2 on BONE1 registration with already matched corresponding SURFACE models (anatomical landmarks as mental foramina and mental spine as registration point). With aligned 3D models, 3D color-coded deviation maps for surface and bone are created. This enables visual and numerical evaluation of temporal surface and bone changes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.5. Validation of method reproducibility

The reproducibility of the method may be affected by segmentation of CBCT images and registration process. For the purpose of assessing reproducibility of the method, obtaining 3D bone models, i.e., segmentation of CBCT images, was repeated by the same operator (intra-operator reliability) and by another one (inter-operator reliability). Segmentation of CBCT images was compared with volume-based metrics by surface comparison between newly segmented and original 3D models with 3D data measurement analysis software (GOM Inspect 2017; GOM GmbH). Volume values were exported into a statistical software program (IBM SPSS Statistics version 25; IBM Corp) where intra- and inter-operator reliability were assessed by the intra-class correlation coefficient (ICC).

BONE1 to BONE2 registration and SURFACE to BONE registration was repeated by the same operator (intra-operator reliability) and by another one (inter-operator reliability) as well. In BONE1 to BONE2 registrations, mean absolute deviations between the two 3D objects were calculated in 4 regions on stable areas where no change was expected and in 4 regions where change was expected, i.e., alveolar ridge, 8 regions total. In SURFACE to BONE registration, mean absolute deviations between the two 3D objects were calculated in 4 regions on alveolar ridge. Deviation values between compared models were exported into a statistical software program (IBM SPSS Statistics version 25; IBM Corp). Intra- and inter-operator reliability for the measurements - mean absolute deviations were assessed by the ICC.

2.6. Analysis of aligned 3D models

The DST changes were quantified with 3D data measurement analysis software (GOM Inspect 2017; GOM GmbH). Visual evaluation was facilitated by the design of 3D color-coded deviations maps obtained by calculating the Euclidean distances between previously aligned 3D STL models, obtained with the same modality. The areas with changes within the arbitrary set threshold limit of ± 0.5 mm were displayed in green, areas with loss were presented with increasingly saturated red and areas with gain were presented by increasingly saturated blue. Additionally, a denture foundation area, obtained from impression with a denture, was marked in 3D images with a line. Two 3D color-coded deviation maps, one for surface (SURFACE) and one for bone changes (BONE) were created (Fig. 3). Such images enable visualization and descriptive analysis of temporal differences between the two 3D models.

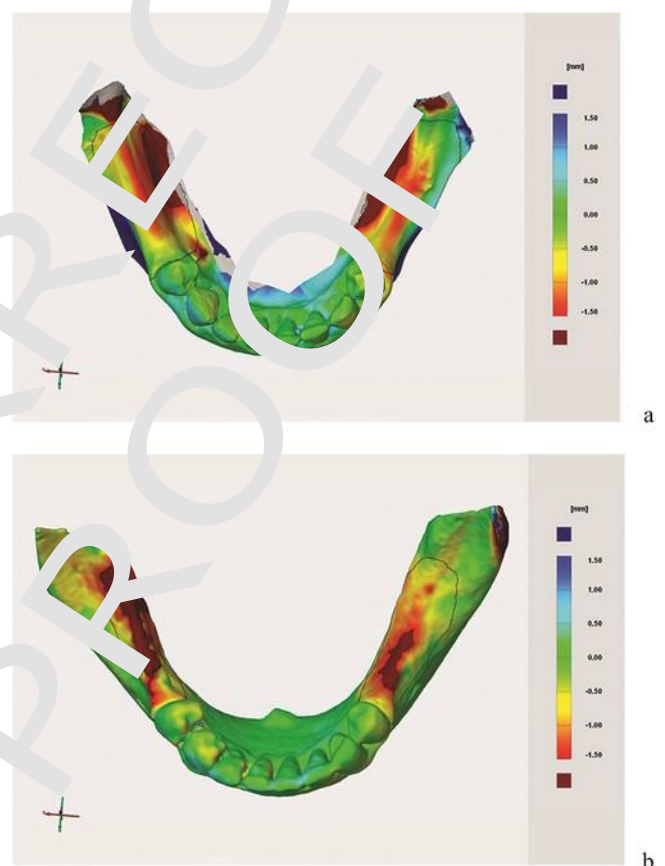


Fig. 3. 3D color-coded deviation maps for surface (a) and bone changes (b). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Three regions of interest (ROI) were defined for numerical evaluation. Beside denture foundation area ROI (dROI), defined with denture borders, wide (wROI) and narrow (nROI) ROI were selected (Fig. 4).

The numerical evaluation was performed for the left and right side separately. The area of surface dROI was measured and expressed in mm^2 . Bone volume changes were measured in wROI and dROI and expressed in mm^3 . A volume to surface ratio was calculated, reporting the average bone change thickness in dROI. Percentage of bone changes within dROI was calculated as well. Bone and soft tissue volume changes were measured and performed at nROI. Positive values

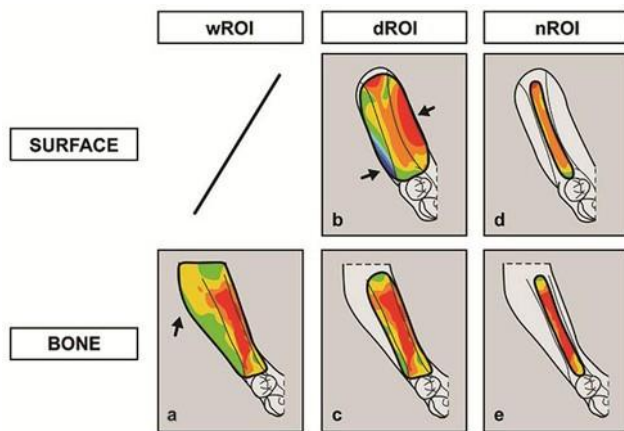


Fig. 4. Regions of interest (ROI) for numerical analysis. First, denture foundation area ROI (dROI) was defined with denture borders on the surface 3D color-coded deviation map (b). dROI on bone 3D color-coded deviation map (c) was defined with the 3D data analysis software-based projection of the denture borders perpendicular to bone surface. Extreme changes due to the mobility of loosely attached alveolar lining mucosa (arrows on b) in denture foundation area were the reason that narrow region (nROI) on central residual ridge area, limited to a firmly attached masticatory mucosa in width of 4 mm was defined, on both surface (d) and bone (e) 3D color-coded deviation maps to avoid misleading data. Bone changes were also present out of the denture foundation area on the bone 3D color-coded deviation map (arrow on a). Therefore, a wider ROI, including all bone changes was defined and referred to as wROI. wROI is defined with last remaining tooth mesially, mylohyoid line medially, laterally oblique line of the mandible and mental foramen laterally, and the transition to ramus distally. Due to the extreme changes caused by the mobility of loosely attached alveolar lining mucosa already in dROI, wROI was not selected on surface model to avoid misleading data. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

represent gain while negative values represent a loss of tissue. For the nROI we assume that the surface changes are a sum of bone changes and mucosal changes, $dV_{\text{surface}} = dV_{\text{mucosa}} + dV_{\text{bone}}$; therefore, the mucosa volume changes were calculated as $dV_{\text{mucosa}} = dV_{\text{surface}} - dV_{\text{bone}}$. All data was saved to a statistical software program (IBM SPSS Statistics version 25; IBM Corp).

Visual evaluation of 3D color-coded deviation maps revealed site-specific changes. Evaluation of changes in mesiodistal direction was calculated in cross-sections, with a distance of 0.5 mm. Values of changes from nROI were exported into spreadsheet software (Microsoft Excel for Mac version 16; Microsoft). For each cross-section mean change was calculated and exported to a statistical software program. Plots with showing change of surface, mucosa, and bone were created and aligned with color-coded 3D deviation maps (Fig. 5). The positive values represent gain in mucosa thickness, the negative values represent loss in mucosa thickness, while 0 indicates that there was no change in mucosa thickness.

For a further understanding of the site-specific relationship between surface, mucosa and bone changes, buccolingual cross-sections were designed at several specific sites. Such cross-sections facilitate measurement of surface, mucosa and bone changes at each point. (Fig. 5).

3. Results

Visual evaluation of 3D color-coded deviation maps of surface and bone models revealed a high diversity of surface and bone changes. Bone models revealed differences between mandibles and also between left and right residual ridge within each mandible. Bone models mostly exhibited no change or small bone loss in restricted parts of the residual ridges, except for mandible 1 and 4, in which extensive bone loss was found. Surface models show even more diversity. Areas exhibiting loss, no change and gain were found in surface models. The biggest

changes, both gain and loss were found at the borders of all surface models.

The surface area of denture foundation area of surface models and volumetric changes of bone models in dROI and wROIs are shown in Table 1. Mean surface area of denture foundation area was 499.69 (range = 317.47 to 719.50) mm². Mean bone volume change in dROI was -135.86 (range = -456.18 to 21.20) mm³. Mean bone volume change in wROI was -183.10 (range = -713.38 to 78.28) mm³. Comparison between bone changes in dROI and wROI revealed that on average 59.60% (range = -12%–113%) of all bone changes occurred inside dROI.

Volumetric changes in nROI for right and left side are shown in Table 2. Mean changes were -38.31 (range = -118.26 to 45.87) mm³, -51.96 (range = -182.54 to 5.6) mm³ and 13.66 (range = -80.62 to 79.46) mm³ for surface, bone and mucosa, respectively.

Average bone change thickness in dROI and nROI are shown in Table 3. Average bone change thickness was -0.26 (range = -0.96 to 0.07) mm in dROI and -0.42 (range = -1.73 to 0.06) in nROI.

Evaluation of changes in mesiodistal direction and on buccolingual cross-sections show site-specific changes (Fig. 5). Mucosa thickness exhibited loss as well as gain, depending on site.

Results of method validation shows excellent intra- and inter-operator reliability for segmentation and registration and are shown in Table 4.

4. Discussion

The proposed method, using a fusion of CBCT and optical 3D images, offers a 3D evaluation of distinct bone and mucosal changes in denture foundation area. Evaluation of those changes can be visual, based on 3D color-coded deviation maps, and numerical, facilitating measurement of thickness, area, and volume.

The originality of the present method is the fusion of 3D image data acquired with two different modalities that were acquired at two time points. Such fusion requires precise spatial registration of four objects in STL file format. The adequately selected sequence of registration together with a selection of representative reference points is crucial for alignment of 3D models. Stable anatomical landmarks outside the denture foundation area were selected for the registration of bone objects (BONE1 to BONE2), because of their resistance to temporal changes. For registration of surface models to previously registered corresponding bone models (SURFACE1 to BONE1 and SURFACE2 to BONE2), teeth are the only possible structures for registration, as they are represented in both 3D objects, limiting the method to partially edentulous patients only. In order to achieve optimal registration, the reference points should be as distant as possible. Automated best fit registration based on ICP algorithm was used in this study, requiring an operator selected reference point and sphere size selection. The method is based on the principle that human is better at coarse alignment, while the computer is better at fine alignment within the sphere. Instead of best fit alignment on the whole surface of 3D models, aiming to minimize the difference between the two 3D models, the limited regions, representing the stable area, were selected (Fig. 1). Furthermore, using the best fit registration based on ICP algorithm minimizes intra- and inter-operator variability due to insensitivity to small variations in reference point positioning [27,28]. This is achieved as long as corresponding reference points are positioned within the user-defined sphere, in other words, the inter- and intra-operator variability should be within sphere limits. In our study, the sphere diameter was set to 1 cm, which is well above inter- and intra-operator variability, proved with excellent ICC values.

The numerical evaluation was performed in three different ROI (Fig. 4). The dROI was an obvious decision because we aimed to quantify changes under the denture. A visual evaluation revealed two phe

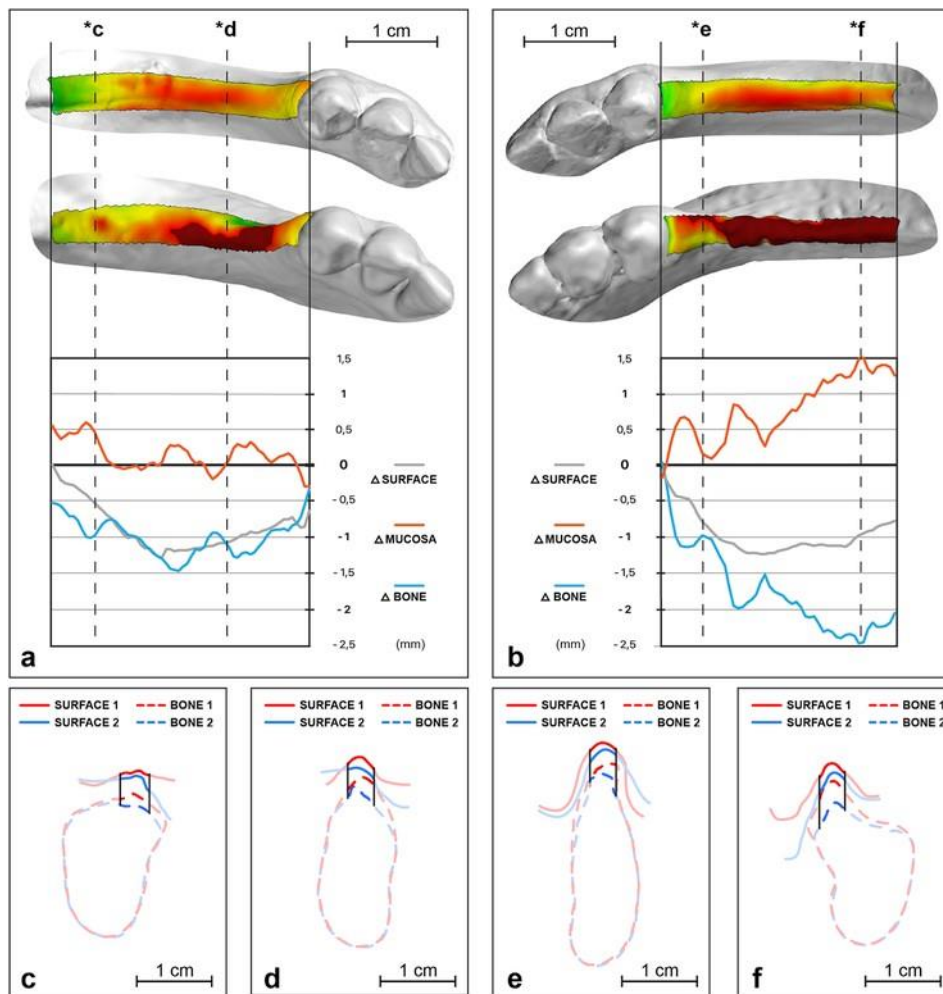


Fig. 5. Evaluation of changes in mesiodistal direction and on buccolingual cross-sections for mandible No. 1. Changes in mesiodistal direction for left (a) and right (b) residual ridges are displayed with, 3D color-coded deviation map of the surface (top image) and bone (middle image), and with a plot with average changes of thickness for surface, mucosa and bone (bottom image) along the left and right alveolar ridge. It should be noted that the positive values represent gain in mucosa thickness, the negative values represent loss in mucosa thickness, while 0 indicates that there was no change in mucosa thickness. Vertical lines, marked with (*c, *d, *e, and *f) are indicating positions of corresponding buccolingual cross-section images (c, d, e, and f). Black vertical lines in cross-section images indicate narrow ROI (nROI) borders. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Results of bone changes in five mandibles, separate for left and right side.

No.	side	Denture foundation area (mm ²)	Δ Volume in dROI (mm ³)	Δ Volume in wROI (mm ³)	Δ Volume within dROI (%)
1	right	473.06	-456.18	-713.38	64
	left	424.83	-317.08	-405.36	78
2	right	479.97	20.07	25.78	78
	left	348.88	-59.78	-147.45	41
3	right	481.96	-72.48	-90.81	80
	left	692.34	-9.29	78.28	-12 *
4	right	694.80	-109.59	-96.75	113 **
	left	719.50	-370.20	-527.08	70
5	right	317.47	21.20	56.98	37
	left	364.07	-5.30	-11.20	47

Table 2
Volumetric changes in nROI at right and left residual ridge for surface, bone, and mucosa.

side	Δ Volume surface (mm ³)	Δ Volume bone (mm ³)	Δ Volume mucosa (mm ³)
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** Δ V in dROI was bigger than Δ V in wROI, due to bone gain outside dROI, resulting in percentage, higher than 100%.

Δ Volume within dROI was calculated as Δ Volume in dROI/ Δ Volume in wROI and represents percentage of bone change occurred within dROI.

* Δ Volume in dROI was negative value, while Δ V in wROI was positive value, resulting in negative percentage value.

<i>M₁</i>	<i>Kuralt</i>	right	-103.08	-182.54	79.46
		left	-108.29	-138.12	29.83
2		right	-33.34	0.27	-33.61
		left	-118.26	-37.64	-80.62
3		right	11.83	-24.68	36.51
		left	45.87	-15.47	61.34
4		right	-49.00	-40.87	-8.13
		left	-49.09	-86.99	37.90
5		right	-18.89	0.84	-19.73
		left	39.20	5.60	33.60

nomena. Firstly, extensive bone changes were observed at and even outside the denture foundation area border of bone model. Therefore, a wROI was selected for bone volume changes evaluation. Secondly, extensive surface changes were observed at the lingual and buccal borders of the 3D surface model. They resulted from high mobility of loosely attached alveolar lining mucosa in these areas. The loosely at

Table 3
Average bone change thickness at dROI and nROI.

	side	dROI (mm)	nROI (mm)
1	right	-0.96	-1.73
	left	-0.75	-0.97
2	right	0.04	0.01
	left	-0.17	-0.20
3	right	-0.15	-0.21
	left	-0.01	-0.11
4	right	-0.16	-0.32
	left	-0.51	-0.72
5	right	0.07	0.01
	left	-0.01	0.06

Table 4

Intra-class correlation coefficients (ICC) to test the repeatability of the presented method within operator (intra) and between operators (inter).

Operator relation	Segmentation	Registration (BONE1 - BONE 2)	Registration (SURFACE - BONE)
Intra	0.997	0.923	0.933
Inter	0.995	0.895	0.915

tached alveolar lining mucosa was also found at the denture foundation area borders, progressively transforming into a firmly attached masticatory mucosa at the central part of the narrow region of the central ridge. Therefore, a nROI, limited to a firmly attached masticatory mucosa in width of 4 mm in a central ridge area, was necessary.

Beside of one-dimensionally measurements limited to arbitrarily defined sites only [10], this method enables advanced numerical evaluation of surface and volume. Volume was rarely used as a parameter in describing the dynamics of DST in previous studies. The bone change was presented as a percentage of total mandible volume [11]. Such parameter depends not only on bone volume change itself but also on whole mandible volume, rendering between subject comparison difficult. Besides reporting the absolute volumes, for left and right side, a volume to surface ratio was calculated, reporting the average thickness of bone loss in denture foundation area. This parameter allows comparison between denture foundation areas of different sizes.

Results of changes after ten months show variability between the subjects, between left and right sides and most importantly high within ROI variability. We found that predominantly bone loss or no change were present. Interestingly, some bone gain was also found in small areas, confirming the result of the previous study, where bone changes, including bone gain, were described and explained as remodeling phenomenon, i.e., resorption and deposition of bone tissues [11]. Interestingly, bone loss was not limited only to the denture foundation area (dROI). Areas of considerable bone loss were in some cases extending outside of denture foundation area as seen on Fig. 3b. This might be explained by a pressure related bone resorption, caused by denture margins. Obviously, the process is gradually diminishing instead of being sharply limited. As direct comparison of bone volume changes at dROI and nROI is not relevant due to the differences of surface areas of dROI and nROI. the average bone change thickness was introduced for comparison. It shows that either more bone remodeling occurred on the top of the residual ridge compared to its sides or that the bone remodeling was similar under whole denture foundation area. The high site variability suggests that bone remodeling is a site-specific process, unequally affecting the bone surface.

posed to solve this with simple equation " $dV_{mucosa} = dV_{surface} - dV_{bone}$ " or with using cross-section analysis. With this in mind, nROI located on firmly attached masticatory mucosa was selected. Evaluation of nROI revealed that bone changes are well compensated by either decrease or increase in mucosa thickness, resulting in a reduction of surface morphology change. Again, this observation shows site-specific variability.

In the present study, image data were acquired by two methods - digitizing gypsum casts and CBCT imaging. Digitizing gypsum casts with the laboratory scanner is a validated method, producing accurate 3D surface models [29,30]. The accuracy of a gypsum cast is influenced by impression taking, i.e., selection of impression material, tray and impression technique, compression of mucosa, and gypsum cast pouring [31]. For obtaining accurate surface information the viscoelastic rheological properties of impression material are very important [32]. In our method, irreversible hydrocolloid impression material was used, which requires significant pressure to record the alveolar ridge. How-

ever, being hydrophilic in nature, it provides good contact with wet surface of oral mucosa. To achieve intimate contact between impres-

sion and oral mucosa at smallest possible loading, viscosity was reduced with automatic mixing [33]. Oral mucosa is highly deformable under compression. Compression might lead to inaccuracy of measurements

Changes in mucosa thickness couldn't be visualized like surface and bone changes with 3D color-coded deviation maps. For qualitative evaluation a simultaneous comparison of surface and bone changes models could be used as in Fig. 5a. For quantitative evaluation we pro

in ~~mucosa~~ thickness and surface changes. However, this deformation is highly site specific [17], which is very clearly seen in 3D color-coded deviation maps for surface changes in Fig. 4 in areas with loosely attached alveolar lining mucosa, i.e., the borders of the surface comparison models. Regions with firmly attached masticatory mucosa are more resistant under compression and this is also why a region limited to a firmly attached masticatory mucosa (nROI), was selected for analysis, which proved to be accurate enough with irreversible hydrocolloid impression material [34]. Intraoral scanning would omit these factors and seems a superior technique. Unfortunately, edentulous areas are lacking anatomic mucosal landmarks [35] and factors like lack of space, patient movement, saliva flow and humidity [36], rendering intraoral scanning less reliable and suitable for this task.

3D bone models were obtained by automated segmentation of CBCT images. The most often used CT image segmentation method in medicine is thresholding, which often requires extensive manual post-processing [37]. In our study a graph cuts segmentation technique was used, requiring an operator selected threshold values and seed points. Although the CBCT is inferior compared to medical CT regarding the stability and reliability of voxel values, due to errors of reconstruction algorithm and artifacts [38], the benefits of CBCT, such as small voxel size, isotropic voxel, and lower dose [39], warrant its use in such studies. With additional seed points selection beside threshold setting, graph cuts segmentation is advanced thresholding approach, which reduces manual post-processing and increases accuracy [26].

Visual representation of comparisons between surface and bone models enables a thorough analysis of the dynamics in DST. 3D color-coded deviation maps show the areas, exhibiting changes as well as the magnitude of changes, represented by hue.

5. Conclusion

Wearing dentures is accompanied by undesirable and irreversible morphological changes of DST [4]. The proposed method facilitates separate evaluation of surface, mucosa and bone changes with a fusion of computed tomography data and optical 3D images. Visual and numerical evaluation of DST opens possibilities for a better understanding of DST remodeling, objective evaluation, and comparison of different treatment options.

Declarations of interest

None.

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Impact of combined non-surgical and surgical periodontal treatment in patients with type 2 diabetes mellitus-a preliminary report randomized clinical study.

Dashnor Bukleta^{1,2}, Shaip Krasniqi³, Giangiacomo Beretta⁴, Armond Daci^{5*}, Arb Nila², Teuta Komoni⁶, Manushaqe Selmani⁶, Brikene Elshani⁷, Rok Schara¹

¹Department of Oral Medicine and Periodontology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

²Department of Oral Surgery, Dental Polyclinic of Peja, Peja, Kosovo

³Institute of Pharmacology Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Prishtina, Prishtina, Kosovo

⁴Department of Pharmaceutical Sciences, Università Degli Studi di Milano, Milan, Italy

⁵Department of Pharmacy, Faculty of the Medicine University of Prishtina, Prishtina, Kosovo

⁶School of Dentistry, Faculty of Medicine, University of Prishtina, Prishtina, Kosovo

⁷Faculty of Medicine, University of Prishtina, Prishtina, Kosovo

Abstract

Background/objectives: Scientific evidence regarding the effects of chronic periodontitis on Type 2 Diabetes Mellitus (T2M) is fragmentary and not definitive. This intervention study was designed to evaluate the effects of a Non-Surgical Procedure (NSP) in addition to a surgical procedure on systemic inflammation and glycaemic control in patients with T2M and periodontitis and Non-Diabetic (ND) patients with periodontitis.

Material and methods: A total of 100 patients with diabetes were randomly allocated to a treatment group and a control group. At least one tooth was extracted from each patient. After extraction, the control group (n=50) did not receive any other treatment until the 3-month follow-up. Patients in the treatment group (n=50) received Full-Mouth Scaling and Root Planing (FM-SRP). The Mean Probing Depth (MPD), Mean Attachment Level (MAL), Plaque Index (PI), Bleeding on Probing (BOP), fasting blood samples for the measurement of high-sensitivity C-Reactive Protein (hs-CRP), and glycated hemoglobin (HbA1c) were measured or taken at baseline and 3 months after treatment. To investigate the effect of diabetes on the therapeutic procedure's outcome, ND patients with periodontitis (n=60) were divided into two equal groups and subjected to the same procedures and analysis.

Results: HbA1c and hs-CRP decreased significantly in the diabetic groups and decreased more markedly when FM-SRP was added to tooth extraction (P<0.001). For ND patients, all of the examined periodontal parameters were in favor of the combination therapy (P<0.001).

Conclusion: Non-surgical periodontal treatment can help improve the outcome of surgical periodontal procedures by reducing systemic inflammatory status and improve glucose metabolism.

Keywords: Diabetes mellitus type 2, Inflammation, Periodontitis, Diabetes, Periodontal treatment, Diabetes.

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Introduction

Type 2 Diabetes Mellitus (T2DM) is a chronic disease that comprises a heterogeneous group of metabolic disorders expressed with altered glucose tolerance or impaired carbohydrate metabolism. In diabetic patients with poorly controlled metabolism, complications develop more rapidly and in more severe forms. Systemic complications

(retinopathy, nephropathy, neuropathy, macrovascular and microvascular disease) are associated with prolonged hyperglycemia [1,2].

The prevalence of diabetes has drastically increased over the past decade, with economic conditions playing an important role due to poor medication adherence [3-5].

Currently, dental professionals play an important role in the process of diagnosing and monitoring DM [6]. A major role was shown to play also dental hygienists in the screening of patients with periodontitis [7].

It has been shown that DM is linked to various oral complications that include periodontal disease, which is classified as the sixth complication of diabetes. Studies have indicated that individuals with type 2 DM are three times more vulnerable to developing periodontal oral complications than individuals without diabetes [8,9].

Moreover, periodontal disease is considered to be one of the major oral complications that are likely to occur in patients with poorly controlled type 2 DM [10]. The prevalence of periodontal oral complications has been shown to increase with age. The loss of teeth or dental formulae is considered to be 15 times higher for individuals with DM than for those without DM [11]. A higher prevalence of periodontal disease is reported in individuals who rarely seek medical attention [12,13].

Individuals with type 2 diabetes also exhibit poor oral health [14]. This implies, therefore, that glycaemic control is generally affected by oral complications such as periodontal infection and oral complications adversely affect periodontal health in individuals [15].

Similarly, periodontal disease increases the severity of DM and complicates glycaemic control. In general, improvements in glycaemic control improvements were shown to decrease the rate of acute and chronic systemic complications [16]. In addition, patients with well-controlled diabetes, as measured by the blood glycated hemoglobin levels, have the less severe periodontal disease than do individuals with poorly controlled diabetes [17]. The elimination of periodontal infection improves the glycaemic control of diabetes, as defined by a reduction in glycated hemoglobin. It was confirmed that even full-mouth tooth extraction raises the improvement magnitude [18].

It is known that inflammation plays a part in insulin resistance in patients with type 2 diabetes [19]. According to this finding, in periodontitis patients without other apparent diseases, CRP levels are higher than in patients without periodontitis [20]. Management of diabetic patients can maintain periodontal health with responding better to the periodontal treatment [21], and on the other side, the control of chronic periodontal infection is essential for achieving long-term control of T2DM [22].

Previous studies found that non-surgical periodontal therapy alone has an impact on the quality of life [23], and also such as full-mouth scaling and root planning resulted in an improvement of glycaemic control [24,25]. Moreover, another study demonstrated that non-surgical periodontal therapy was not proven to be effective in patients with T2DM with underlying obesity [26].

However, recent meta-analyses have suggested that this conclusion must be interpreted with care due to the limited

robustness and heterogeneity of the studies available in the literature [27,28].

Taking this into consideration together with the necessity for further clinical trial investigations for T2DM and periodontal disease, the aim of the present study was to evaluate the effects of surgical treatment (tooth extraction) alone or in combination with non-surgical periodontal treatment (tooth cleaning) on periodontal status, systemic inflammation and glycaemic control in diabetic and non-diabetic patients with periodontal disease.

Material and Methods

Study design

In this study conducted during 2015-2016, based on a literature review, the data for 200 patients who were admitted to the endocrinology department of "Peja's Regional Hospital" and Dental Polyclinic in the city of Peja were assessed and examined for eligibility by oral surgery specialists. All procedures performed in our study involving human participants were in accordance with the ethical standards. The study was approved by the Ethics Committee of the University Clinical Centre of Kosovo (Nr.4212/2) and informed consent was obtained from all individual participants included in the study. A priori sample size calculation was performed given: Effect size $d=0.5$, alpha error probability 0.08 and power 0.8 resulting in 26 patients for the group.

Personal interviews were used to collect baseline data from each participant using a pre-structured questionnaire. Patients were selected if they met the following inclusion criteria: had been diagnosed with type 2 DM; had a baseline HbA1c $\geq 6.5\%$, had at least 10 teeth in the functional dentition (excluding third molars); and had a clinical diagnosis of periodontal disease with at least one site with a Probing Depth (PD) ≥ 5 mm, two teeth with attachment loss ≥ 6 mm and no modification in the pharmacological treatment of diabetes during the study period. Exclusion criteria included pregnancy or lactation, major diabetic complications, and the use of antibiotic therapy or non-steroidal anti-inflammatory drug therapy within 4 months before the first visit. Patient Characteristics and Baseline characteristics of biochemical and clinical data's from the patients are shown in (Tables 1 and 2).

After clinical examination, 160 patients aged 30-70 y old were selected for this open-label, randomized clinical trial. These were further divided into four groups: type 2 DM groups (with or without initial periodontal therapy) and non-diabetic groups (with or without initial periodontal therapy) (Figure 1).

All the patients had periodontal disease, and at least one tooth extraction was performed for each patient. Prior to the surgical procedures, an adjunctive, non-surgical periodontal treatment to achieve a full-mouth tooth cleaning was performed for the patients in the treatment groups: Full-Mouth Scaling and Root Planing (FM-SRP) using an ultrasonic device (UDS-J Ultrasonic Scaler, Guilin Woodpecker Medical Instrument) and

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periodontal curettes for the mechanical debridement of supra- and subgingival plaque and calculus.

Post-operative rinsing was followed by the use of the antiseptic solution Listerine® (ethanol 21.6%, methyl salicylate 0.06%, menthol 0.042%, thymol 0.064% and eucalyptol 0.092%) as a mouthwash thrice a day for 3 w. The study was registered on Clinical.Trials.gov (NCT02874963).

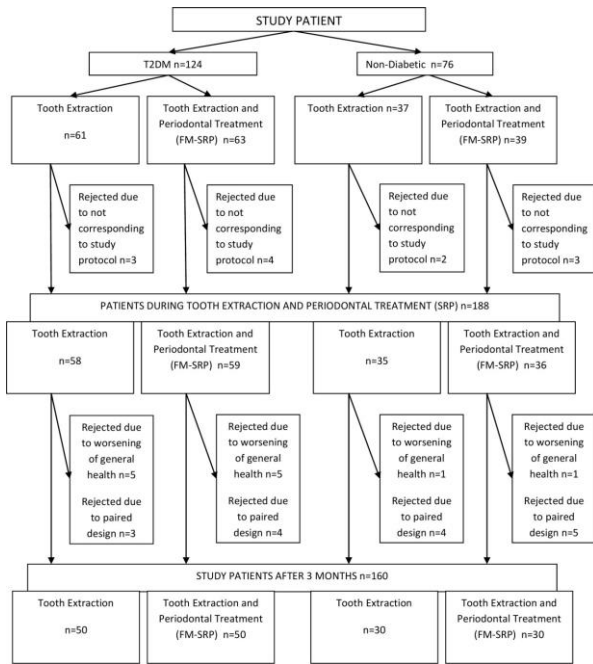


Figure 1. Study population flowchart.

Clinical data collection

The periodontal clinical examinations were performed according to the online periodontal chart (Department of Periodontology, School of Dental Medicine (ZMK), University of Bern) at baseline and 3 months after the periodontal treatment. All the parameters were recorded for six measurement points (mesial buccal, buccal, distal buccal, mesial lingual, lingual, distal lingual) on all teeth (excluding 3rd molars) during each of two visits.

Laboratory investigations

The levels of glycosylated hemoglobin (HbA1c) and of high-sensitivity C-reactive protein (hs-CRP) were measured using venous blood collected from patients at baseline and 3 months after the study procedures. All biochemical analyses were conducted in a biochemical laboratory in Peja (Laboratory Diagnostic Center, Peja, Kosovo) using a biochemistry analyzer (Select Pro XS, ELITech Clinical Systems, Paris, France) and enzyme-linked immunoassay kits (ELITech Clinical Systems, Paris, France).

Statistical analyses

All data were expressed as the means and Standard Deviations (SDs). Before the statistical analysis was performed, the normal distribution and homogeneity of the variances were tested. Associations between the experimental parameters were investigated using one-way ANOVAs, followed by t-tests of pairwise comparisons with the Least Square Difference (LSD) post hoc adjustment for multiple comparisons. Significant differences were also evaluated using the Mann-Whitney U-test, paired Student's t-test or the Wilcoxon rank-sum test. The results were considered significant when the P value was $P < 0.05$ (GraphPad Prism 5.0 software).

Results

Glycemic control and inflammation

The FM-SRP treatment after tooth extraction-induced significant improvements in the serum HbA1c values in T2DM patients with periodontal disease.

No significant difference was found in the HbA1c plasma serum levels of ND patients ($5.56 \pm 0.62\%$ vs. $5.65 \pm 0.65\%$; $P = 0.099$, $n = 30$), (Figure 2A) or in ND patients treated with the combined surgical/non-surgical therapy ($5.79 \pm 2.92\%$ vs. $5.58 \pm 0.74\%$, (-3.6%); $P = 0.036$, $n = 30$) (Figure 2B).

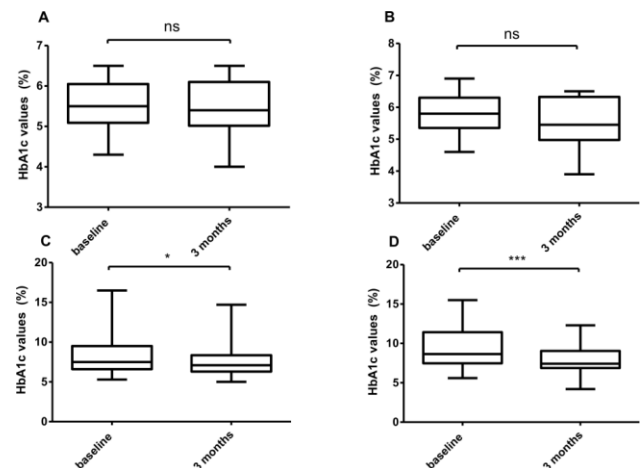


Figure 2. Graphical representation (box plot) of HbA1c values (%) at baseline and 3 months after tooth extraction in (A) non-diabetic patients with periodontal disease, (B) non-diabetic patients with periodontal disease treated with FM-SRP, (C) T2DM patients with periodontal disease and (D) T2DM patients with periodontal disease treated with FM-SRP. * indicates $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS: Not Significant.

With regard to periodontal disease, only the T2DM patients showed a significant HbA1c reduction after tooth extraction ($8.82 \pm 3.01\%$ vs. $7.69 \pm 2.09\%$, (-13%); $P = 0.018$, $n = 50$), (Figure 2C) and after tooth extraction followed by tooth cleaning with FM-SRP ($9.59 \pm 2.57\%$ vs. $8.03 \pm 1.67\%$, (-16.3%); $P = 0.0003$, $n = 50$) (Figure 2D). The variation in the serum levels of hs-CRP in ND patients was not statistically significant after tooth extraction (1.64 ± 1.06 vs. 1.64 ± 0.92 ;

P=0.913, n=30) (Figure 3A), but it was significant for patients with extracted teeth combined with FM-SRP ($3.63 \pm 2.19\%$ vs. $3.11 \pm 1.95\%$, (-14%); P=0.0006, n=30) (Figure 3B). In T2DM patients with periodontal disease, the reduction of hs-CRP after treatment with either tooth extraction only or with tooth extraction combined with FM-SRP was significant and had a higher magnitude than that of the ND patients (4.05 ± 2.20 vs. 3.24 ± 1.67 , (-20%); P=0.0003, n=50) and 4.44 ± 2.55 vs. 2.85 ± 1.96 , (-36.2%); P=0.0009, n=50) (Figures 3C and 3D, respectively).

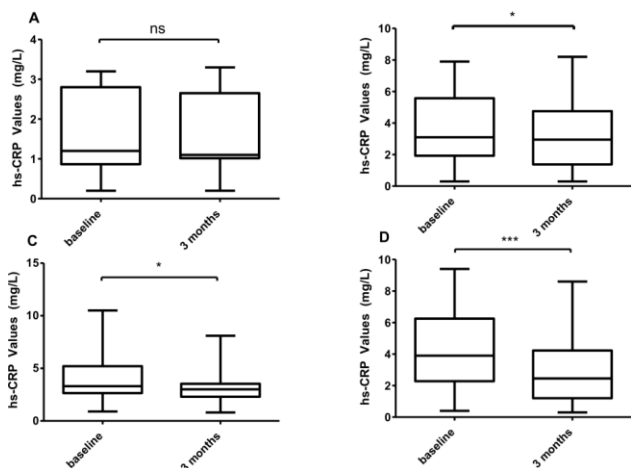


Figure 3. Graphical representation (box plot) of hs-CRP values (mg/L) at baseline and 3 months after tooth extraction in (A) non-diabetic patients with periodontal disease, (B) non-diabetic patients with periodontal disease treated with FM-SRP, (C) T2DM patients with periodontal disease and (D) T2DM patients with periodontal disease treated with FM-SRP. *indicates $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Periodontal conditions

The MPD value decreased, although not significantly, in ND patients after tooth extraction without FM-SRP treatment, while the decrease observed in non-diabetic patients reached the level of statistical significance in the FM-SRP treated group. This variation was significant and slightly higher in both T2DM groups: extraction only (6.60 ± 1.47 mm vs. 5.24 ± 2.17 mm; P=0.0004, n=50) and extraction followed by FM-SRP (6.52 ± 0.82 mm vs. 5.08 ± 1.10 mm; P=0.0001, n=50) (Figure 4A). The MAL value decreased significantly only in T2DM patients after tooth extraction followed by FM-SRP (-8.51 ± 1.55 vs. -7.32 ± 1.31 ; P=0.0001, n=50) (Figure 4B). Although the other groups showed a similar trend, those differences did not reach the level of statistical significance.

As expected, a significant reduction of PI was found in all groups with different magnitudes of reduction, depending on the FM-SRP application.

The lowest reductions were found in ND and T2DM patients after tooth extraction only ($95.07 \pm 7.14\%$ vs. $86.23 \pm 12.51\%$; P=0.0014, n=30) and ($93.02 \pm 15.78\%$ vs. $79.41 \pm 20.97\%$;

P=0.0001, n=50 respectively); the differences were enhanced by the FM-SRP treatment in both the ND and T2DM groups (96.57 ± 8.28 vs. 61.07 ± 25.27 (n=30); P=0.0028 and $98.06 \pm$

5.92 vs. 58.67 ± 22.76 ; P=0.0001, n=50 respectively) (Figure 5A).

BOP was reduced in all groups: ND patients after tooth extraction ($70.36 \pm 22.58\%$ vs. $55.70 \pm 20.57\%$; P=0.011, n=30) ND patients after tooth extraction followed by FM-SRP ($73.31 \pm 32.26\%$ vs. $48.57 \pm 29.72\%$; P=0.0031, n=30), T2DM patients after tooth extraction only ($71.55 \pm 34.65\%$ vs. $51.01 \pm 34.02\%$; P=0.0023, n=50), and T2DM patients after tooth extraction in combination with FM-SRP ($87.76 \pm 23.89\%$ vs. $28.94 \pm 21.65\%$; P=0.0001, n=50) (Figure 5B).

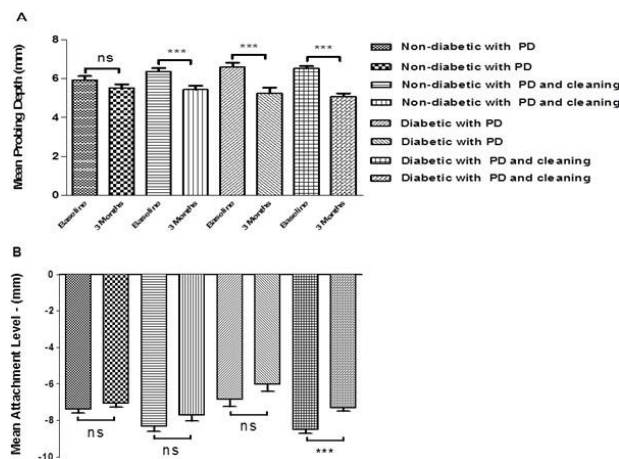


Figure 4. Comparison of mean probing depth (mm) (A) and mean attachment level (mm) (B) among groups with respect to time at baseline and 3 months after tooth extraction or extraction accompanied by periodontal treatment (FM-SRP). *indicates $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

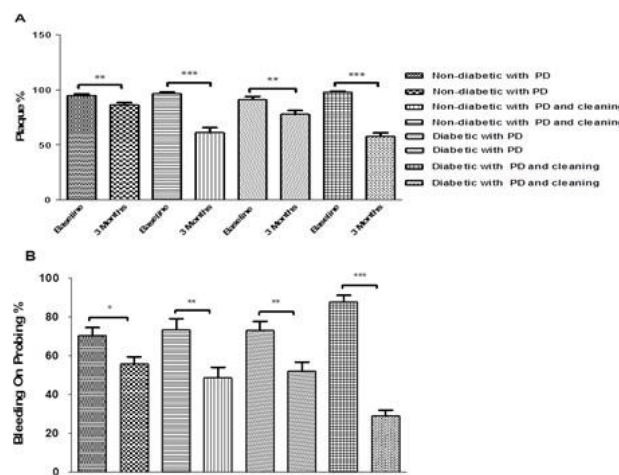


Figure 5. Comparison of plaque (%) (A) and bleeding on probing (%) (B) among groups with respect to time at baseline and 3 months after tooth extraction or extraction accompanied by periodontal treatment (FM-SRP). *indicates $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table I. Patient characteristics.

Characteristics	T2DM	Controls
Total patients (n)	100	60

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Sex (Male/female)	50/50	33/27	BMI	26.01 ± 3.64	26.49 ± 2.20
Age (y)	59.49 ± 10.82	56.66 ± 12.22	No. of teeth extracted	175 (1.75)	103 (1.72)
Weight (Kg)	76.03 ± 9.76	78.3 ± 9.76	Oral therapy	48	-
Height (cm)	174.64 ± 13.96	171.86 ± 6.81	Insulin	52	-

Table 2. Baseline characteristics of biochemical and clinical data's from the patients.

Parameters	Patients with diabetes		Patients without diabetes		Smokers
	Tooth extraction and FM-SRP	Tooth extraction	Tooth extraction and FM-SRP	Tooth extraction	Diabetes/without diabetes
No. of teeth extracted	87	88	54	49	88/37
PD (mm)	5.92 ± 1.06	6.37 ± 0.93	6.59 ± 1.47	6.53 ± 0.83	6.55 ± 0.75/6.08 ± 0.864
mAL (mm)	7.38 ± 1.31	6.8 ± 1.68	6.85 ± 2.74	8.52 ± 1.56	7.40 ± 2.79/7.75 ± 1.41
P (%)	95.07 ± 7.14	96.57 ± 8.28	93.02 ± 15.78	98.06 ± 5.92	96.41 ± 9.22/93.73 ± 10.05
BOP (%)	70.36 ± 22.58	73.31 ± 32.26	71.55 ± 34.65	87.76 ± 23.89	79.57 ± 30.99/72.74 ± 27.33
HbA1c (%)	8.82 ± 3.01	9.59 ± 2.57	5.56 ± 0.62	5.79 ± 2.92	8.98 ± 2.48/5.76 ± 0.63
hs-CRP (mg/L)	4.05 ± 2.20	4.44 ± 2.55	1.64 ± 1.06	3.63 ± 2.19	4.2 ± 2.07/2.37 ± 1.54

Discussion

The present study investigated the effects of surgical and non-surgical treatments on the periodontal status, glycaemic control (HbA1c) and systemic inflammation (hs-CRP) of T2M and ND subjects with periodontal diseases. This study was conducted to detect the beneficial properties of full-mouth tooth cleaning after tooth extraction in T2DM patients with moderate to severe periodontitis.

The critical analysis of different periodontal parameters in the T2DM group and the control group enabled us to find valuable evidence that indicates that the study interventions are effective in reducing oral complications, particularly in T2DM patients.

Our results show that the all measured periodontal clinical parameters (MPD, MAL, BOP and PI) improved significantly in T2DM patients. These results are consistent with those from previous studies in which the effect of FM-SRP, subgingival curettage, and tooth cleaning treatments on glycaemic control has been evaluated [26,29-32].

Our results demonstrated that concomitant cleaning and tooth extraction is more beneficial than tooth extraction alone in the course of periodontal disease treatment for both non-diabetic and T2DM patients.

It is known that the progression of the periodontal disease as a chronic infection is a risk factor for the development of diabetes, and this was also observed in our study groups [33].

In addition, we used the HbA1c and hs-CRP levels as indicators of diabetic control and inflammation in both groups. Other studies have evaluated periodontal treatments such as topical antibiotics and surgical procedures and have shown significant improvements in HbA1c through the reduction of

hs-CRP levels, and these results are consistent with our findings [31,34,35].

Similarly, following tooth extraction alone, patients tended to have improved HbA1c and hs-CRP values [17,36]. Oral hygiene plays an important factor in the control of periodontal status and in metabolic control [37].

It is well known that dental prophylaxis alone plays a role in periodontal health but does not affect the improvement of HbA1c levels [38].

Interestingly, in another study, full-mouth disinfection in type 1 diabetes patients has shown a positive effect on metabolic control [39]. In another meta-analysis, non-surgical periodontal treatment improved metabolic control in patients with periodontitis and diabetes [25]. Moreover, another recent study demonstrated that better oral hygiene improved periodontal health and metabolic control, which also confirms our findings [40].

These studies suggest a beneficial effect of surgical and non-surgical treatments on glycaemic control and periodontal status. Hence, taking this into consideration, we confirm that tooth cleaning through FM-SRP increases the positive effect of tooth extraction in patients with the periodontal disease with or without T2DM, and we shed light on their importance in dental oral practice.

Conclusion

By showing the critical effects of tooth cleaning accompanied by tooth extraction in the response to and efficacy of treatment for periodontal disease patients with T2DM and without DM, our work may set the stage for larger investigational studies aimed at evaluating the impact of non-surgical and surgical

approaches to the clinical management of periodontal disease and T2DM.

Compliance and Ethical Standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

"All procedures performed in our study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards". The study was approved by the Ethics Committee of the University Clinical Centre of Kosovo (Nr.4212/2).

Informed consent

"Informed consent was obtained from all individual participants included in the study".

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Authors' Contributions

The study design was planned by RC, DB, AD, and GB. Data collection was performed by DB, AN, TK, MS. Data analysis was conducted by RC, DB, AD, SK. All authors contributed to the interpretation of the data and manuscript preparation.

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***Correspondence to**

Armond Daci
Department of Pharmacy
Faculty of Medicine
University of Prishtina
Kosovo

Effects of Lower Third Molar Angulation and Position on Lower Arch Crowding

By Mimoza E. Selmani; Julijana Gjorgova; Manushaqe E. Selmani; Mirsad Shkreta; Shkelzen B. Duci

Abstract: The role of the third molars in lower arch crowding has been debated for more than a century. Objectives: The aim of this study was to determine the relationship between lower arch crowding and the presence of angulation and position of lower third molar. Methods and Materials: The measurements of the dental arch were made in 120 subjects aged 16 to 21 years, with average age to 18 years. The subjects were divided into two groups: Class I normal occlusion comprised 35 male and 25 female with mean age 18, 87 years, whereas Class I crowding comprised 27 males and 33 females with mean age 18, 5 years. The dental pantomogram (DPT) were used to calculate the ratio of retromolar space (Ganss ratio), angulation of third molar to second molar and third molar to the base of the mandible. Results: The results showed that measurements of Ganss ratio, third molar angulation to the base of the mandible, and third molar to second molar inclination, was statistically significant between crowded and normal groups. Conclusion: It can be concluded that there was a strong relationship between angulation and position of third molars and lower arch crowding.

Key words: Third molars, angulation, lower arch, crowding

Introduction

Crowding of teeth is considered as the most common type of malocclusion,¹ which is one of the most frequent reasons why people consult an orthodontist, taking into account the high aesthetic demand expressed by patients.² Dental crowding can be simply defined as the overlap of teeth caused by insufficient space within the dental arch.³ Crowding may occur due to different reasons, for example growth, decrease in dental arch length, maturation, aging of dentition, mesial drift, soft tissue pressures, and tooth morphology.⁴ According to Richardson,⁵ during the teenage years, pressure from the back of the arch is an important cause of late mandibular incisor crowding. Such pressure because of physiologic mesial drift, the anterior component of the force of occlusion on mesially-inclined teeth, or the presence of a developing third molar may cause forward movement of the buccal teeth, with shortening of the arch and increase in crowding. A large number of researches have been aimed to reveal influence of potential factors on the stability of the obtained orthodontic results.⁶⁻⁹ Robinson¹⁰ claimed that late lower incisor crowding is caused by the erupting third permanent molar teeth in the lower jaw. A study of 60 dental students with unilateral aplasia of a third permanent molar showed less crowding on the side where the third permanent molar was missing than on the contralateral side.¹¹ Bjork's and Skiller's¹² implant studies explain late crowding and its relationship to the growth pattern of the mandible. They had confirmed that the changes in anteroposterior of the incisors have a major influence on arch length changes than the molars but found no evidence to confirm a relationship between late lower labial segment crowding and third molar eruption. Richardson¹³ conducted the Belfast third molar study that is in

support of the pressure from behind the theory. She examined 51 subjects (21 females and 29 males) with intact lower arches and bilateral third molars present in the age group of 13 to 18 years.

Tufekci, et al¹⁴ compared the opinions of Swedish orthodontists and American orthodontists regarding the association between third molar eruption and dental crowding. The results showed that both Swedish and American orthodontists believed that lower third molars were more likely than upper third molars to cause force (65% and 58% for Swedish and American orthodontists, respectively) and crowding (42% and 40%, respectively).

Some authors have tried to explain the arch crowding through third molar position and angulation,¹⁵ whether the presence of third molars is capable of causing alterations in the positioning of other teeth.

Therefore, the purpose of this study was to determine the relationship between lower arch crowding and the presence of angulation and position of lower third molar

Methods and Subjects

The study groups consisted of 120 subjects aged 16 to 21 years, with average age of 18 years. The sample was divided into two groups: Class I normal occlusion comprised 35 male and 25 female with mean age 18.87 years, whereas Class I crowding comprised 27 males and 33 females with mean age 18.5 years. The crowded group (Figure 1) selected from patients seen in the Department of Orthodontics, Dental University Clinical Center, Kosovo. The inclusion criteria were: (1) presence of all permanent teeth (including un-erupted third molars); (2) Angle Class I molar relationships; (3) no artificial dental crowns and no anomalies of crown morphology; and, (4) no orthodontics treatment in maxillary and mandibular arch.



Figure 1: a) Class I molar relationship on crowding group; b) Crowding of the lower arch (unerupted third molars).



Figure 2: a) Class I molar relationship on noncrowding group; b) Noncrowding group of the lower arch (erupted third molars).

The normal group (Figure 2) was selected from the students in Dental University of Kosovo, and they have all permanent teeth in both jaws (including erupted third molars) with normal occlusion, which had not undergone orthodontic treatment.

The study was approved by the Local Research Ethics Committee in our country, and the participants were informed by the purpose of the study. Once participants have given their consent, the panoramic radiograph was taken to obtain the data of the study.

The mandibular dental arch was examined clinically, and each subject had a panoramic radiograph taken (Figures 3, 4) All the measurements were made by a single investigator and were assessed at least twice. On each panoramic radiograph (Figure 5), the following parameters were traced on an acetate sheets, and measurements were carried out by the same examiner:

1. The retromolar space to lower third molar crown width (Ganss ratio). A- Distance between distal border of second molar crown and anterior border of ramus measured on occlusal plane, B- width of third molar crown. The Ganss ratio = A/B.
2. Third molar to second molar inclination (angle A).
3. Third molar angulation to the base of the mandible (angle B).

All the radiographic measurements were statistically compared between the two groups on the right and the left side. Differences between these measurements of the lower arch "crowded" and "normal" (right and left side) groups were made by Mann-Whitney U test (Z/U). Frequency and percentages distribution of gender were calculated statistically on crowding and normal group. Descriptive studies such as mean and standard deviation were calculated for numerical data age. Values less than 0.05 was considered as significant.

Results

Results related to differences in measurements of Ganss ratio between crowded and normal groups are reported in Table 1. Values of distances A/Br in the normal group are greater than in the crowding group; the difference for $Z=-9.38$ and $p<0,001$



Figure 3: Panoramic radiograph of crowding group (unerupted) lower third molars.



Figure 4: Panoramic radiograph of noncrowding group (erupted) lower third molars.

($p=0,000$) is significant. The average value of Ganss Ratio A / B1 in the group of subjects with normal occlusion ($x = 1, 13$) is significantly higher than the average of Ganss Ratio A / B1 in the group of subjects with crowding ($x = 0.61$), the difference $Z = -9.34$ and $p<0,001$ ($p = 0,000$) is significant.

Results related to differences in the angle of inclination between crowded and normal group are reported in Table 2. Angle Ar and Al in the crowded group in relation to the non-crowded group is significantly greater $p<0,001$ ($p=0,000$). Angle of inclination Br and Bl in the non-crowded group is significantly greater than in crowded group $p<0,001$ ($p=0,000$).

Table 3 shows the distribution of gender on crowding and normal group. From 60 subjects, 27 (45, 00%) were male, and 33 (55, 00%) were female in crowding group. In the normal group, 35 (58.33%) were male and 25 (41. 67%) were female. The Pearson chi-square =0, 12 and $p<0, 05$ (0.69) shows that there is no significant difference in the distribution of subjects by gender.

Table 4 shows descriptive statistics for age. In the crowding group, the mean age of subjects was 18.05 with a standard deviation of $\pm 1. 57$ years, while the mean age in the normal group was 18.87 with a standard deviation of ± 1.52 years. Subjects in the normal group are significantly older, $Z=-2.75$ and $p<0, 01$ ($p=0,006$).

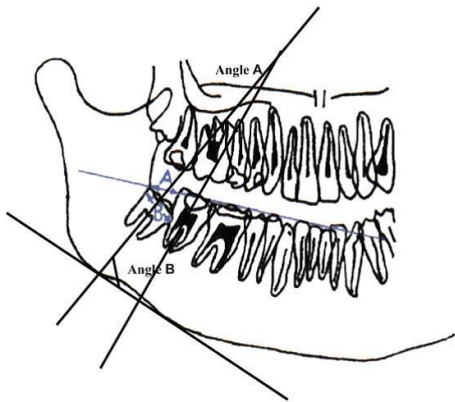


Figure 5: Panoramic radiographic measurements. A: Distance between distal border of second molar crown and anterior border of ramus measured on occlusal plane, B: width of third molar crown. The Ganss ratio= A/B. Angle A: Third molar to second molar inclination. Angle B: Third molar angulation to the base of the mandible.(31).

Discussion

Lower arch crowding that develops after finalization of the permanent dentition during the teenage period as well as post orthodontic treatment relapse is a common clinical problem faced by many orthodontists. Mandibular third molars have attracted the interest of the orthodontic professionals, not only because of their position and eruptive pattern but also because of their possible role in the development of late incisor crowding.^{16,17} Many studies have been conducted to determine relationship between dental crowding and gender dimorphism.¹⁸ According to R ta et al,²⁰ dental crowding is more frequent in females because males had significantly longer and wider dental arch dimensions than females. Males generally showed higher values in all arch dimensions, but the differences were not statistically significant in comparison to females.¹⁹ Frequency and percentage distribution by gender in our study were more in female subjects than in male for crowding group, while in the normal group, the percentage and frequency were greater in male subjects than female; however, there was no statistically significant differences between males and females for the crowding and the normal group. Numerous research studies have been conducted to determine the effect of third molars on lower incisor crowding.²¹⁻²³

Lindauer et al²⁴ reported significant differences between the opinions of American orthodontists and those of oral and maxillofacial surgeons regarding the role of erupting third molars in causing crowding of anterior teeth. More surgeons than orthodontists generally believed that erupting third molars produce an anterior component of force and cause crowding of the anterior dentition; thus they were more likely to recommend prophylactic removal of third molars to prevent such problems.

Sidlauskas and Trakiniene²⁵ evaluated the correlation between third molar presence and lower incisor crowding in 91 subjects. They reported that although differences between the groups were not statistically significant, a greater number of

Table 1 - Differences of the measurements of retromolar region (Ganss ratio) in "crowded" and "normal" clusters.

Parameters	Rank Sum Crowding	Rank Sum Normal	U	Z	p-level	Valid N Crowding	Valid N Normal
Ganss Ratio A/B _r	1843,00	5417,00	13,00	-9,38	0,000	60	60
Ganss Ratio A/B _i	1851,000	5409,000	21,00	-9,34	0,000	60	60

Table 2 - Differences between of the Angle A and Angle B/"crowded" and "normal" clusters.

Parametar	Rank Sum Crowding	Rank Sum Normal	U	Z	p-level	Valid N Crowding	Valid N Normal
Angle A _r	5310,00	1950,00	120,00	8,82	0,000	60	60
Angle A _i	5250,00	2010,00	180,00	8,50	0,000	60	60
Angle B _r	2731,00	4529,00	901,00	-4,72	0,000	60	60
Angle B _i	2495,00	4765,00	665,00	-5,96	0,000	60	60

Table 3 - Frequency and percentage distribution of gender

Gender	Crowding group		Normal group	
	Frequency	Percent	Frequency	Percent
Male	27	45,00	35	58,33
Female	33	55,00	25	41,67
Total	60	100,00	60	100,00

Table 4 - Age statistics

	Age in Years	
	Crowding group	Normal group
Number of subjects	60	60
Mean Age	18,05	18,87
Std. Deviation	1,57	1,52

tendencies for crowding in the mandibular anterior teeth were expressed in groups with third molars present than in groups with these teeth missing, supporting the concept of an anterior component of force.

Third molars generally erupt between 16 and 24 years of age, and the position of the mandibular third molar changes during the eruption and development period.^{26, 27} If space is insufficient, they can cause complications such as difficulties during their eruption, lower arch crowding, inflammatory processes, and temporomandibular joint dysfunction.²⁸⁻³⁰ Angulation of the third molar and the available space in the third molar region reportedly affect the possibility of third molar eruption.^{31,32} A large number of studies have measured third molar angulation and space on panoramic radiographs³³⁻³⁵ and have shown that panoramic radiograph are a reliable indicator in evaluating third molar angulations^{36,37}. Thus, we decided to use panoramic radiographs to determine the angulation and position of third molars. Niedzielska et al³¹ concluded that based on the measurement of the ratio, third molar angulation to the base of the mandible, and third molar to second molar inclination, it is

possible to predict lower third molar position in the dental arch and make an early decision concerning its removal or retention. Thus, in the present study, the angle between third molars and the mandibular plane is smaller in the crowding group than in the normal group with significant differences while the angle of third molar to second molar inclination is higher in the crowding group than in the normal group with significant differences. Through this data, the third molars in the crowding group were inclined mesially, and we expect that they will have the upright position of the eruption, and this shows it possible to exert mesial force in the teeth of the lower arch.

Ades et al.³⁸ conducted a long term study to determine the relationship between the third molars and changes in lower dental arch parameters. They concluded that the third molar removal to decrease or prevent incisor crowding might not be justified. Similarly, Hasegawa, et al.³⁹ found no significant correlation between the angulation of the lower third molar and the angulation of the other teeth in the lateral segment. Findings in our study show that there were statistically significant differences between lower arch crowding and third molar angulation and position. Niedzielska¹⁵ suggested that, when a sufficient space is available for the eruption of the third molars, the tooth assumes a normal position in the dental arch and does not cause displacement of the other teeth; conversely, when the space is deficient, third molars may aggravate dental crowding.

Zachrisson⁴⁰ reported that a mesially-directed force is an important cause of increased mandibular incisor crowding in early teenagers and young adults and that the presence of a developing mandibular third molar with insufficient space could be a cause of late mandibular arch crowding. This is in agreement with our study because at this age, we have an insufficient retromolar space since values of Ganss ratio were smaller in the crowding group when compared with the normal group, with significant differences, and this could be a contributing factor for lower arch crowding. All these measurements can be useful in providing information regarding future development of lower arch crowding. The early prediction of third molar eruption will be a great help and relief during orthodontics treatment planning and results in a timely decision for third molar removal.

Conclusion

The results of the present study have shown substantial evidence to assert that third molars are a contributing factor for lower arch crowding. Therefore, it can be concluded that there was a strong relationship between angulation and position of third molars and lower arch crowding

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MANDIBULAR ARCH DIMENSIONS IN RELATION TO ERUPTION OR IMPACTION OF THIRD MOLARS

DIMENSIONET E HARKUT MANDIBULAR NE RAPORT ME ERUPTIMIN APO IMPAKSIONIN E MOLAREVE TE TRETË

Selmani E. M.¹, Gjorgova J.², Selmani E. M.³, Duci Sh.⁴

¹Dental University Clinical Center of Kosova, Department of Orthodontist.

²Dental University Clinical Center of Macedonia, Department of Orthodontist.

³Dental University Clinical Center of Kosova, Department of Prosthetic.

⁴University Clinical Center of Kosova, Clinic of Plastic Surgery.

Corresponding autor e-mail: dr.moza_79@hotmail.com

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ABSTRACT

Objective: The aim of this study is to determine the relationship between lower arch dimensions and the presence of third molars impaction and eruption.

Methods: The study groups consisted of 240 subjects aged 16 to 21 years. For the measurement of arch length and arch width were used Korkhaus caliper. Arch perimeter was measured by Lundstrom method's on the right and left side, using Vernier caliper.

Results: Based in our study data the impacted group was found to have shorter length arch and narrower width when compare with erupted group, and also arch perimeter was found greater in impacted group with significant differences.

Discussion: The changes in the dental arch dimensions that occur as a result of impacted third molars and treatment are of interest to the orthodontist and require careful consideration during treatment planning

Conclusion: Presence of impacted and erupted third molars were strongly correlated with lower arch dimensions.

Key words: Arch dimensions, impacted third molars, erupted third molars

INTRODUCTION

The start of the 21st century and the information revolution has pushed the human population towards maximizing aesthetics, which has become the main concern for seeking orthodontic treatment.¹ In orthodontics, the role of the third molars is whether they can contribute to the development of malocclusion or relapse after orthodontic treatment. The size and shape of the arches have considerable implications in orthodontic diagnosis and treatment planning, affecting the space available, dental esthetics, and stability of the dentition.²

Information regarding mandibular arch dimensions in human populations is important to clinicians in orthodontics, prosthodontics, and oral surgery. It is also of interest to anthropologists and other students of human oral biology.³

Arch dimension is explained by arch width, arch length and arch perimeter. Arch perimeter is the distance from the mesial contact of one first permanent molar to its antimere as measured through the contact points or buccal cusp tips of all of the intervening teeth, ignoring

those teeth that are malpositioned or blocked out so that the measurement represents an ideal arch form.⁴

Investigators have studied the growth of arch widths in persons with normal occlusion, and compared these values with those of different malocclusion samples.^{5,6}

Crowding of the teeth, the most common type of malocclusion at present undoubtedly is related in part to the continuing reduction in jaw size in human evolutionary development. Jaw dimension do seem to have a strong genetic control, and the transverse dimensions directly affect the amount of space for the teeth.⁷

Some investigators^{8,9,10} suggests that other morphological characteristics such as tooth shape and arch dimensions play an important role in space discrepancies and these parameters have great implications in orthodontic diagnosis and treatment planning.

Most of previous studies in the twentieth century suggested that lower third molars have a large influence on dental crowding and these teeth cause pressure from the back of the arch.¹¹⁻¹⁵

However, recent studies indicate the contrary: lower third molars did not exert any significant influence on the length and width of the dental arches and did not contribute to incisor crowding.¹⁶⁻¹⁹

Also lower third molars cause little or no influence in the mesiodistal angular positioning of the teeth in lateral segment.²⁰

Disproportionate sizes of the teeth, arch, and jaws are influenced by both environmental factors and genetic make-up of the individual.^{21,22}

Therefore the aim of this study is to determine the relationship between lower arch dimensions and the presence of third molars impaction and eruption.

METHODS AND SUBJECTS

This is retrospective study conducted in the Department of Orthodontics, Dental University Clinical Center, Kosovo. The study groups consisted of 240 subjects aged 16 to 21 years, with average age of 18 years. The sample was divided into two groups: 120 clusters with erupted third molars comprised 70 male and 50 female with mean age 18, 17 years, whereas 120 clusters with impacted (unerupted third molars) comprised 54 males and 66 females with mean age 18, 05 years. Ethical clearance was

obtained from the Ethics Committee and the participants also gave informed consent.

Subjects with Angle Class I molar relationship, complete lower dental arch (including unerupted third molars) with good state of care were included in the study.

An impacted tooth (unerupted third molars) is one that is not in the normal upright position, such that the occlusal surface of its crown does not reach the occlusal level line of the dentition, after the processes and stages of eruption are completed.²³

The erupted group was selected from the students and also from the patients who have made orthodontic examination rather than orthodontic treatment with normal transversal relationship and optimal intercuspitation (Fig.1).

Subjects of impacted group were assessed from patients mainly by clinical evaluation (Fig.2). The mandibular dental arch was examined clinically, each subjects had taken alginate impressions to allow casting of study models. The following measurements were taken on both sides of the dental arch:

1. ARCH LENGTH: (a) distance between mesial contact point of medial incisors and the middle point of the mesial surface of the lower first molar; (b) distance between mesial contact point of the medial incisor and the middle point of the canine distal surface; (c) distance between the middle point of the mesial surface of the canine and the middle point of the distal surface of the lower first molar; (d) distance between the mesial contact point of the lateral incisor and the middle point of the distal surface of the first premolar.

These measurements were made in segments of anterior and posterior for right and left sides (Fig.1).

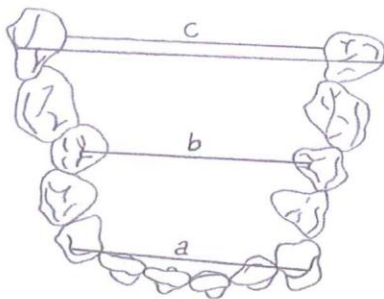
Three dimensions of the six were used by Lavelle and Foster 24 however dimension (d) and method of measurements of the arch width were used by Niedzielska et al 25. For the measurement of arch length and arch width were used Korkhaus caliper (Fig.2).

2. ARCH WIDTH : (a) distance between the canine cusp; inter-canine width, (b) distance between the second premolars measured in the middle of the central fissure; inter-premolar width, (c) distance between the buccal surfaces of the lower second molars + distance between the lingual second molar surfaces, divided by two; inter-molar width (Fig.3)

Fig.2. Korkhaus Caliper



Fig.3. Measurements of arch width: a- intercanine width, b-interpremolar width, c-intermolar width(modified by Niedzielska et al ²⁵).



3. ARCH PERIMETER was measured by Lundstrom ²⁶ method's on the right and left side, using Vernier caliper with an accuracy of 0,01 mm (Fig.4). The arch perimeter measurements were made from the distal aspect of the permanent first molar on one side to the distal aspect of permanent first molar on the other side. Lundstrom segmental analysis was carried out in the following method: The dental arch was divided in to six straight line segments, including two teeth per segment, starting from distal aspect of permanent first molar to the distal aspect of permanent first molar on the other side.

S1 arch segment: first molar and second premolar of the right side of lower arch; S2 arch segment: first premolar and the canine of the right side; S3 arch segment: lateral and central right incisors; S4 arch segment: central and lateral left incisors; S5 arch segments: canine and first premolar in the left side;

S6 arch segment: second premolar and first molar in the left side of the lower arch.

Figure 4. Vernier Caliper

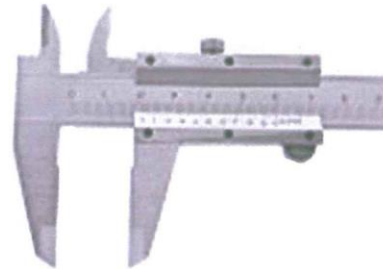
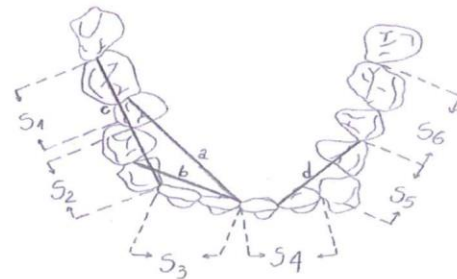


Figure 5. Measurements of the lower arch dimensions: Length: a, b, c, d; Arch perimeter: S1-S6.



Differences of analyzed parameters between two independent samples (right side and left side) impacted and erupted group on the series with numerical characters were tested by Mann-Whitney U test (Z/U).

RESULTS

In table (1) was shown differences between distances of analyzed parameters of the arch length (a, b, c, d) in the right and left side in the subjects of impacted and erupted groups.

Average distances of all parameters of arch length in the right and left sides on erupted group were higher in relation with corresponding parameters of the subjects in the impacted group.

Differences of parameters a/r, b/r, in the right side and b/l in the left side on erupted group were significantly higher in relation with impaction group, while the other parameters of arch length were insignificantly higher when compare with impacted group.

Table 1. Differences of lower arch dimensions between crowding and normal group / Length of the lower arch.

Parameters	Rank Sum Impacted	Rank Sum Erupted	U	Z	p-level	Valid N Impacted	Valid N Erupted
a/r	13107,50	15812,50	5847,50	-2,52	0,01	120	120
b/r	12331,00	16589,00	5071,00	-3,96	0,000	120	120
c/r	13964,00	14956,00	6704,00	-0,92	0,36	120	120
d/r	14503,00	14417,00	7157,00	0,08	0,94	120	120
a/l	13645,00	15275,00	6385,00	-1,52	0,13	120	120
b/l	11978,50	16941,50	4718,50	-4,61	0,000	120	120
c/l	14783,50	14136,50	6876,50	0,60	0,55	120	120
d/l	14579,50	14340,50	7080,50	0,22	0,82	120	120

In table (2) was shown differences of analyzed distances of the arch width (a, b, c) and arch perimeter (S1-S6) between erupted and impacted group.

Average distances of the width parameters (a, b, c) of the erupted group were significantly higher in relation with

corresponding distances in the subjects of the impacted group; $p < 0,001$.

Average values of segments S1-S6 in the right and left side of the arch in subjects of the impacted group were higher when compare with erupted group, differences were significant $p < 0,001$.

Table 2. Differences between distances of the width and arch perimeter of the in impacted group and erupted group

Parameters	Rank Sum Impacted	Rank Sum Erupted	U	Z	p-level	Valid N Impacted	Valid N Erupted
a	11072,50	17847,50	3812,50	-6,30	0,000	120	120
b	11301,00	17619,00	4041,00	-5,87	0,000	120	120
c	10067,50	18852,50	2807,50	-8,17	0,000	120	120
S1	16589,50	12330,50	5070,50	3,96	0,000	120	120
S2	16494,00	12426,00	5166,00	3,78	0,000	120	120
S3	15597,50	13322,50	6062,50	2,12	0,03	120	120
S4	16010,00	12910,00	5650,00	2,88	0,004	120	120
S5	17011,00	11909,00	4649,00	4,74	0,000	120	120
S6	17602,00	11318,00	4058,00	5,84	0,000	120	120

DISCUSSION

According to Haralabakis is doubtful whether the third molar in reality interferes with the occlusion of the teeth and is responsible for disturbing the structural arrangement of the alveolar bone of the jaws or the balance of inclination of the long axis of the tooth, though he observes that the third molar poses a problem for the orthodontist.²⁷

Many factors may influence development or changes in the anterior alignment during growth. Factors may include: growth²⁸, erupting third molars²⁹, anterior component of force during third molars eruption²⁵, presence or congenitally missing third molars¹⁷.

Some investigators⁹⁻¹⁰ suggests that other morphological characteristics such as tooth shape and arch dimensions play an important role in space discrepancies and these parameters have great implications in orthodontic diagnosis and treatment planning. In this study, we aimed to determine the differences between groups with erupted and impacted third molars in terms of lower arch dimensions to better understand the morphological relationships of these parameters with dental malocclusion.⁹

It seems to be generally expected that dental arch length starts to decrease after eruption of the second molars and incisor irregularity appears during teenage years.^{30,31}

Bjork and Skieller³² seemed contradictory in saying: 'More probably the shortening of the arches is to some extent due to the pressure exerted by the erupting second molars. The part played by the third molars in this connection, however, cannot be judged reliable until completion of jaw growth and root development.'

According to M Waheed-ul-Hamid³³ arch length was found greater in noncrowded arches as compared to crowded group and the differences were statistically significant ($P < 0.05$).

In our study the impacted group was found to have shorter length arch and narrower width when compare with erupted group and well aligned teeth of the lower arch.

Burns et al were used measurements of tooth size and arch dimensions on lower casts of adult subjects who had bilaterally impacted lower third molars and bilaterally erupted third molars. Their study analysis showed that differences between impacted and erupted group were small but impacted group tend to have longer arches with greater circumference.³⁴ This is compatible with our findings because the arch perimeter was found significantly greater in impacted group when compared with erupted group and this show us the importance of the relation between the two groups.

According to Al- Zubair³⁵ studying the descriptive analysis for the mandibular arch width revealed that the strongest relationships were observed between the inter-canine distance and total arch length, molar arch length and anterior arch length. He concluded that among studied mandibular dimensions in subjects with normal dento-skeletal relationship, the inter-canine distance showed the strongest linear relationship with the mandibular arch sizes.

Sanin and Savara³⁶ evaluated 150 children and reported that children without crowding in the permanent dentition had larger anterior and posterior widths of the mandibular dental arch. Nordeval et al³⁷ compared 27 adults with ideal occlusion with slight mandibular crowding and reported no differences in intercanine width between the groups.

The changes in the dental arch dimensions that occur as a result of impacted third molars and treatment are of interest to the orthodontist and require careful consideration during treatment planning. In addition, it is important to consider that changes, even though

statistically significant, would be unnoticeably small on examination and not visual comparison but such a change is only detectable on measurements. This leads us to explain that such measurements are very important in orthodontic treatment planning.

CONCLUSION

The following conclusions were made from present study:

1. Presence of impacted lower third molars shows a strong tendency to decrease the length of the arch.
2. Narrower arch width was predisposed in impacted group of third molars when compare with erupted group and well aligned teeth of the lower arch with significant differences.
3. Arch perimeter was found significantly greater in impacted group when compared with erupted group.

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DIMENSIONET E HARKUT MANDIBULAR NE RAPORT ME ERUPTIMIN APO IMPAKSIONIN E MOLAREVE TE TRETË

Selmani E. M.¹, Gjorgova J.², Selmani E. M.³, Duci Sh.⁴.

¹Qendra Klinike Stomatologjike Universitare e Kosovës, Klinika e Ortodontisë.

²Qendra Klinike Stomatologjike Universitare e Maqedonisë, Klinika e Ortodontisë.

³Qendra Klinike Stomatologjike Universitare e Kosovës, Klinika e Protetikës.

⁴Qendra Klinike Universitare e Kosovës, Klinika e Kirurgjisë Plastike.

ABSTRAKTI

Qëllimi i punimit është që të përcaktoj marrëdhëniet në mes të dimensioneve të harkut të poshtëm dhe prezencës së molarëve të tretë të impaktuar apo të eruptuar.

Metoda: Grupet e studimit përbëhen nga 240 subjekte të moshës 16 deri 21 vjeç. Për matjen e gjatësisë dhe gjërësisë së harkut është përdorur kompas Korkhaus. Perimetri i harkut është matur me metodën e Lunstrom-it në anën e djathtë dhe të majtë duke përdorur kompasin Vernier.

Rezultatet: Bazuar nga të dhënat e punimit është gjetur se në grupin e molarëve të impaktuar gjatësia e harkut është më e shkurtër dhe gjërësia e harkut është më e ngushtë kur janë krahasuar me grupin e molarëve të eruptuar, dhe gjithashtu perimetri i harkut është gjetur më i madh në grupin e molarëve të impaktuar me dallim signifikantë.

Diskutimi : Ndryshimet në dimensionet e harkut dentar që ndodhin si pasojë e impaktimit të molarit të tretë dhe trajtimit janë me interes për të ortodontin dhe kërkon vëmendje të veçantë gjatë planifikimit të trajtimit

Konkludimi: Prezenca e molarëve të impaktuar dhe të eruptuar janë të lidhura apo ekziston një korelacion i fortë me dimensionet e harkut të poshtëm.

Fjalet kyç: Dimensionet e harkut, molaret e trete te impaktuar, molaret e trete te eruptuar.

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Approaches And The Management Of Patients In Oral Surgery During COVID- 19 Pandemic Kosovo Region.

Bukleta D¹, Cena Muhaxheri G², Krasniqi E³, and Selmani Bukleta M^{4*}.

¹Department of Oral Surgery. School of dental medicine. College of Rezonanca. Kosova.

²Department of Oral Medicine. School of dental medicine. College of Rezonanca. Kosova.

³Private dental practice. Pejë. Kosova.

⁴Department of Prosthodontics. School of dental medicine. College of Rezonanca. Kosova.

ABSTRACT

Treatment of Covid 19 positive patients in Oral surgery has a very high risk transmission of SARS Covid 2 because it is associated with the specific nature of dental interventions, which includes: face to face communication, usage of sharp instruments, saliva and other oral fluids that the patient might contain. Aspect discussions, data collection and the purpose of the review of managing in oral surgery during the COVID-19 pandemic is to provide protection for both, technical and human resources and to avoid unnecessary exposure to infected patient. The research methods are used in electronic bibliographic databases of relevant scientific literature such as, world health organization, Scisearch, etc. Practical Guidelines for Dentistry professional during the pandemic COVID-19 virus should be adjusted in accordance with the directions of the Center for Diseases Control and Prevention. Administration for Occupational Health and Safety, American Dental Association and Alberta Dental Association & Collage, Canada.

Keywords: Covid-19, Sars-Cov-2, Pandemic, Protective Equipment, Oral Surgery, Practical instructions for Dentistry Professionals.

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**Corresponding author*

INTRODUCTION

Treatment of Covid Positive Patients in Oral Surgery is associated with a high risk of transmission of COVID – 19, as it is associated with the specific nature of Dental Interventions which include face-to-face communication, use of sharp instruments and extensive exposure to saliva, and other oral fluids of the patient. It was obvious already at a very early stage of the pandemic that healthcare workers are affected in 29% of the cases, which is disproportionately high. During the SARS-CoV-2 pandemic, the specialty must organize patient treatment in such a way that infection transmission is reduced to a minimum, while all relevant treatment options are at hand to provide adequate patient care. Concepts have to be developed that take into account the possible need for triaging patients according to the degree of urgency of treatment in the field of Oral Surgery. Currently, specific guidelines and recommendations are just evolving (1).

MATERIALS AND METHODS

The research method is the electronic bibliographic databases of the relevant scientific literature such as: MEDLINE, EMBASE, CINAHL, ScienceDirect and Google search engine.

Literature searches were conducted for English language articles using index terms (e.g., Medical Entities Titles [MoH], Emtree) and free text keywords to identify acceptable reports.

The search terms used were "coronavirus 19 disease, COVID-19, severe coronavirus severe respiratory syndrome 2, SARS-CoV-2, transmission, pandemic, oral surgical procedures, oral surgery, dental equipment, personal protective equipment, prevention and infection control "The last search was conducted on 23.03 2021."

RESULTS

It is important to have a clear and well-communicated concept of the benefits of procedures in Oral Surgery.

Although it may be easy to distinguish between elector and emergency procedures, the boundaries between intermediate priority and urgent and emergency interventions can sometimes be blurred.

Therefore, relevant guidelines may vary between institutions or may depend on the individual characteristics of an institution.

If a conservative therapy is equivalent to surgery, it should be preferred at times with limited capacity for surgery.

Therefore, following the protective protocols in the COVID-19 crisis is of great importance for a dental environment where it can be done as much as possible to prevent the spread of the virus as well as to perform the necessary Dental services.

WHO through "Open data Kosova" (update) daily presents statistical data (collected by the National Institute of Public Health of Kosovo) on the number of infected persons and their gender within 24 hours (2).

The data presented in figure 1 are official data from the National Institute of Public Health of Kosovo.

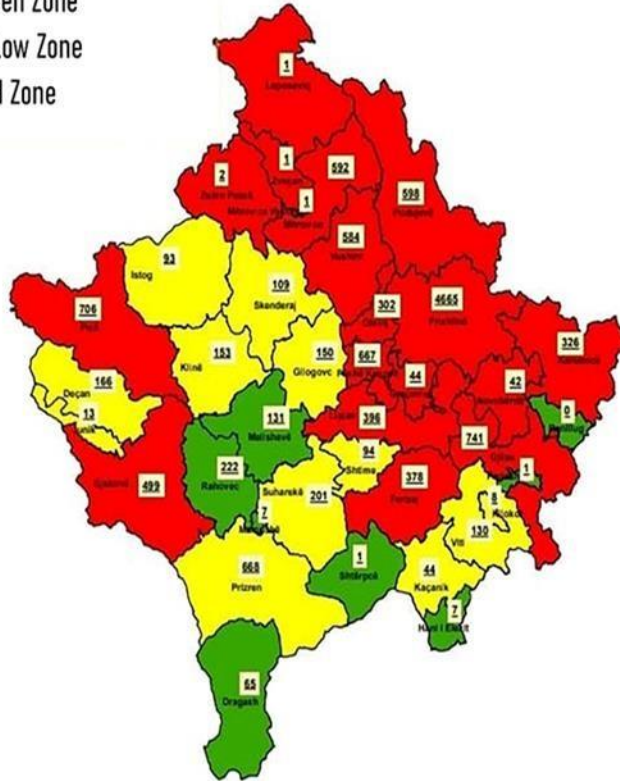
The following data, such as preventive measures and acquaintance with the patient's status, can be obtained through telephone or other electronic equipment's.

Epidemiological overview of active cases with COVID 19 in the Republic of Kosovo from 13 March - 22 March 2021

Day: 375	Infected during the last 24 hours	Total infected	Recovered during the last 24 hours	Total recovered	Dead during the last 24 hours	Total dead	Active cases	Tested during the last 24 hours	Total tested	Infected in 100k habitants	Recovered in 100k habitants	Dead in 100k habitants	Population
Cases	705	83777	522	69123	10	1786	12808	3922	393713	4698	3879	100	1782115
%	17,98	21,26	0,62	82,57	0,01	2,13	15,30	0,22	22,09	0,26	0,22	0,01	

Municipality	Infected	Recovered	Dead	Active cases	Ratio cases to 100k habitants
Prishtina	32755	27823	255	4665	2151
Gjilan	4634	3754	109	741	972
Prizren	4027	3153	206	668	346
Peje	3897	3092	99	706	719
Fushë Kosovë	3870	3154	69	667	1712
Mitrovicë e Jugut	3164	3054	49	692	660
Podujevë	3055	3002	56	538	734
Ferizaj	3634	3145	105	378	340
Gjakovë	3588	2969	120	499	537
Vushtrri	2789	2132	73	584	942
Lipjan	2436	2006	34	396	690
Gjogj	1489	1291	48	150	248
Obiliq	1402	1074	28	302	187
Viti	1396	1211	55	130	274
Shkup	1263	1071	71	201	351
Kamenice	1166	703	47	326	172
Skenderaj	1048	909	30	109	208
Deçan	968	777	25	166	393
Rahovec	959	675	64	222	294
Malishevë	932	754	47	131	234
Kiçine	900	711	36	153	387
Shitme	701	596	21	94	348
Istog	676	553	30	93	277
Kaçanik	481	421	16	44	128
Dragash	373	275	33	65	191
Gracanicë	384	39	1	44	361
Novobërdë	127	84	1	42	590
Hani i Elezit	95	86	2	7	70
Jurine	92	77	2	13	204
Mamuzhe	63	50	6	7	120
Kilicët	58	50	0	8	296
Zubin Potok	58	54	2	2	30
Mitrovicë e Veriut	49	45	3	1	8
Zveçan	34	32	1	1	14
Shitërracë	31	27	3	1	15
Leposaviq	26	21	4	1	8
Ranilug	8	8	0	0	0
Partesh	5	4	0	1	5
Total	83777	69123	1786	12808	

Green Zone
Yellow Zone
Red Zone



Total cases hospitalized with COVID 19 on 23.03.2021		
Confirmed with COVID 19	Cases	%
Hospitalized	706	9,29
O ₂	669	94,76
CPAP	25	3,54
Respirator	11	1,56

Source: MSH, IKSH, AKK and ASK

1:500000

Figure 1: Number of infected residents with COVID -19 by Cities of Kosovo. Graphs and Statistic updated on 03/23/2021 07:25:10.

Urgency and Emergency in Oral Surgery

Dental urgency refers to conditions that require necessary interventions to stop major, intense pain and infections (3).

These conditions include:

1. Severe pain caused by inflammation of the pulp & apical periodontium;
2. Pericoronitis;
3. Postoperative osteitis, alveolitis.

Guide for applying dental interventions to patients with COVID 19:

- Localized abscess, pain and swelling;
- Soft and hard tissue trauma;
- Tooth traumas such as: avulsion, luxation;
- Dental interventions that precede medical interventions;
- Tissue biopsy;
- Patient interventions should be minimally invasive.

From rapid measures it is important to distinguish non-emergency dental interventions such as:

- First or systematic visit;
- Application of regular preventive measures;
- Removal of soft and hard deposits;
- Orthodontic interventions, except when they do not cause pain, infection or trauma;
- Asymptomatic tooth extraction;
- Restorative procedures which include asymptomatic carious teeth;
- Aesthetic dental interventions.

Measures to prevent health professional infection and nosocomial transmission at dental clinics (4):

- Obligatory, only one patient and / or attendant can stay in the waiting room;
- At the entrance / reception, there should be disinfectant and instructions for patients on how to use;
- All magazines, toys, etc. should be removed as a precaution against contamination / pollution;
- Given that the coronavirus stays on the surface for 24+ hours, it is very important to keep all surfaces clean and disinfected. This applies to the dental office, toilet, waiting area, and it is especially important to disinfect the operating room as often as possible;
- Commonly used disinfectants, such as 0.1% sodium hypochlorite or 62% -71% ethanol, have been proven to be very effective;
- The ventilation of the environment should be as natural as possible and the ventilation with air conditioner should be avoided;
- Extra care for instruments by applying the principles of Aseps and Antiseptics (2).

Precautions in the operating room

Prior to taking a patient to the surgical room, a test for SARS-CoV-2 should be performed. An emergency patient that leaves no time for testing should be treated as being infective.

In the operating room, negative pressure must be established with the aim to reduce dissemination of the virus. Before entering the operating room, every staff member needs to put on personal protective equipment. Besides an FFP3 respirator, it is also important to wear a face shield. At any time, the number of staff members in the operating room should be minimal.

The personal protective equipment of the surgical team should be completed by a water-tight sterile gown. Whenever possible an experienced team should perform the surgery. During the procedure, leaving or entering the operating room should be limited to a minimum.

If an extra oral approach is a relevant alternative to an intraoral one, it should be preferred. Reducing aerosol formation to a minimum should be a priority. Excessive water cooling for dental drill, saws, ultrasonic devices, and piezoelectric devices should be avoided. Instead of drilling screw holes, self-drilling screws should be used. The use of osteotomies should be considered wherever possible. Electric cautery should be avoided or performed with the lowest power possible and a smoke evacuation system (1).

Some dental procedures favor the creation / spread of aerosols, which can be potentially hazardous to Covid-19 transmission. Therefore, it is recommended that dentists limit such procedures to protect patients, staff and themselves. Pandemic Protective Equipment (PPEs) that do not create aerosols.

Current standards for infection prevention and control are applied with appropriate personal protective equipment: gloves, surgical mask, and goggles.

PPEs that create aerosols for dental procedures that create aerosols, in addition to current standards for the prevention and control of infection, additional personal protective equipment is needed: protective clothing, gloves, mask N-95 / respirator, suitable goggles or face shield (4).

Additional measures before treatment:

1. Use 1% 5cc hydrogen peroxide for rinsing for 30 seconds before examining the oral cavity;
2. Use Cofferdam for isolation;
3. Use high volume aspirators during dental procedures (4).

DISCUSSION

The COVID-19 pandemic puts pressure on the healthcare system, because of this there is a need for continuous adaptation of recommendations and guidelines.

In oral surgery elective procedures such as urgent and emergent procedures are performed.

When treating patients during the SARS-CoV-2 pandemic, a major issue is disease transmission from the patient to the medical staff. Patients with symptomatic COVID-19 should be treated in the field of oral and maxillofacial surgery only when the indication is urgent or an emergency. Symptomatic patients are a major source of viral transmission and therefore must be treated in an adequate infrastructure with personal protective equipment.

Additionally, asymptomatic patients and patients undergoing the incubation period can be carriers of SARS-CoV-2 and can be responsible for infection transmission. It is even debated whether patients in the recovery phase are potential sources of virus transmission. As with every other infectious disease, the approach to the situation must be, that the patient must be considered infective as long as the opposite is not proved. Obviously, there is a need for sensitive, reliable, and rapid testing of patients who enter the private practice or the hospital for an urgent or emergency treatment. The potential patients should be advised to first opt for a consultation on the telephone before they come for a face-to-face consultation. A relevant number of issues can be clarified by telephone, helping to avoid face-to-face contact (1).

CONCLUSION

The major aim is to protect patients as well as the medical team from unnecessary infection and to keep the healthcare system running effectively.

Although it might be easy to distinguish between elective and emergency procedures, the boundaries between interventions of intermediate and urgent priority might be blurred sometimes. Therefore, respective guidelines might differ between institutions or might be dependent on individual characteristics of an institution.

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RESEARCH ARTICLE

Comparison Of The Effectiveness Of Antibiotic Prophylaxis In Oral Surgery Department.

Bukleta D¹, Rexhaj E², Nila A², Berisha M², and Selmani-Bukleta M^{3*}.

¹Department Of Oral Surgery. School Of Dental Medicine. College Of Rezonanca. Kosova.

²Dental Polyclinic Oral Surgery. Peje. Kosova.

³Department Of Prosthodontics. School Of Dental Medicine. College Of Rezonanca. Kosova.

ABSTRACT

Antibiotic prophylaxis means the administration of antibiotics before surgical procedures in those cases when there is a possibility of postoperative complications or the possibility of transient bacteraemia. The methods are based on electronic literature, scientific papers which are researched in MEDLINE, PUBMED, LIBGEN, SCI-HUB and Google search engine. These studies were selected based on 2009 to the present time, surgical intervention, type of therapy and antibiotic dose. Three surgical procedures such as the extraction of third molar, the placement of dental implant and infective endocarditis has been researched with similar studies where a comparison has been made between them and the prescribed antibiotic therapy to see which of the therapies is more effective. Almost all studies have recommended antibiotic prophylaxis in these surgical procedures. In all three surgical procedures antibiotic prophylaxis is recommended in order to reduce the chances of postoperative complications, provided that these patients have a higher risk of these complications.

Keywords: antibiotic prophylaxis, dental implant, third molar surgery, oral surgery, endocarditis.

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**Corresponding author*

INTRODUCTION

The oral cavity contains a microflora with about 700 different microbial species.

The purpose of Antibiotic Prophylaxis in dentistry is to protect patients with a certain specific risk from local microbial contamination or systemic spread of oral bacteria during dental procedures which induce bleeding and a transient bacteraemia.

Dental procedures can cause a transient bacteraemia, with higher values in oral surgery (third molar extraction, periodontal surgery) (1). But is very important that the prescription of antibiotics should always be done after we have seen the general condition of the patient and see if there is an allergy to antibiotics (2).

Antibiotic prophylaxis to have effective results, we must adhere to antibiotic administration protocols such as determining the correct antibiotic and at the time of surgery, the dose of antibiotic should be present in the systemic circulation in order to prevent postoperative complications. The extraction of third molar is one of the most common surgical interventions requiring the need for antibiotic prophylaxis (3). The most common complications that may occur after third molar surgery are swelling, pain, trismus, infection etc. (4).

Dental implant placement is another surgical procedure that requires antibiotic prophylaxis to prevent postoperative infections and increase the success of implant longevity (5). Infective endocarditis (IE) also is a disease with high mortality. Most guidelines recommend giving antibiotics as prophylaxis to prevent IE in patients with specific predisposing cardiac conditions (6).

MATERIALS AND METHODS

The research method is the electronic bibliographic databases of the relevant scientific literature such as: MEDLINE, PUBMED, LIBGEN, SCI-HUB and Google search engine.

The first surgical procedure that we have been studied is the prophylaxis before and after surgical intervention related to the third molar. The research from Martin-Ares et al (7) has divided patients into two groups. One group of patients was administered 750 mg amoxicillin every 8 hours for 5 days after surgery and the second group placebo which means the administration of anti-inflammatory 50 mg diclofenac sodium every 8 hours for 4 days after surgery and analgesic 575 mg magnesiummetamizol every 6-8 h in cases when pain occurs.

The research from Milani et al is related also with the third molar (8). Here the patients were divided into three groups, the first group used 1 g amoxicillin 1 h before surgery + 500 mg every 8 h for 7 days. The second group used 1 g amoxicillin 1 h before surgery plus placebo, every 8 h for 7 days, while the third group used placebo 1 h before surgery and 500 mg every 8 h for 7 days.

The second surgical procedure that we have been studied is for the prophylaxis before the surgical procedure of the placement of dental implant. In the both mentioned studies, patients were divided into two groups. In the research of Arduino et al (9) the comparison of preoperative and postoperative antibiotics is made to see which is more effective in dental implants. The first group of patients were administered 2g of amoxicillin orally 1h prior to implant placement and the second group were administered 2g of amoxicillin orally 1h prior to implant placement; 1g in the evening of the day of surgery and 1g twice a day for 2 days following surgery.

In the research from El-Kholy (10) antibiotics were administered before and after surgery and a comparison was made to see as to which group of therapy was most effective. The first group of patients were administered 1g of amoxicillin preoperatively and in the second group were administered 1g of amoxicillin preoperatively and the antibiotic continued after surgery for three days.

The third surgical procedure that we have studied is the prevention of infective endocarditis before surgical intervention by prescribing antibiotics as prophylaxis. Articles are from American Heart Association and European Society of Cardiology (11, 12).

Both researches that we have been studied related with infective endocarditis, point that only a small number of cases of infective endocarditis might be prevented by antibiotic prophylaxis. Recent studies suggest prescribing of antibiotics only for patients with the highest risk for infective endocarditis. In both studies the preferred dose was 2 g amoxicillin 30-60 min before the procedure.

RESULTS

In this study, three surgical cases were researched and compared with similar studies. These studies were selected based on 2009 to the present time, then based on the surgical procedure, type and dose of antibiotics.

In table 1 were presented two cases with surgical intervention in the third molars. Here is a comparison between similar studies to determine which type of therapy is most effective in preventing the occurrence of infection after surgery in the third molars.

In the first case the patients were divided into three groups in which different therapies are given in order to compare which type of therapy is most effective in the case of third molar surgery. In the second case the patients are divided into three groups with different therapies and the comparison is made to determine which of the therapies is more effective.

In table 2 were presented two cases for surgical interventions of dental implants placement. Here is a comparison of these two studies with relevant therapy in order to prevent postoperative complications.

In the first case patients are divided into two groups and the comparison of preoperative and postoperative antibiotics is made, which is more effective to prevent infections during the procedure of dental implants placement. In the second case patients were divided into two groups and antibiotics were administered before and after surgery and a comparison was made to see which group of therapy was most effective.

Table 3 presents two researches on infective endocarditis with doses of antibiotics for prophylaxis.

The first case is from the organization of the American Heart Association while the second case is from the organization of the European Society of Cardiology.

These researches show the recommendations for antibiotic prophylaxis.

Transient bacteremia appears while working with teeth and periodontal tissues, and there is a wide variation in reported frequencies of bacteremia in patients resulting from dental procedures: tooth extraction (10% to 100%), periodontal surgery (36% to 88%), teeth cleaning (up to 40%), rubber dam matrix/wedge placement (9% to 32%), endodontic procedures (up to 20%), scaling and root planning (8% to 80%) (11).

DISCUSSION

The purpose of this study is to show efficiency of antibiotic prophylaxis in dental procedures, more precisely in three dental procedures which are: the extraction of the third molar, the placement of dental implants and infective endocarditis. In each of these surgical procedures a comparison was made with similar researches but having different therapies for antibiotic prophylaxis.

After detailed study related with antibiotic prophylaxis before and after surgical intervention related to the third molar, it has been confirmed that the group of patients who have used antibiotic therapy have shown higher efficacy in avoiding complications such as pain, inflammation etc. In other way it recommends that antibiotics should not be used for a long time because they can cause serious problems (7). However, they recommend that the use of antibiotics should not be done in cases when patients are healthy and adhere to strict antiseptic rules with good oral hygiene. Because the patient's defense mechanisms are sufficient and effective in preventing the occurrence of postoperative complications.

The use of antibiotics is indicated in patients with general immunosuppressive diseases such as uncontrolled diabetes, in patients who do not adhere to the strike rules of good oral hygiene (8). Based on the research related for prophylaxis before the placement of dental implants, can be concluded that it is sufficient to prescribe antibiotic preoperatively than to prescribe postoperatively (9, 10).

In other way prevention of infective endocarditis during oral surgery procedure it is very important. The ability of antibiotic therapy to prevent bacteremia associated with a dental procedure is controversial. Some studies reported that antibiotics administered before a dental procedure reduced the frequency, nature, and duration of bacteremia where as others did not (11).

Amoxicillin is the preferred choice for oral therapy because it is well absorbed in the gastrointestinal tract and reaches high serum levels. For individuals who are allergic to penicillins or amoxicillin, the use of cephalexin, clindamycin, azithromycin, or clarithromycin is recommended (11).

Table 1. Third molar case intervention and compared with similar researches

Authors	Year	Intervention	Dosage of antibiotics	Conclusions
Martin-Ares et al ⁷	2017	Third molar	Group 1- 750 mg amoxicillin p.o every 8 h for 5 days postoperative Group 2- Placebo	The first group has shown higher efficiency in presenting pain and inflammation that reduces the need for analgesics
Milani et al ⁸	2015	Third molar	Group 1- 1 g amoxicillin 1 h before surgery + 500 mg every 8 h for 7 days Group 2- 1 g amoxicillin 1 h before surgery plus placebo, every 8 h for 7 days Group 3- Placebo 1 h before surgery and 500 mg every 8 h for 7 days	No difference which group of antibiotics is better than the other in treatment

Table 2. Dental implant case intervention and compared with similar researches

Authors	Year	Intervention	Dosage of antibiotics	Conclusions
Arduino et al ⁹	2015	Dental implant	Group 1- 2 g of amoxicillin administered orally 1h prior to implant placement Group 2- 2 g of amoxicillin administered orally 1h prior to implant placement, 1 g the evening of the day of surgery and 1 g twice a day for 2 days following surgery	There are no statistical differences between the two groups but based on this study it is sufficient to prescribe 2 g of antibiotic preoperatively than to prescribe postoperatively
El-Kholey ¹⁰	2014	Dental implant	Group1 – 1 g amoxicillin preoperatively Group 2- 1 g amoxicillin preoperatively and the antibiotic continued after surgery for 3 days	No statistical differences between the two groups but based on this study 1 g amoxicillin preoperatively is sufficient

CONCLUSION

Based on the elaborations on this study, we can conclude that all three surgical procedures antibiotic prophylaxis is recommended in order to reduce the chances of postoperative complications, provided that these patients have a higher risk of these complications.

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KOLEGJI I SHKENCAVE MJEKËSORE “REZONANCA”
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Prishtinë

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NJOHURITË E INFERMIEREVE NË PARANDALIMIN
E INFEKSIONEVE SPITALORE NË SPITALIN
SHEIKH ZAYED VUSHTRRI

Dorëzuar nga

M.s.c. Mexhit Mustafa

Mentore

Prof. Dr. sc. Sadije Namani

Prishtinë, 2017

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REZYME

Infeksionet spitalore përbëjnë sfidën kryesore të shëndetësisë moderne. Ato janë treguesi më i mirë i cilësisë së shërbimeve të ofruara nga stafi infermieror në një institucion shëndetësor.

Qëllimi i punimit: është hulumtimi i njohurive të infermiereve për infeksionet spitalore, për masat e higjienës personale të personelit shëndetësor (larjen e duarve), për zbatimin e ciklit të dekontaminimit dhe menaxhimin e mbeturinave infektive në parandalimin e infeksioneve spitalore në Spitalin Sheikh Zayed në Vushtrri.

Materiali dhe metodat: Ky hulumtim paraqet një studim prospektiv të njohurive të infermiereve për infeksionet spitalore dhe parandalimin e tyre. Struktura e të anketuarve është e përbërë nga 40 Infermier/e të njësive të ndryshme të Spitalit Rajonal në Vushtrri.

Rezultatet: Nga 40 infermier/e të anketuar dominoi gjinia femerore (94%), grup-mosha nga 37 deri 45 vjece (62%), niveli i mesëm i edukimit infermieror (65%) si dhe përvoja e punës mbi 30 vite (32%).

Nga hulumtimi i bërë konstatoam se rreth 70% të stafit infermieror kanë njohuri të knaqshme për infeksionet spitalore dhe rolin e perkujdesjes infermiere në parandalimin e infeksioneve spitalore ndërsa vetëm 55% kanë njohuri të mjaftueshme për rrugët e përhapjes së infeksioneve spitalore. Mbi 77% të të anketuarve kanë njohuri të mjaftueshme për masat dhe parandalimin e infeksioneve spitalore. Për rolin e infermiereve në parandalimin e infeksioneve spitalore kanë njohuri mbi 70% e të anketuarve, ndërsa 94% mendojnë se praktika infermiere në parandalimin e infeksioneve spitalore ka nevojë për përmirësim. Për rolin e higjienës së duarve si masë kryesore në parandalimin e përhapjes së infeksioneve spitalore kanë njohuri vetëm 45% e stafit infermieror ndërsa vetëm 75% e të anketuarve i njohin teknikat dhe kohëzgjatjen e larjes së duarve. Rreth 75% e pjesëmarrësve në hulumtim, kanë njohuri të mjaftueshme për ciklin e dekontaminimit me theks të veçantë për sterilizimin dhe dezinfektimin si element kyç në parandalimin e infeksioneve spitalore ndërsa 90% e të anketuarve i klasifikojnë dhe menaxhojnë drejtë mbeturinat intrahospitalore.

Diskutimi Një tematikë e tillë rreth hulumtimit të njohurive infermiere në parandalimin e infeksioneve spitalore nuk është hulumtuar më parë në spitalin tonë, dhe kjo na pamundëson realizimin e krahasimeve të gjetjeve tona të hulumtimit me të dhëna paraprake në Spitalin e Vushtrrisë, por sipas krahasimit me literaturën e përdorur. Ajo që duhet potencuar shumë praktika infermiere duhet avancuar, prandaj hulumtimi ynë ishte shumë i qëlluar në mënyrë

që të vërehen lëshimet e mundshme që mund të ndodhin gjatë praktikave dhe punën infermierore.

Përfundimi. Punimi paraqet një pasqyrë reale të njohurive infermierore në spitalin Sheikh Zayed Vushtrri. Njohuritë e infermiereve për infeksionet spitalore dhe parandalimin e tyre janë të qëndrueshme, pasi që përgatitjet dhe njohuritë e tyre ndihmojnë në uljen e infeksioneve spitalore. Edhe pse studimi tregon se të anketuarit kanë njohuri rreth disa masave (ndërhyrjeve) infermierore në parandalim të infeksioneve spitalore, po ashtu tregon edhe për disa intervenime të cilat do duhej të përmirësohen.

Fjalët Kyçe: Njohurit infermierore, infeksionet spitalore, larja e duarve, dekontaminimi, menaxhimi i mbeturinave spitalore

Resume

Hospital infections are the main challenge of modern healthcare. They are the best indicator of the quality of services provided by nursing staff in a health institution.

Purpose of the thesis: is the research of nursing knowledge for hospital infections, the personal hygiene measures of the personnel (hand washing), the implementation of the decontamination cycle and the management of infectious waste in preventing hospital infections at Sheikh Zayed Hospital in Vushtrri.

Material and Methods: This research presents a prospective study of nursing knowledge on hospital infections and their prevention. The structure of the respondents is comprised of 40 Nurses of different units of the Regional Hospital in Vushtrri.

Outcomes: Out of 40 nurses surveyed dominated female gender (94%), age group 37 to 45 (62%), secondary nursing education (65%) and work experience over 30 years (32 %).

From the research conducted, we found that about 70% of nursing staff have satisfactory knowledge of hospital infections and the role of nursing care in preventing hospital infections, while only 55% have sufficient knowledge about the ways of spreading hospital infections. Over 77% of respondents have sufficient knowledge about the measures and prevention of hospital infections. The role of nurses in the prevention of hospital infections is knowledgeable over 70% of respondents, while 94% think that nursing practice in preventing hospital infections needs improvement. For the role of hand hygiene as the main measure in preventing the spread of hospital infections, only 45% of the nursing staff have knowledge, whereas only 75% of the respondents know the techniques and length of the handwashing. About 75% of the research participants have sufficient knowledge of the decontamination cycle with special emphasis on sterilization and disinfection as a key element in preventing hospital infections, while 90% of respondents classify and manage intraspinal waste.

Discussion. A topic like this relating the investigation of nurses knowledge in preventing the hospital infections has not been conducted before in our hospital, and this disables us from making comparisons of our findings with earlier data in the Hospital of Vushtrri, but comparing to the used literature. What should be highlighted is that the nurse practices should be advanced a lot, that's why our survey is very important in order to detect the mistakes that might happen during practices and in nursing.

Conclusion. This paper shows a true reflection of the nursing knowledge in the Sheikh Zayed Hospital Vushtrri. The knowledge of nurses regarding the hospital infections and their prevention in this hospital, are stable, since their preparation and their knowledge help in decreasing the number hospital infections. Even though the study shows the surveyed have knowledge about some of the nursing intervention in preventing the hospital infection, it also shows some of the interventions that should be improved.

Key words: Nursing knowledge, Hospital infections, hand-washing, hospital waste managing.

I. Hyrje

Ne tematikën që do ta shtjellojmë ka të bëjë me hulumtimin e njohurive, përvojat, si dhe punën e infermiereve dhe ndërhyrjet e tyre në parandalimin e infeksioneve spitalore në spitali rajonal të Vushtrrisë. Infeksionet spitalore përbëjnë sfidën kryesore të shëndetësisë moderne. Ato janë tregues të cilësisë së shërbimeve të ofruara të një spitali apo institucioni tjetër shëndetësor. Infeksionet spitalore i përkasin edhe infeksionet gastrogjenë, të cilat janë rrjedhojë e gabimeve të punëtorëve shëndetësorë gjatë procedurave të ndryshme diagnostiko-terapeutike. Në fund të shekullit XIX obstetri hungarez I. Semelvajsi i vuri themelet e disiplinës së kontrollit të infeksionit dhe epidemiologjisë spitalore duke rekomanduar metodën e pastrimit të duarve me ujë klorik për parandalimin dhe reduktimin e incidentesh së etheve puerperale në mesin e lehonave. Ndërkaq në fillin të shekullit XX fillon të zbatohet hapi i parë i kontrollit të infeksionit, në trajtë të izolimit të pacientëve me sëmundje ngjithëse në karantinë.

Për çdo vit infeksionet e shkaktuara gjatë kujdesit shëndetësor prekin qindra milionë njerëz përreth globit dhe janë kërcënimi kryesor për sigurinë e pacientit. Në vendet e zhvilluara, 5-15% e pacientëve të hospitalizuar marrin një apo më shumë infeksione gjatë kujdesit shëndetësor. Ndërkaq në vendet me burime të ultë dhe të mesme këto shifra janë disa herë më të larta. Këto infeksione shkaktojnë sëmundje edhe më serioze, zgjasin qëndrimin e pacientëve në spital, japin invaliditet afatgjatë, shtojnë koston për pacientët dhe familjet e tyre, ndikojnë në shpenzimet masive shtesë në sistemin e mbrojtjes shëndetësore dhe shpesh përfundojnë me vdekje. Infeksionet gjatë kujdesit shëndetësor shkaktohen nga shumë faktorë, të cilët ndërlidhen me sistemin dhe procesin e ofrimit të mbrojtjes shëndetësore. Ato, po ashtu, ndërlidhen edhe me sjelljet e njerëzve që kushtëzohen nga kufizimet edukative, ekonomike e politike në vetë shtetet, sistemet e mbrojtjes shëndetësore, e shpesh edhe në normat shoqërore dhe besimet. Për definimin e tyre në mbarë botën përdoren përkufizimet e infeksioneve spitalore sipas Qendrës për Kontroll të Sëmundjes-CDC (Centers for Disease Control) nga Atlanta [1, 2, 3].

Një prej sinonimeve për infeksionet spitaleve është edhe emërtesa “infeksione nozokomiale”; ajo është më e gjerë dhe përfshin edhe infeksionet në institucionet e tjera të përkujdesjes shëndetësore, siç janë qendrat e mjekësisë familjare, qendrat e rehabilitimit, mjekimin shtëpiak

dhe institucionet speciale shëndetësore. Sot në literaturën shkencore gjithnjë e më shumë po zë vend emërtimi infeksione të bashkëshoqëruara nga kujdesi shëndetësor (healthcare-associated infections). Infeksionet spitalore janë të njohura para 150 viteve. Të gjitha infeksionet origjina e të cilave është në spital nënkuptohen si infeksione intrahospitalore. Shpesh ende quhen infeksione nozokomiale ose shtëpiake.

Infeksionet spitalore mund të parandalohen nëse zbatohen masat adekuate mbrojtëse nga ana e të gjithë profesionistëve shëndetësor.

Parandalimi i infeksioneve spitalore kërkon ndërprerjen e zinxhirit të infeksionit spitalor në cilindo qoftë nivel të tij. Parandalimi i infeksioneve intrahospitalore po ashtu kërkon identifikimin dhe aplikimin e drejtë të ciklit të dekontaminimit, menaxhimin e drejtë të mbeturinave spitalore, zbatimin e saktë të procedurës së larjes së duarve nga ana e punëtorëve shëndetësor. Infeksionet spitalore përbëjnë kërcënim serioz për shëndetësinë moderne, si në vendet e industrializuara, ashtu edhe tek vendet në zhvillim. Për fat të mirë shumica e këtyre infeksioneve mund të parandalohen. Higjiena e duarve është masë parësore për uljen e infeksioneve. Higjiena e duarve nënkupton pastrimin e duarve në kohën e duhur, në mënyrën e duhur dhe në vendin e duhur, bashkë me të gjitha masat e tjera të rëndësishme të parandalimit dhe kontrollit të infeksionit që kryhen në praktikën e përditshme të kujdesit shëndetësor. Higjiena e duarve duket një masë e thjeshtë, por në mbarë botën problem të madh përbën mungesa e aderentës me udhëzimet për higjienë tek punëtorët shëndetësorë [1, 2, 3, 4].

I. 1 Infeksionet Spitalore

Infeksioni spitalor përkufizohet si infeksion që shfaqet te një pacient gjatë kujdesit shëndetësor në spital apo në ndonjë institucion tjetër shëndetësor; infeksioni i tillë nuk ka qenë prezent në momentin e pranimit të pacientit në spital e as në fazën e inkubacionit.

Shkalla e infeksioneve spitalore është treguesi më i mirë i cilësisë së shërbimeve të ofruara nga institucioni përkatës shëndetësor. Spitalet bartin rrezikun më të lartë për marrjen e një infeksioni gjatë ofrimit të mbrojtjes shëndetësore. Në vendet e industrializuara të botës infeksionet spitalore ndërlikojnë 5-10% të pranimeve të pacientëve në spital. Prevalenca mesatare në Bashkimin Evropian është 7.1% (rang 3.5-9.3%), kurse në disa vende të pazhvilluara të Azisë, të Amerikës Latine dhe të Afrikës Sub-Saharike shkalla e këtyre infeksioneve shkon deri në 40%. Kosova dhe Shqipëria kanë shkallën më të lartë të prevalencës së infeksioneve spitalore në Evropë me 17.4%, respektivisht 19.1%. Shumë faktorë ndërlidhen në patogjenezën e infeksioneve spitalore. Ato varen nga agjencitë infektive (virulenca, rezistenca ndaj antibiotikëve, qëndrueshmëria në mjedisin spitalor), strehuesi (sëmundja shoqëruese, keq ushqyeshmëria, ekstremet e moshës, imunosupresioni, sëmundjet kronike, trauma, intervenimi kirurgjik) dhe mjedisi spitalor (pranimi në kujdes intensiv, procedurat invazive). Faktorët kryesorë të rrezikut për marrjen e infeksionit në spital janë: faktorët e brendshëm të kushtëzuar nga nevoja për trajtim spitalor, procedurat invazive diagnostike-terapeutike dhe përdorimi i pakontrolluar i antibiotikëve. Qasja moderne në mbikëqyrjen dhe kontrollin e infeksioneve spitalore fillon në vitet e 60-ta, pas shpërthimit të epidemive me *S.aureus* në pavijonet e të porsalindurve, të shoqëruar me sëmundshmëri dhe vdekshmëri shumë të lartë. Ato përshpejtuan hartimin e programeve të para të kontrollit të infeksionit, të cilat lindën në Britani të Madhe dhe Amerikën Veriore [1, 2, 5].

Definimi.-Infeksionet spitalore përkufizohen si infeksione që nuk kanë qenë të pranishme apo nuk janë në fazën e inkubimit në momentin e pranimit të pacientit në spital. Termi nozokomial ka origjinë nga greqishtja *nosos* (sëmundje) dhe *komein* (përkujdesje) dhe nënkupton infeksionet që janë marrë si rrjedhojë e përkujdesjes mjekësore. Emërtesa Infeksione Nozokomiale përdoret si sinonim për infeksionet brenda spitaleve por ajo është më e gjerë dhe përfshin edhe nivelet tjera të përkujdesjes shëndetësore siç janë ambulancat, qendrat e rehabilitimit, mjekimin shtëpiak dhe institucionet speciale shëndetësore.

Korniza kohore për diagnostikën e këtyre infeksioneve është së paku 48 orë pas pranimit në spital; 3 ditë pas lëshimit nga spitali apo 30 ditë pas ndërhyrjes kirurgjike. Për definimin e tyre në mbarë botën përdoren përkufizimet e infeksioneve spitalore sipas Qendrës për Kontrollë të Sëmundjeve-CDC (Centers for Disease Control) nga Atlanta.

Frekuenca.-Studimet nga literatura botërore kanë dëshmuar se shpeshtësia e shfaqjes së infeksioneve spitalore shkon prej 5-10% në Evropën Perëndimore dhe Amerikën Veriore, deri në 40% në disa pjesë të Azisë, Amerikës Latine [1, 2, 3].

infeksioneve spitalore kanë shkallë të lartë të sëmundshmërisë dhe të vdekshmërisë ato :

- Zgjasin kohën e qëndrimit të pacientëve në spital;
- Rrisin koston e mjekimit;
- Shoqërohen me mungesë në punë, e nganjëherë edhe me invaliditet;
- Mund të përhapen nga institucionet
- Shëndetësore në komunitet dhe shpesh përcillen me denoncime gjyqësore dhe me imazh negativ për vet institucionin ku paraqiten. këto infeksione.

Çmimi i infeksioneve spitalore është substancial kudo në botë. Ato kanë kosto të lartë për pacientin, familjen shoqërinë dhe sistemin shëndetësor [12.5]

I. 1. a. Faktorët që ndikojnë në përhapjen e infeksioneve spitalore

Faktorët kryesorë të rrezikut për marrjen e infeksionit në spital janë: faktorët e brendshëm të kushtëzuar nga nevoja për trajtim spitalor, procedurat invazive diagnostike-terapeutike dhe përdorimi i pakontrolluar i antibiotikëve. Rruga më e shpeshtë e përhapjes së infeksioneve spitalore është kontakti i drejtpërdrejtë dhe transferimi fizik i mikrobeve ndërmjet personit të infektuar apo të kolonizuar dhe strehuesit të ndjeshëm. Infeksionet spitalore përhapen më së shumti përmes kësaj rruge, në të cilën duart e personelit mjekësor e luajnë rolin kryesor. Rrugë tjetër e përhapjes së infeksioneve spitalore është rruga ajrore, përmes thërmizave të traktit respirator. Kjo ndodh gjatë kollitjes, teshtitjes, të folurit dhe instrumentimit të aparatit respirator (bronkoskopia dhe sukcionit).

Nganjëherë infeksionet spitalore përhapen edhe përmes aparaturës dhe pajisjeve (me kontaminimin e pajisjeve mjekësore, medikamenteve, infuzioneve, prodhimeve biologjike, dializatorëve, ajrit të kondicionuar, etj. [1, 2, 3, 5, 12].

Gjatë shtrimit në spital pacienti ekspozohet ndaj një numri të madh mikroorganizmash por kontakti midis tyre jo gjithmonë rezulton në shfaqjen e një sëmundjeje klinike. Ka disa faktorë të tjerë që influencojnë natyrën dhe shpeshtësinë e infeksioneve spitalore.

Mundësia që ekspozimi ndaj një mikroorganizmi të shkaktojë sëmundje pjesërisht varet nga karakteristikat e mikroorganizmit që përfshin rezistencën ndaj agjentëve antimikrobik, virulencës dhe sasisë së materialit infektues (inokulimit). Infeksionet mund të shkaktohen në spital nga transmetimi i një mikroorganizmi nga një person tek tjetri ose mund të shkaktohen nga flora e vetë pacientit (infeksion endogjen). Disa mikroorganizma mund të merren nga objekte ose substanca të cilat janë kontaminuar nga një pacient tjetër (infeksion mjedisor). Progresi në trajtimet me antibiotikë të infeksioneve bakteriale ka bërë që vdekshmëria nga sëmundjet infektive të ulët ndjeshëm. Predispozicioni i Pacientit për të marrë Infeksione Spitalore Moshë, imuniteti i dobësuar, sëmundjet kronike, procedurat e shumta terapeutike dhe diagnostike janë disa nga faktorët që e bëjnë pacientin më të predispozuar për të marrë infeksione spitalore. Fëmijët dhe të moshuarit janë më pak rezistentë ndaj infeksioneve.

Pacientët me sëmundje kronike si: tumore malinje, leucemi, diabet, insuficiencë renale, AIDS janë më të predispozuar ndaj infeksioneve nga patogjenët oportunist. Këto të fundit mikroorganizma të cilët në përgjithësi nuk shkaktojnë sëmundje (p.sh.: mikroorganizma që

janë pjesë e florës bakteriale të njeriut) por që shndërrohen në patogjen kur imuniteti është i kompromentuar. Barnat imunosupresore ose rrezatimi e ulin rezistencën ndaj infeksioneve. Dëmtime të lëkurës ose mukozave si dhe kequshqyerja janë gjithashtu faktorë risku. Shumë procedura terapeutike dhe diagnostike moderne si: biopsitë, ekzaminimet endoskopike, ventilimi dhe procedurat kirurgjikale e rrisin ndjeshëm riskun për infeksione [1, 2, 3, 5].

Tabl. paraqet Krahasimin e shkallës së infeksioneve të ndërlidhura nga përdorimi i pajisjeve mjekësore në vendet e pazhvilluara dhe në SHBA e BE

	INICC 2003–2008	U.S. NHSN 2006- 2007	HELICS BE
NjKI/ Mjekësore - kirurgjike			
Sepse nga kateterat vaskularë qendrorë	7.4	2.0	3.1
Infeksionet e traktit urinar nga kateterat	6.1	3.3	3.2
Pneumonia ventilatore	14.7	3.3	5.7
NjKI / Të porsalindurit (1501-2500 gr)	13.9	2.4	
Sepse nga kateterat vaskularë qendrorë			
Pneumonia ventilatore	9.5	1.0	

NJKI- Njësitë e Kujdesit Intensiv

INICC= Konzorciumi Ndërkombëtar i Kontrollit të Infeksioneve Nozokomiale

NHSN- Sistemi Nacional i Mbikëqyrjes së Infeksioneve Nozokomiale, SHBA

HELICS= Spitalet Evropiane të lidhur në kontrollin e infeksioneve përmes mbikëqyrjes [12].

I. 1. b. Faktorët Mjedisor

Shërbimi i kujdesit mjedisor në funksionin e tij synon krijimin e kushteve për të ndikuar drejtpërdrejt apo tërthorazi në rezultatin e trajtimit të pacientëve. Në mënyrë të veçantë, aktiviteti i këtij shërbimi për higjienës mjedisore, synon parandalimin e sëmundjeve kryesisht infektive. Kujdesi ndaj mjedisit mbart peshën më të madhe në trajtimin efikas të çdo infeksioni, apo rreziku për infeksion për të cilin mund. Shërbimi i mjedisit përmes zbatimit të masave rutinë për parandalimin e kontrollin e infeksioneve, mbron jo vetëm pacientët, por edhe personelin e vizitorët ndaj rrezikut të infektimit, për shkaqe të ndryshme të mosrespektimit të udhëzimeve dhe praktikave të pastrimit siç përshkruhen në manualin e shërbimeve të mjedisit si dhe në manualin për parandalimin dhe kontrollin e infeksioneve.

Kujdesit i mjedisit në spital është me rëndësi kryesore për kontrollin e infeksioneve dhe është përgjegjës për pastrimin e rregullt rutinë të të gjitha sipërfaqeve, si dhe për mbajtjen e një niveli të lartë të higjienës në spital.

Pacientët me infeksione ose që mbartin mikroorganizma patogjen janë burim infeksioni për pacientët e tjerë si dhe për stafin mjekësor. Pacientët të cilët infektohen në spital janë një burim tjetër infeksioni. Mbipopullimi në ambientet spitalore, transferimet e shpeshta të pacientëve nga një repart në tjetrin si dhe përqendrimi i pacientëve më të predispozuar për infeksione në të njëjtën hapësirë, ndikojnë në përhapjen e infeksioneve spitalore [1, 2, 12].

Shërbimet shëndetësore përfshirë këtu institucionet shëndetësore publike dhe private duhet të plotësojnë standardet e cilësisë (ISO 9000 dhe ISO 14000). Është i njohur fakti që ndërtesat e vjetra ose ndërtesat e vendeve në zhvillim nuk i plotësojnë këto standarde me gjithat këto standarde duhet të njihen dhe duhet të jenë në qendër të planifikimeve lokale për mundësinë e zbatimit të tyre në të ardhmen.

I. 1. c. Rezistenca Bakteriale

Mbi përdorimi i antibiotikëve për terapi ose në formë profilaksie ka bërë që në shumë raste ato të jenë më pak të efektshëm për shkak të zhvillimit të rezistencës ndaj tyre. Kur një antibiotik përdoret gjerësisht, bakteret rezistonte ndaj këtij bari mund të përhapen në të gjithë spitalin duke shkaktuar kështu endemi. Ky problem është veçanërisht kritik në vendet në zhvillim ku antibiotikët më të shtrenjtë të linjës së dytë mund të mos jenë të disponueshme.

Rezistenca bakteriale ndodh kur bakteri ndryshon gjenet e tij pas rënies në kontakt me antibiotikun , dhe pas këtij veprimi ky antibiotik nuk vepron me kundër këtij bakteri. Ne mund të ndihmojmë në reduktimin e përhapjes së rezistencës bakteriale në radhë të parë duke parandaluar infeksionet dhe në radhë të dytë duke i përdorur antibiotiket në mënyre të zgjuar dhe vetëm në rastet kur ata janë të domosdoshëm .

Pse rezistenca bakteriale është një problem serioz?

Shume infeksione bakteriale dikur lehtësisht të durueshëm janë tani shumë të vështira për tu trajtuar ose edhe të pa durueshme për shkak të rezistencës bakteriale. Bakteret rezistence mund të kalojnë gjenet e tyre tek bakteret e tjera për të formuar shtame bakteriale rezistence të cilat mund të përhapen nga një person tek tjetri dhe të kontribuon në paraqitjen e infeksioneve spitalore në masë të madhe duke rritur kështu ditet e mjekimit dhe koston e shërimit në spitale [1, 2, 3].

I. 2. Epidemiologjia e infeksioneve spitalore

Infeksionet spitalore mund të klasifikohen në bazë të disa kriterëve klinike, biologjike dhe vendit të infektuar. Infeksionet spitalore gjithashtu mund të klasifikohen si endemike ose epidemike. Infeksionet endemike janë më të shpeshtat. Me infeksione epidemike kuptojmë që shpeshësia e infeksionit e ka kaluar normën e zakonshme për atë tip infeksioni ose për atë lloj mikroorganizmi. Tipi i infeksioneve spitalore Kriteret Infeksion ne zonën kirurgjikale Çdo lloj abscesi, mahisje ose celuliti që shfaqet në zonën ku është kryer ndërhyrja kirurgjikale gjatë muajit pas operacionit me infeksion urinar, Kulturë pozitive në urinë (1 ose 2 specie), me të paktën 10⁵ baktere/ml, me ose pa simptoma klinike Infeksion respirator simptoma respiratorë të shoqëruara menjë ose më shumë nga shenjat e mëposhtme gjatë qëndrimit në spital: Kollë, Sputum Infeksion në hyrjen e kateterit venoz Inflammacion, limfangit ose mahisje në vendin e hyrjes së kateterit Septicemi Temperaturë dhe të paktën një kulturë e marrë nga gjaku të rezultojë pozitive.

I. 2. a. Infeksionet Urinare

Infeksionet Urinare janë infeksionet spitalore më të shpeshta. 80% e infeksioneve lidhen me përdorimin e një kateteri urinar afatgjatë. Në përgjithësi këto lloj infeksionesh shkaktojnë më pak morbozitet në krahasim me infeksionet e tjera por në disa raste të rralla ato mund të çojnë në bakteremi dhe në vdekje. Bakteret që shkaktojnë këtë lloj infeksioni vijnë nga flora e zorrëve ose janë baktere që janë prezent në trupin e njeriut si p.sh.: E. Koli ose të marra në spital (Klebsiella multirezistente).

I. 2. b. Infeksionet në vendin e ndërhyrjes kirurgjikale

Infeksionet në vendin e ndërhyrjes kirurgjikale janë gjithashtu të shpeshta. Këto janë një problem shqetësues që komprometojnë suksesin e ndërhyrjes kirurgjikale. Pasojat që ato kanë mbi koston e shërbimit shëndetësor dhe kohëzgjatjen e qëndrimit të pacientit në spital janë të konsiderueshme. Në përgjithësi infeksioni merret gjatë ndërhyrjes kirurgjikale dhe mund të

shkaktohet ose nga jashtë (si p.sh.: nga ajri, pajisjet mjekësore, kirurgu, ose pjesëtare të tjerë të stafit) ose nga brenda (si p.sh.: nga flora në lëkurë ose në vendin ku kryhet ndërhyrja kirurgjikale); në raste të rralla mund të shkakatohet dhe nga gjaku i marrë gjatë operacionit. Sa do të jetë kontaminimi gjatë procedurës kirurgjikale? Kjo do varet kryesisht nga kohëzgjatja e ndërhyrjes kirurgjikale si dhe nga gjendja e përgjithshme shëndetësore e pacientit [1, 2, 3, 7, 8].

I. 2. c. Pneumonia Nozokomiale

Pneumonia nozokomiale ndodh në disa grupe të ndryshme pacientësh. Grupi më i rëndësishëm është ai i pacientëve në reanimacion, të cilët marrin frymë me anë të ventilimit mekanik. Mikroorganizmat kolonizojnë stomakun, pjesën e sipërme të traktit respirator si dhe bronket dhe shkaktojnë infeksion në mushkëri (pneumonia). Këto mikroorganizma zakonisht janë endogjen (nga aparati tretës, hunda ose fyti) por mund të jenë dhe ekzogjen, zakonisht me prejardhje nga pajisjet respiratorë të kontaminuar.

Faktorët e riskut përfshijnë llojin dhe kohëzgjatjen e ventilimit të përdorur, cilësinë e kujdesit shëndetësor respirator, shkallën e rëndesës së sëmundjes së pacientit dhe përdorimin e mëparshëm të antibiotikëve [1, 2].

I. 2. d. Bakteremia nozokomiale

Këto infeksione nuk janë shumë të shpeshta por vdekshmëria është mjaft e lartë.

Incidenca është në rritje veçanërisht për mikroorganizmat si: Stafilokoku multirezistent dhe speciet Candida. Infeksioni mund të ndodh në vendin e hyrjes së pajisjes intravaskulare ose në rrugën subkutane të kateterit. Mikroorganizmat që kolonizojnë kateterin brenda në enët e gjakut mund të shkaktojnë bakteremi pa shfaqur një infeksion të jashtëm të dukshëm. Në këtë rast burim infeksioni është flora kutane. Faktorët e riskut më të rëndësishëm janë kohëzgjatja e mbajtjes së kateterit, niveli i sterilitetit gjatë vendosjes së kateterit dhe kujdesi i vazhdueshëm për mirëmbajtjen e tij.

I. 2. e. Infeksione të tjera nozokomiale

Infeksione të tjera nozokomiale përshijnë: Infeksione të lëkurës ose indeve të buta: plagët e hapura si ulcerat, djegiet, etj. Që nxisin kolonizimin bakterial dhe mund të shkaktojnë një infeksion sistematik. Gastroenteriti është infeksioni respirator më i shpeshtë tek fëmijët, i cili shkaktohet në të shumtën e rasteve nga patogjeni rotavirus. Në vendet e zhvilluara shkaktari kryesor i infeksioneve spitalore tek të rriturit është *Clostridium Dificile*. Sinozitin dhe infeksione të tjera enterike, infeksione të syrit dhe konjunktivave Endometritin dhe infeksione të tjera të organeve riprodhuese pas lindjes [1, 2, 3, 7, 8].

I. 3. Mikroorganizmat dhe Bakteret

Ka disa lloj patogjenësh që mund të shkaktojnë infeksione spitalore. Mikroorganizmat infektues janë të ndryshëm në popullata pacientësh të ndryshëm, në ambiente shëndetësore dhe vende të ndryshme të botës. Bakteret më të shpeshtë që shkaktojnë infeksione spitalore janë ato patogjene. Bakteret mund të ndahen në baktere të padëmshme dhe baktere patogjene. Bakteret e padëmshme gjenden në florën bakteriale të njerëzve. Ato kane një rol të rëndësishëm mbrojtës duke parandaluar kolonizimin nga mikroorganizma patogjen. Por disa baktere të këtij lloji mund të shkaktojnë infeksion nëse imuniteti i bartësit (njeriut) është i kompromentuar. Për shembull *E. Koli* është shkaktari kryesor i infeksioneve urinare [1] infeksioni që shfaqet nën kushte të caktuara janë kushtimisht baktere patogjene që jetojnë në trup në vazhdimësi. Kështu, imuniteti i dobët mund të shkaktojë rritjen e kultivimit të llojeve të caktuara të mikroflora natyrore të baktereve që çojnë në sëmundje dhe përhapjen e infeksioneve spitalore.

I. 3. a. Bakteret Patogjene

Bakteret patogjene janë më virulente dhe shkaktojnë infeksione (sporadike ose epidemike) pavarësisht nga gjendja e bartësit. Për shembull bakteri gram pozitiv. Stafilokoku Aureus shkakton një numër të madh infeksionesh të mushkërive, kockave, zemrës dhe infeksione në gjak dhe zakonisht janë rezistencë ndaj antibiotikëve. Bakteret gram negative si p.sh.: E. Koli, Klebsiella, Enterobakteri, etj.; mund të kolonizojnë vendet ku mekanizmat mbrojtës të bartësit janë të kompromentuar (vendosja e kateterit, vendosja e kaçulave) dhe të shkaktojnë infeksione të rënda (infeksione të zonave kirurgjikale, infeksione të mushkërive, bakteremi). Gjithashtu ato mund të jenë shumë rezistence. Organizmat gram negative si speciet pseudomonas zakonisht gjenden në ujë ose vende të lagështa. Ato mund të kolonizojnë aparatit tretës të pacientëve të shtruar në spital.

I. 3. b. Viruset dhe Parazitët dhe Myku

Ka disa viruse që mund të transmetohen në spitale, përfshirë këtu viruset e hepatitit B dhe C (me anë të transfuzioneve, dializës, injeksioneve, endoskopisë), rotavirusi dhe enteroviruset (që mund të transmetohen nëpërmjet kontaktit dorë-gojë ose me anë të rrugës fekale-orale). Edhe viruse të tjerë si: HIV, virusi influencës, virusi herpes simpleks dhe virusi varicela zoster gjithashtu mund të transmetohen në spital. Disa parazitë si Giardia Lamblia transmetohen lehtësisht midis të rriturve ose fëmijëve. Shumë myqe dhe parazitë të tjerë janë organizma oportunist që mund të shkaktojnë infeksion gjatë përdorimit për një kohë të gjatë të antibiotikëve dhe në imunodeficiencë të rëndë. Këto janë shkaktarët kryesor të infeksioneve sistematike tek pacientët me imunitet të kompromentuar. Kontaminimi nga organizma që transmetohen me rrugë respiratorë si për shembull speciet aspergilus, të cilat vijnë nga pluhuri dhe dheu, janë gjithashtu shqetësuese, veçanërisht gjatë kryerjes së punimeve në spital [1, 3, 7, 8].

I. 4. Transmetimi i infeksioneve spitalore

Bakteret që shkaktojnë infeksione respiratorë mund të merren në mënyra të ndryshme: Mund të merren nga flora bakteriale e zakonshme e pacientit (infeksion endogjen). Bakteret që janë prezentë në florën normale shkaktojnë infeksion për shkak të transmetimit të tyre në zona të tjera jashtë habitatit të tyre të zakonshëm (traktin urinar), për shkak të dëmtimit të indeve (plagë) ose për shkak të terapisë së papërshtatshme me antibiotikë.

Mund të merren nga flora e një pacienti tjetër ose i një anëtari të stafit (infeksione ekzogjen). Bakteret transmetohen midis pacientëve:

a) nëpërmjet kontaktit të drejtpërdrejtë midis pacientëve (duarve, pikëzave të pështymës ose lëngjeve të tjera të trupit), b) nëpërmjet ajrit (pluhuri i kontaminuar nga baktere të një pacienti tjetër), c) nëpërmjet stafit që mund të jetë kontaminuar gjatë ofrimit të kujdesit shëndetësor (duarve, rrobave, hundës ose fytit) dhe që më pas i transmetojnë këto baktere të pacientë të tjerë gjatë një kontakti të drejtpërdrejtë me ta, d) nëpërmjet objekteve të kontaminuar nga pacienti, stafi, vizitorët ose nga burime të tjera mjedisore (si p.sh.: uji, ushqimet).. Mund të merren nga flora e mjedisit në spital (infeksione endemike ose epidemike ekzogjene mjedisore). Ka disa mikroorganizma që rezistojnë mjaft mirë në ambientet spitalore. Në ujë zona me lagështirë dhe në disa raste në produkte sterile ose dezinfektant, në pajisje të përdorura për të ofruar kujdes shëndetësor [1,7].

I. 5. Parandalimi i Infeksioneve Spitalore

Parandalimi është çelësi i zgjidhjes së problemit të infeksioneve spitalore. Programet efikase të kontrollit të infeksioneve mund ta reduktojnë shkallën e infeksioneve spitalore mesatarisht për 32%, me rangun e parandalimit për disa prej infeksioneve që shkon prej 10-70%. Disa institucione dhe shtete kanë arritur ta reduktojnë shkallën e infeksioneve spitalore, por shumica e tyre nuk ia kanë dalë mbanë në këtë fushë. Disa zgjidhje parandaluese janë të thjeshta dhe nuk kërkojnë burime të mëdha. Përveç higjienës së duarve, e cila mbetet masa më e thjeshtë.

Parandalimi i infeksioneve spitalore duhet të mbështetet në programin e hartuar nga njësia për kontrollin dhe parandalimin infeksioneve spitalore kërkon një program të integruar monitorimi në të cilin të përfshihen këto komponentë kyç: Kufizimi i transmetimit të organizmave mikrobike ndërmjet pacientëve duke iu kushtuar rëndësi kujdesit të larjes adekuate të duarve, përdorimit të dorezave dhe metodave të duhura aseptike, strategjive izoluese, metodave të sterilizimit dhe dezinfektimit dhe higjienës së rrobave. Kontrolli i faktorëve ambientale riskantë për infeksion. Mbrojtje të pacientëve me përdorimin e duhur të profilaksisë antimikrobiale, ushqyerjes së përshtatshme dhe vaksinimit. Kufizim të riskut për infeksione endogjene me anë të minimizimit të procedurave invazive dhe përdorimit të duhur të antimikrobialëve. Mbikëqyrje, identifikimi i infeksioneve dhe kontrolli i shpërthimeve epidemike. Parandalimi i infeksioneve në anëtarët e stafit. Shtimi i stafit që kujdeset për pacientët dhe vazhdimi i edukimit të tyre. Kontrolli i infeksioneve është një përgjegjësi e të gjithë personelit shëndetësor-doktorë, infermier, trapistë, farmacistë, etj. [1, 2, 3, 8].

I. 5. a. Masat e duhura aseptike që ndikojnë në parandalimin e infeksioneve spitalore

Shkalla e infeksionit spitalor përcaktohet nga faktorë individual të tillë si: shkalla e komprometimit të imunitetit dhe lloji i ndërhyrjeve me risk të lartë.

Tabela 2. Masat e Duhura Aseptike për nivele të ndryshme të riskut të infeksionit

Risku i infeksionit	Asepsia	Aseptikët	Duart	Rrobat	Pajisjet
1. Minimal	Pastërti	Jo	Larje e thjeshtë ose dezinfektim	Rroba sa më paktë rrudhosura	Të pastra dhe të dezinfektuara
2. Mesatar	Asepsi	Produkt antiseptik standard	Pastrim higjienik ose dezinfektim	Mbrojtje nga ndotja me gjakun dhe lëngjet e tjera	Të dezinfektuara dhe të sterilizuara në nivel të lartë
3. Rëndë	Asepsi Kirurgjikale	Produkte specifike madhore	Pastrim ose dezinfektim kirurgjikal	Veshje kirurgjikale (maska doreza etj..)	Të dezinfektuara dhe të sterilizuara në nivel të lartë

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Në qoftë se i referohemi “The Oxford Popular English Dictionary” - për Parandalimi është reduktimi i infeksioneve të plagëve pasoperative është objektiv i përkujdesjes cilësore për të sëmurin. Sipas IFIC (2004), parandalimi kërkon ndërprerjen e zinxhirit të infeksionit që do të thotë të ndërpritet rruga normale e përhapjes së infeksionit.

Parandalimi i infeksionit të plagës kërkon uljen e ekspozimit ndaj mikroorganizmave dhe mbajtjen e pacientit rezistent ndaj infeksioneve. Larja e mirë e dorës, përdorimin e doreza sterile, ndërrimi dhe kujdesi për të parandaluar futjen e organizmave infektivë. Hidratim të mirë dhe të ushqyerit e mirë për të mbështetur shërimin e pacientit që të bëhet rezistencë ndaj infeksionit (Linton, Matteson & Maebius 1995) [1, 2, 3, 4, 7].

I. 5. b. Parandalimi i infeksioneve spitalore më të shpeshta

Katër infeksionet më të shpeshta janë: infeksionet e traktit urinar, infeksionet e plagëve kirurgjikale, pneumonia dhe infeksionet me rrugë vaskulare.

Shpeshësia e tyre është paraqitur grafikisht në grafikun nr. 1. Secila nga këto është e lidhur me një procedurë mjekësore invazive. Për të minimizuar këto infeksione duhen zbatuar praktika specifike, të rishikuara, përditësuara dhe monitoruara sistematikisht për përshtatshmërinë e tyre.

Parandalimi Infeksionet e traktit urinar. Shumica e infeksioneve të traktit urinar tek të sëmurët e hospitalizuar është rrjedhojë e përdorimit të pajisjeve drenuese të urinës, siç janë kateterat urinarë. Në kushte normale, flora bakterore uretrale, e cila tenton të migrojë në drejtim të fshikëzës urinare, vazhdimisht shpëlahet gjatë urinimit. Nëse është vënë kateteri, atëherë ky mekanizëm mbrojtës mungon dhe flora perineale dhe uretrale (zakonisht mikroorganizmat aerobe nga zorrët) mund të mbërrijnë në fshikëzën urinare nëpërmjet shtresës së lëngut që ndodhet ndërmjet murit të jashtëm të kateterit dhe mukozës së uretrës. Për këtë arsye, kolonizimi i fshikëzës urinare është pothuajse i pashmangshëm, nëse kateteri qëndron për një kohë të gjatë. Përveç kësaj, infeksioni i fshikëzës urinare mund të shkaktohet edhe nga refluksi i baktereve që kanë origjinë nga urina e kontaminuar në qeskën drenuese. Hulumtimet e shumta kanë dëshmuar qartë se përdorimi i sistemeve të mbyllura të drenazhit e redukton dukshëm kontaminimin e urinës dhe njëkohësisht edhe përqindjen e infeksioneve. Prandaj kateterat duhen përdorur në minimum apo evituar fare përdorimi i sistemeve të hapura.

Në institucionet shëndetësore që përdorin kateter, infeksionet e aparatit urinar mund të jenë infeksionet më të shpeshta nozokomiale. Shumica e të sëmurëve të kateterizuar do të kenë bakteriuri asimptomatike apo infeksione të lehta, të cilat largohen me heqjen e kateterit. Kurse, tek disa të sëmurë të tjerë mund të zhvillohen infeksione të rënda që shkaktojnë pielonefrit, sepsis dhe vdekje. Përveç kësaj, infeksionet e traktit urinar shkaktojnë kohëzgjatjen e hospitalizimit dhe rrisin shpenzimet e mjekimit për shkak të nevojës për procedurat plotësuese diagnostike dhe antibiotikoterapeutike [1,2].

Për fat të mirë, pjesa dërmuese e infeksioneve urinare parandalohen lehtë përmes reduktimit të kateterizimit urinar të panevojshëm apo në kohëzgjatje joadekuate si dhe përmes përdorimit të sistemeve të mbyllura drenuese dhe teknikave standard aseptike.

Infeksionet e traktit urinar janë infeksionet më të shpeshta. 80% e këtyre infeksioneve janë të shoqëruara me një kateter urinar që përdoret për një periudhë të gjatë kohe.

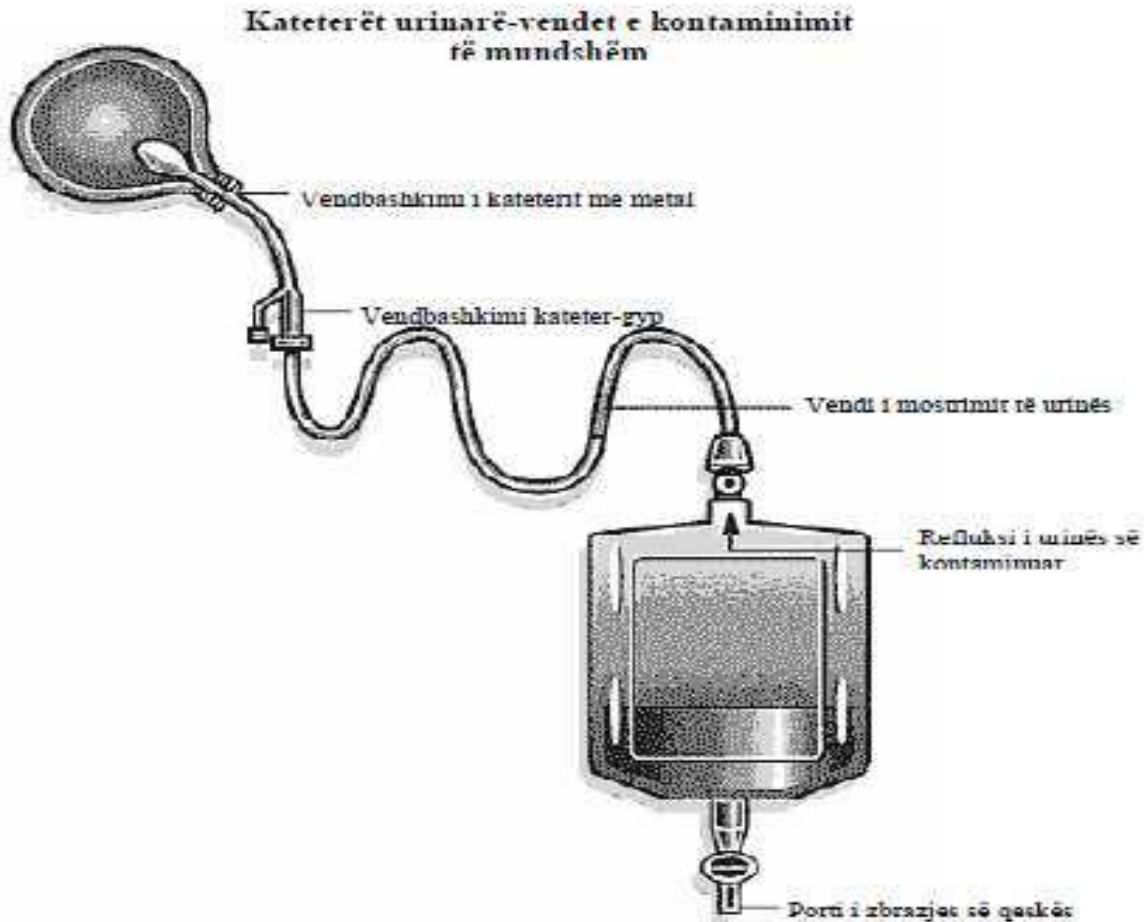


Figura. 1 Porta Hyrëse e mikroorganizmave në Sistemin e Drenazhit Urinar:

I. 5. c. Parandalimi i infeksionit dhe masat e përgjithshme

Të mbahen shënime të sakta me qasje të shpejtë për sëmundjet profesionale;

Çdo punëtori të ri t'i merret anamneza për sëmundjet ngjitëse. Ata duhet të vaksinohen kundër sëmundjeve që mund të parandalohen me vakcina;

Të mbahen shënime në "librin e aksidenteve" për secilin rast aksidental të shpimit me gjilpërë në vendin e punës dhe për lëndimet e tjera; të dhënat epidemiologjike për ekspozimin ndaj prodhimeve të gjakut duhet analizuar periodikisht për të kontrolluar praktikatat e punës dhe për identifikimin e rreziqeve që mund të parandalohen. Të bëhet vlerësimi dhe t'u jepet pushim mjekësor punonjësve që lëngojnë nga sëmundje ngjitëse apo janë të ekspozuar ndaj infeksionit, të mundësohet që çdo punëtor shëndetësor ta mbulojë lëndimin në lëkurë me lidhëse nga materiali që i reziston ujit.

Përkufizimi i rrugëve të përhapjes së infeksionit:

- **Kontakti:** përfshin kontaktin e drejtpërdrejtë prej personi në person (p.sh. gjaku i të sëmurit drejtpërdrejt në plagën e hapur të punëtorit shëndetësor) dhe kontakti I tërthortë (bartja nga një person në tjetrin përmes objektit ndërmjetës, siç janë duart e personelit apo pajisjet e ndryshme, p.sh. gjilpërat).
- **Spërklat:** përhapja e infeksionit përmes spërklave mund të ndodhë nëse i sëmuri është i larguar nga personi i ndjeshëm në distancë rreth 1 metër. Sekretet e gojës dhe ato respiratorë mund të barten në drejtim të syve apo të mukozave përmes kollitjes, qoftë me përhapjen e drejtpërdrejtë të spërklave, apo tërthorazi përmes kontaminimit të sipërfaqeve, të cilat pastaj preken nga personi tjetër.
- **Ajri:** bëhet përmes shpërndarjes së bërthamëzave të spërklave (grimcat me madhësi < 5 mikronë), të cilat mund të mbesin të shpërndara në ajër gjatë një periudhe të gjatë kohore. Kufizimi i personelit të ndjeshëm ndaj ekspozimit është strategjia parandaluese më e dobishme, e shpesh edhe e vetmja kundrejt sëmundjeve të cilat përcillen pjesërisht apo plotësisht përmes ajrit. Maskat kirurgjike të rëndomta ofrojnë mbrojtje minimale. Maskat respiratorë të efikasitetit të lartë, mund të ofrojnë njëfarë mbrojtjeje të personelit që ka kontakt të ngushtë me të sëmurët nga tuberkulozi. Mirëpo, maskat e tilla janë të shtrenjta dhe nuk janë çdo herë në dispozicion. Nuk është sqaruar ende efikasiteti i tyre në mbrojtjen e personelit gjatë përhapjes masive të viruseve të morbillit dhe variçellës.

Përhapja përmes artikujve të përdorimit të gjerë: mikroorganizmat mund të përcillen përmes artikujve të ndryshëm siç janë: ushqimi, uji, barnat dhe pajisjet e ndryshme.

- **Përhapja përmes vektorëve:** mushkonjat, mizat, minjtë dhe bartësit e tjerë që përhapin mikroorganizma.
- **Kërkesat minimale për mbrojtjen e personelit dhe të sëmurëve** Parandalimi i përhapjes së infeksionit shpesh kërkon “ndërprerjen e zinxhirit të infeksionit”, d. m. th. ndërpritet rruga normale e përhapjes së infeksionit.
- **Kontakti:** Të pastrohen duart rregullisht pas kontaminimit të mundshëm si dhe para fillimit të përkujdesjes për të sëmurin. Nëse duart nuk janë shumë të papastra, atëherë për pastrim mund të përdoren edhe antiseptikët. Çdo herë që kontaktohet mukoza apo lëkura e dëmtuar, duhet mbajtur dorëza, të cilat duhet të jenë të pastra në momentin e përdorimit. Gjatë kontaktit me lokacione sterile të organizmit duhet përdorur dorëza sterile. Nëse ka nevojë, duhet përdorur edhe pajisjet e tjera plotësuese për mbrojtje në punë: syzet mbrojtëse të gjitha procedurat që mund të shoqërohen me stërpikje dhe dorëzat adekuate gjatë kontaktit me substancat trupore të lëngshme. Para procedurave të punës ndërmjet dy të sëmurëve të njëpasnjëshëm, duhet dezinfektuar të gjitha pajisjet. Të gjitha mostrat klinike duhet të përpunohen asisoj që të konsiderohen si të infektuara. Gjatë manipulimit me rroba të ndyra dhe mbeturina duhet shmangur kontaktin me lëkurë.
- **Gjaku:** Për shkak të mundësisë së ekspozimit ndaj virusit të hepatitit B nga gjaku i të sëmurëve, rekomandohet imunizimi i gjithë personelit shëndetësor të ekspozuar ndaj gjakut dhe lëngjeve trupore. Mirëpo, imunizimi nuk e pakëson nevojën për procedura të sigurta praktike, të cilat reduktojnë lëndimet me mjete të mprehta dhe ekspozimet e tjera ndaj gjakut. P.sh. vendosja e kapakut në gjilpërë, pas përdorimit duke shfrytëzuar teknikën me një dorë. Mjetet e mprehta pas përdorimit dhe para dispozimit, duhet të vendosen në kuti që i rezistojnë shpuarjeve. Gjatë manipulimit me gjak apo me material të kontaminuar me gjak duhet të përdoret teknika e mosprekes (pinceta ose Parandalimin e infeksionit plagë nga profesionistët mjekësor është i bazuar në disa faktorë të tjerë që janë përmendur më poshtë:

a) Kategoritë kirurgjikale kjo do të thotë se çfarë lloj të operacioneve janë (kontaminuar apo jo), implant ose operacion transplantim. b) Të gjithë procedura kirurgjikale që do të thotë

teknikë të dobët kirurgjikale, kohëzgjatje të gjatë të operacionit, hemorragji dhe hematoma, përdorimin ose kullon. c) profilaksia antibiotik Do të thotë një antibiotik relevant duhet të jepet në kohën e duhur, pra në induksion të anestezisë. Nuk duhet të jepet në rreth 24 orë, mundësisht një çështje dose. Tjetër në parandalimin e infeksionit plagë është profilaksia antibiotik që përmendet më sipër, kjo masë parandaluese të ulet shumë mundësia e infeksionit të plagëve kirurgjikale kjo është me kosto efektive (Shoqata Amerikane Mjekësore në vitin 2006).Në këtë artikull, e cila është shumë me rëndësi nga aspekti mjekësor, theksojnë rëndësinë e profilaksisë antibiotikut dhe llojin e kirurgjisë në parandalimin e infeksionit kirurgjikale. Përdorni i koncepteve nga mjekësia është shumë i lidhur me kujdesin infermierior, por dallimet janë në atë se si ata qasen në këtë koncept . Më tej, ne do të shohim aspekte të ndryshëm në pikëpamjen e këtyre koncepteve nga pikëpamja shëndetësore .

Ekspozimi ndaj virusit HIV dhe mbrojtja e personelit Probabiliteti i infeksionit HIV që pason dëmtimet me age prej pacientëve HIV pozitiv është 0.2% - 0.4% për çdo dëmtim. Për të ulur riskun për të gjithë patogjenët që përhapen me anë të gjakut duhet:Zbatimi i masave parandaluese rutinë me përdorimin e barrierave mbrojtëse të përcaktuara. Përdorimi i pajisjeve të sigurt dhe sistemeve të posaçme për hedhjen e mjeteve të mprehta për të limituar ekspozimin ndaj tyre. Vazhdimin e trajnimit të personelit shëndetësor në lidhje me sigurinë ndaj mjeteve të mprehta. Faktorët që shoqërohen me një mundësi të lartë të marrjes së infeksionit HIV nga personeli shëndetësor përfshijnë:

Dëmtimet e thella intramuskulare gjak i dukshëm në pajisjen dëmtuese Pajisje dëmtuese që është përdorur për injeksion Pacientët me ngarkesë të lartë virale. Shpimet me age Stafi mjekësor me potencial të lartë ekspozimi ndaj gjakut dhe produkteve të tij duhet të informohet në lidhje me masat mbrojtëse parandaluese. Në këto masa përfshihet ekzaminimi pacientëve, hedhja e mbeturinave të mprehta, veshjet mbrojtëse,menaxhimi i aksidenteve të shpimit gjatë injeksionit, sterilizimi dhe dezinfektimi. Profilaksia pas ekspozimit duhet të fillojë jo më larg se katër orë pas ekspozimit. Rekomandohet përdorimi pas ekspozimit i barnave antiretrovirale. Rekomandohet kombinimi i barnave antiretrovirale. Në personat e ekspozuar duhet të merret një kampion gjaku për testimin për HIV sa më shpejt që të jetë e mundur pas ekspozimit dhe në intervale të rregullta më pas për të dokumentuar një serokonversion të mundshëm.

Punonjësit e shëndetit duhet të informohen në lidhje me paraqitjen klinike të sindromit retroviral akut i ngjashëm me mononukleozën akute, e cila ndodhet në 70% - 90% të rasteve të

pacientëve me infeksion HIV dhe të raportojnë menjëherë ndonjë sëmundje që ndodh brenda 3 muajve të dëmtimit. Një ekspozim profesional mund të ndodhë në çdo kohë; këshillimi, trajtimi dhe testimi duhet të jenë të mundshme në çdo kohë 24 orë në ditë. Mënyra e ndjekjes së ekspozimit ndaj infeksionit HIV duhet të standardizohet me teste serologjike të përsëritura për një vit.

I. 5. d. Ekspozimi ndaj Hepatit B

Probabiliteti i infeksionit ndaj Hepatit B në rastet e dëmtimit me agje është 1.9% - 40%. Në rast të dëmtimit me mjete të mprehta duhet të bëhet testimi në kohën e ekspozimit për të identifikuar nëse personi është i infektuar apo jo. Infeksioni i personelit shëndetësor me infeksion të hepatit B mund të ndodhi në rastet e pacientëve me HBsAg ose HBeAg pozitive. Për imunizimet e mëparshme me antikorpet anti HBs më tepër se 10mIU/ml nuk rekomandohet trajtim i mëtejshëm. Për të tjerët rekomandohet profilaksia që konsiston në injektimin intramuskular të imunoglobulinave për Hepatitin B dhe kursi i plotë i vaksinimit për hepatitin B. Imunoglobulinat për Hepatitin B duhet të administrohen sa më shpejt të jetë e mundur brenda 48 orësh dhe jo më larg se 1 javë pas ekspozimit. Serologjia pas ekspozimit duhet të merret për të identifikuar një përgjigje serologjike adekuate. Hepatiti D ndodh vetëm në personat e infektuar me hepatitin B dhe transmetohet me të njëjtën rrugë. Masat parandaluese për sa i përket hepatitit janë gjithashtu efektive për agjentin delta.

I. 5. e. Ekspozimi ndaj Hepatitit C

Rruga e infektimit është e njëjtë me atë të hepatitit B. Nuk është i mundshëm trajtimi pas ekspozimit por mund të bëhet serokonvertimi. Si në rast të hepatitit B duhet të bëhet testimi për hepatitin C për pacientin që mendohet se mund të ketë qenë burimi.

I. 6. Parandalimi i infeksioneve nga aspekti infermieror

IFIC (2004) definojnë mbikëqyrjen si regjistrim ose vështrim sistematik dhe i vazhdueshëm i shpeshëtisë dhe përhapjes ndonjë infeksioni dhe ngjarjeve që shtojnë ose ulin rrezikun e shfaqjes së infeksionit.

Parandalimi i infeksioneve nënkupton procesin aktiv dhe të vazhdueshëm të zbatimit planifikimit të ndërhyrjeve dhe procedurave infermierore me qëllim të zvogëlimit të predisponimit ose prirjes së paraqitjes së infeksionet të rastet pasoperative në spital. Kontribut të jashtëzakonshëm në parandalimin e infeksioneve e jep mbikëqyrja aktive e infeksioneve të plagëve dhe raportimet e rregullta të rezultateve të kirurgët.

Prevenimi i infeksionit është përgjegjësi e gjithë ekipit profesional shëndetësor. Janë pra masat e përgjithshme dhe specifike të cilat duhet të ndermirën.

Parandalimi i infeksionit nënkupton, mbikëqyrje dhe menaxhim të drejt nga ekipi profesional multidisciplinar (ekip për pengimin e infeksioneve).

Ndërhyrjet infermierore në parandalimin e infeksioneve intrahospitalore definohej si “çdo trajtim, i bazuar në gjykim klinik dhe në njohuri, të cilat përformohen nga infermierja për të pasur rezultat tek pacienti”.

Një aspekt i kujdesit infermieror është të monitoroj progresin e plagës nëse ka identifikim të hershëm të ndonjë problemi.

Ndërhyrjet infermierore nënkuptojnë implementimin e njohurive dhe aftësive infermierore për të realizuar me efikasitet objektivat e parashtruara gjatë punës së përditshme të praktikës profesionale. Po ashtu ndërhyrjet infermierore përfshijnë një aktivitet të përbashkët me pacientët dhe me profesionist të tjerë shëndetësor për të realizuar një kujdes të mirëfilltë shëndetësor për pacientët.

Ndërhyrjet infermierore nënkuptojnë zbatimin e veprimeve infermierore në përputhshmëri me nevojat dhe reagimet e pacientëve duke u fokusuar në nevojat prioritare të tyre. Intervenimi infermieror është trajtim për së afërmi i pacientit dhe familjes së tij/saj.

Ndërhyrjet infermierore mund të definohej edhe si masa, veprime, aktivitete si dhe strategji të planifikuara dhe të hartuara lidhur me parandalimin e infeksioneve të rastet post-operative

më qëllim të ruajtjes, mbrojtjes avancimit dhe përparimit të gjendjes shëndetësore të pacientëve si dhe krijimit të ambientit të sigurve dhe të shëndosh spitalor për të gjithë.

Roli i infermierëve në infeksionet spitalore është vendosja e praktikave të kujdesit për pacientin në lidhje me kontrollin e infeksioneve. Infermierët duhet të jenë në dijeni dhe t'i njohin mirë këto praktika në mënyrë që të shmangin përhapjen e infeksioneve.

Detyrat dhe përgjegjësitë e infermierit:

1. Të marrë pjesë në planifikimin e mbikëqyrjes prospektive të infeksioneve spitalore,
2. Të zbatojë programin përmes regjistrimit ditor të pacientëve, kontrollit të dhënave klinike e laboratorike, konsultimeve me mjekë e infermierë në reparte.
3. Të regjistrojë dhe validojë të dhënat e grumbulluara në datotekën kompjuterike,
4. Të përgatis raporte (ditore, javore, mujore) të frekuencës së shfaqjes së infeksioneve spitalore nëpër reparte të caktuara,
5. Të marrë pjesë në analizën e të dhënave dhe identifikimin e faktorëve të rrezikut,
6. Të instalojë masat parandaluese të shfaqjes së infeksioneve,
7. Të dizajnojë dhe të marrë pjesë aktive në hulumtimin e shpërthimeve të epidemive apo rasteve sporadike të infeksioneve,
8. Të inspektojë departamentet e ndryshme në lidhje me kriteret e përmbushjes së rekomandimeve të dhëna nga programi i kontrollit dhe parandalimit të infeksioneve,
9. Të mundësojë trajtimin dhe edukimin e personelit të mesëm në lidhje me parimet dhe praktikat e kontrollit të infeksioneve,
10. T'i raportojnë përgjegjësit për kontroll të infeksioneve dhe drejtorit mjekësor të spitalit [1, 2].

I. 6. a. Higjiena e duarve në parandalimin e infeksioneve

Instrumenti që përdoret 24 orë në ditë janë pikërisht duart e punëtorëve shëndetësorë.

Bartja e tërthortë e mikroorganizmave në mjediset e kujdesit shëndetësor është e zakonshme dhe ndodh përmes duarve. Njohja e burimeve të infeksionit dhe agjensëve infektivë si dhe rrugët e bartjes së tyre do t'iu mundësojnë punëtorëve shëndetësorë të punojnë të sigurt.

Transmetimi i infeksionit nëpërmjet duarve si një nga rrugët më të rëndësishme mund të minimizohet nëpërmjet higjienës së duhur të tyre.

- Duart luajnë rolin kryesor në bartjen e mikrobeve patogjene
- Higjiena e duarve e ul shkallën e infeksioneve spitalore
- Higjiena e duarve për çdo pacient- 24orë në ditë, 7 ditë në javë, parandalon infeksionet spitalore [12].
- Higjiena e duarve luan rol me rëndësi edhe në sigurinë e pacientit. Qëllimi i sigurisë së pacientit është të reduktojë rreziqet e shumta, që ndërlidhen gjatë kujdesit shëndetësor ndaj pacientit. Siguria e pacientit është një disiplinë e re që fuqizon raportimin, analizën dhe parandalimin e gabimeve mjekësore që shpesh qojnë në përfundime fatale.

Edukimi dhe trajnimi nuk mjaftojnë për t'u siguruar se punëtorët shëndetësorë duhet t'u përmbahen kritereve dhe rekomandimeve për higjienën e duarve. Njerëzit kanë norma të ndryshme personale, qëndrime, sjellje dhe besime. Sjellja është një çështje e ndërlikuar dhe për përmirësimin e aderentës duhet auditim dhe kontrolli i rregullt. Në dinamikën e sjelljeve të tyre përfshihen shumë faktorë si edukimi, motivimi dhe ndryshimet e sistemit. Higjiena e duarve është zgjedhje e dobishme dhe kursyese në parandalimin e infeksioneve. Investimi në edukimin e punëtorëve shëndetësorë dhe pajisjet optimale për higjienë të duarve është më i lirë sesa çmimi i infeksioneve spitalore. Përhapja e shpejtë e mikrobeve multirezistente është një realitet. Koha është për përmirësimin e aderentës së higjienës së duarve. Strategjia është vetëm fillimi - në jetën reale matet vetëm rezultati.

Një program efikas i higjienës së duarve i kontribuon drejtpërdrejt sigurisë për pacientin.

Higjiena e duarve është e dobishme për pengimin e përhapjes së sëmundjeve edhe në komunitet (në shkolla, çerdhe fëmijësh, shtëpi). Promovimi i higjienës së duarve përmirëson shëndetin e fëmijëve pasi që redukton infeksionet e traktit të sipërm respirator, diarrenë, gripin dhe impetigon [1, 2, 3, 12].

Higjiena e duarve shpesh është jo optimale kjo për shkaqe të ndryshme si: mungesa e mjeteve të duhura, alergjive të ndryshme ndaj produkteve të larjes së duarve, njohurive të pamjaftueshme të stafit rreth rreziqeve dhe procedurave, mos zbatimit të kohëzgjatjes së rekomanduar për larje të duarve, mungesës së kohës, etj..

Higjiena Optimale e Duarve, Për larjen e duarve duhet të kemi ujin i rrjedhshëm si dhe produktet e duhura që janë të nevojshme për larjen e duarve si: sapun ose antiseptik në varësi të procedurës. Mjetet e duhura të pa kontaminuar përfshirjen dhe tharjen e duarve

Për dezinfektimin e duarve Dezinfektantë specifik për duar, Procedurat e thjeshta të higjienës kufizohen vetëm për duart dhe kyçin e dorës dhe ato kirurgjikale përfshijnë duart dhe parakrahun. Procedurat ndryshojnë në varësi të vlerësimit të riskut.

Sa here duart janë dukshëm të ndotura. Përpara: Kontaktit me pacientin, veshjes së dorezave, vendosjes së pajisjes invazive Manipulimit të pajisjes invazive, Pas kontaktit me lëkurën e pacientit. Kontaktit me likuidet trupore, sekrecionet, ndërrimit të plagës, pajisjeve të kontaminuar, Kontaktit me objektet e pacientit si dhe Heqjes së dorezave, Efektshmëria e Higjienës së duarve; Preparatet në vrasjen e Baktereve

Pse gjeli me bazë alkooli është kaq i mirë ?

Vret më shumë dhe më shpejt, është më pak i dëmshëm për lëkurën, e than më pak dhe është më pak irritues, kërkon më pak kohë .

Kontenieret/pajisjet e tyre mund të vendosen në pikat e kujdesit dhe janë të arritshme për këdo. Kujdesi Rutinë Higjiena e duarve me sapun jo dezinfektantë, Dezinfektim higjenik i shpejtë i duarve me solucion alkoolik, Pastrim antiseptik i duarve- kujdes aseptik i pacientëve të infektuar, Higjiena e duarve me sapun antiseptik (duke ndjekur instruksionet e prodhuesit p.sh.: 1minutë) - Ose higjienë e shpejtë dezinfektuese: si më parë [1, 2, 3, 4].



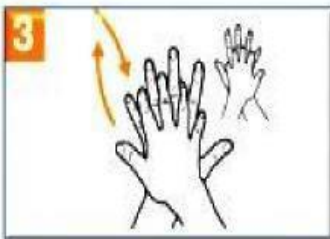
Lagen duart me ujë



Përdoret sapun mjaftueshëm sa të mbulohet e gjithë sipërfaqja e duarve



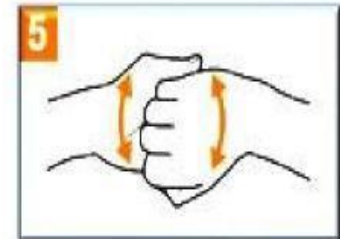
Fërkohen pëllëmbët



Pëllëmba e djathtë fërkon shpinën e dorës së majtë me gishta të ndërthurur dhe anasjelltas



Fërkohen pëllëmbët me gishta të ndërthurur



Fërkohen gishtat e puthitur me pëllëmbën e dorës



Fërkohet gishti i madh i dorës së majtë me pëllëmbën e dorës së djathtë dhe anasjelltas



Fërkohet pëllëmba e majtë me gishtat e dorës së djathtë dhe anasjelltas



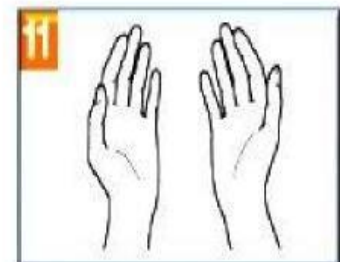
Shpëlahen duart me ujë



Fshihen duart me picetë letre me një përdorim



Përdoret e njëjta picetë letre për të mbyllur rubinetin



Tani, duart tuaja janë të pastra dhe të sigurta

Fig 2. Manuali për Teknikën e Larjes së Duarve, OBSH 2009

I. 6. b Pastrim Kirurgjikal

Pastrim kirurgjikal i duarve dhe parakrahut me sapun antiseptik dhe kohën e mjaftueshme (3-5 min) Ose dezinfektim kirurgjikal i duarve dhe parakrahut: larje dhe tharje e thjeshtë e duarve të ndjekur nga dy aplikime dezinfektuese të tyre. Pajisjet dhe produktet që përdoren për higjienën e duarve janë të ndryshme në vende dhe institucione të ndryshme. Ato duhet të përshtaten me nevojat lokale [6, 7, 8].

Në të gjitha rastet duhet të zbatohet procedura më e mirë e mundshme.

1. Duart duhet të dekontaminohen menjëherë para dhe pas çdo kontakti direkt me pacientin dhe pas çdo situatë që mund të rezultojë me kontaminim të tyre. (Grada B)
2. Duart të cilat janë dukshëm të ndotura ose të kontaminuar me materiale organike duhet të lahen me ujë dhe sapun. (Grada A)
3. Në rastet kur duart nuk janë dukshëm të pista, preferohet që duart të dekontaminohen me një solucion me përmbajtje alkooli para se të kalohet nga një pacient tek tjetri dhe midis dy aktiviteteve të ndryshme tek i njëjti pacient. (Grada A)
4. Përpara se të fillojë pastrimi i duarve, duhet që të hiqen të gjitha bizhuteritë që mbahen në dorë. Thonjtë duhet të mbahen të shkurtër, të pastër dhe pa manikyr. (Grada D)
5. Që pastrimi i duarve të jetë i efektshëm duhen ndjekur 3 hapat e mëposhtme: përgatitja, larja e shpëlarja, dhe tharja e duarve. Përgatitja kërkon që duart të lagen me ujë të vakët përpara se të aplikohet sapuni i lëngshëm ose ndonjë preparat antimikrobik. Solucioni që përdoret duhet të ketë kontakt me çdo pjesë të sipërfaqes së dorës. Duart duhet të fërkohen mirë për rreth 10-15 sekonda, duke u siguruar që janë pastruar dhe majat e gishtërinjve, gishtat e mëdhenj dhe zonat midis gishtërinjve. Duart duhet të shpëlahen me ujë të bollshëm përpara se të thahen me letra të një kualiteti të mirë. (Grada D)
6. Kur duart pastrohen me anë të një solutioni me përmbajtje alkooli, ato nuk duhet të kenë pisllëk ose materiale organike. Solucioni duhet të prekë të gjithë sipërfaqen e dorës. Duart duhet të fërkohen mirë derisa solutioni të avullojë, duke u siguruar që janë pastruar siç duhet dhe majat e gishtërinjve, gishtat e mëdhenj dhe zonat midis gishtërinjve. (Grada D)
7. Një krem zbutës duhet të përdoret rregullisht për të mbrojtur lëkurën nga tharja që mund të shkaktojë larja e shpeshtë e duarve. (Grada D) [1, 2, 3, 4, 7].

Tabela 2. Përmbledhje e vetive të antiseptikëve që përdoren për higjienën e duarve

Antiseptiku	Përqendrimi Tipik	Shpejtësia e veprimit	Aktiviteti rezidual	Përdorimi
Alkoolet	60-70%	Shpejt	Jo	Fërkim i duarve (FD)
Kloroksilenoli	0.5-4%	Ngadalë	Kundërthënës	Pastrim i duarve (PD)
Klorheksidina	0.5-4%	Mesatare	PO	FD, PD
Heksaklorofeni	3%	Ngadalë	PO	PD, por nuk rekomandohet
Jodoforet	0.5-10%	Mesatare	Kundërthënës	PD
Triklosan	0.1-2%	Mesatare	PO	PD, rrallë
Komponimet kuaternare të amonit		Ngadalë	Jo	PD,FD; rrallë

Higjiena e duarve në kujdesin shëndetësor/ LulRaka...[et al.]. - Prishtinë: [Autoret], 2010.

I. 6. c. Mbrojtja e personelit shëndetësor dhe përdorimi i dorezave

Përdorimi i dorëzave nuk mund të konsiderohet si zëvendësim për higjienën e duarve. Dorëzat nuk janë gjithmonë barrierë plotësisht e padepërtueshme (20-30%), e dorëzave shqyhen gjatë intervenimeve kirurgjike). Dorëzat duhet të përdoren si masë shtesë e higjienës së duarve dhe jo si zëvendësim për të. Dorëzat duhet vënë gjatë përkujdesjes për rastet që parashihen të përcillen me kontakt me gjak dhe material potencial infektiv të pacientit (qelbi, fecesi, sekretet respiratorë ose eksudatet e lezioneve të lëkurës).

Dorëzat sterile vihen tek procedurat me të cilat depërtohet në inde apo zgavrë sterile trupore; Pas përkujdesjes për pacientin të hiqen dorëzat dhe të pastrohen duart; Nuk rekomandohet ripërdorimi i dorëzave. Megjithatë nëse resurset janë të kufizuara, dorëzat mund të ripërdoren, por vetëm pas një riprosesimi të sigurt. Mos përdorni dorëza nëse nuk ka nevojë për to (p.sh. gjatë matjes së shtypjes së gjakut, bisedës telefonike, punës në kompjuter, shkrimit në tabelë etj.). Nëse personeli është alergjik në dorëzat e lateksit, atëherë mund të përdoren dorëzat e virilit, nitrimit, neoprenit apo polietilenit.

Sidoqoftë, përdorimi i dorëzave e zvogëlon dukshëm numrin e mikroorganizmave që mund të barten te i sëmuri apo te punëtori shëndetësor. Dorëzat gjithashtu ofrojnë deri diku mbrojtje edhe ndaj viruseve që barten përmes gjakut; Gjatë shpërthimeve të epidemive brenda spitalore, higjiena e duarve dhe bartja e dorëzave janë masa të rëndësishme mbrojtëse për parandalimin e përhapjes së agjentëve infektivë te të sëmurët dhe punëtorët e ndjeshëm shëndetësorë.

Gjatë përkujdesjes prej një të sëmuri në tjetrin apo prej procedurave të pastra në ato të ndyra te i njëjti i sëmurë, nuk bën të barten të njëjtat dorëza. Fërkimi i duarve me alkool apo pastrimi i duarve duhet të bëhen pas heqjes së dorëzave dhe para vënies së dorëzave sterile.

Në vendet që kanë mungesë dorëshash, dorëzat nga lateksi mund të lahen me sapun dhe ujë, të thahen, pluhurosen, sterilizohen apo dezinfektohen në nivel të lartë dhe të ripërdoren. Për procedurat kirurgjike preferohet sterilizimi Personeli duhet të vendosi maskë kur kujdeset për pacientët me infeksione që transmetohen me rrugë ajrore ose kur kryejnë procedurën e bronkoskopisë ose procedura të ngjashme. Në këto raste rekomandohet një maskë me

eficiencë të lartë. Pacientët me infeksione që transmetohen me rrugë ajrore duhet të përdorin maskë kur dalin jashtë dhomës së tyre të izolimit.

Dorezat përdoren për mbrojtjen e pacientit, personeli duhet të përdorë doreza sterile në rast ndërhyrjesh kirurgjikale, në kujdesin për pacientë me imunitet të kompromentuar, në rast të procedurave invazive që hyjnë në kavitetet e organizmit.

Dorezat jo sterile vishen në të gjitha rastet e kontaktit me pacientët dhe kur duart mund të kontaminohen, ose për ndonjë kontakt me membranat mukoze.

Mbrojtjen e stafit: Stafit mjekësor përdor doreza sterile gjatë kujdesit për pacientët me sëmundje që transmetohen me kontakt të drejtpërdrejtë, kur kryen ekzaminimin e bronkoskopisë ose ndonjë procedure ose ekzaminim tjetër të ngjashëm.

Mbasi dorezat hiqen ose ndërrohen duart duhet të lahen, Dorezat e hedhura nuk duhet të ripërdoren.

Lateksi ose klorid polivinili janë materialet që përdoren më shpesh për doreza.

Cilësia e tyre në lidhje me porozitetin mungesën e birave dhe rezistenca e tyre në përdorim është e ndryshme nga një lloj tek një tjetër

Mund të ndodh sensitivitet ndaj lateksit ndaj është i rëndësishëm krijimi i një programi me një linjë veprimi të përcaktuar për të menaxhuar këtë problem.

.Teknika të sigurt dhënia e injeksionit dhe parandalimi i infeksionit

Për të parandaluar transmetimin e infeksioneve duhet:

- Të eliminohen injeksionet e panevojshme
- Të përdoren age dhe shiringa sterile
- Përdorimi i shiringave dhe ageve të përshtatshme për çdo procedurë,
- Të parandalohet kontaminimi i barnave
- Të ndiqet metoda e sigurt e hedhjes së mjeteve të mprehta [1, 7, 8].

I. 7. Cikli i dekontaminimit

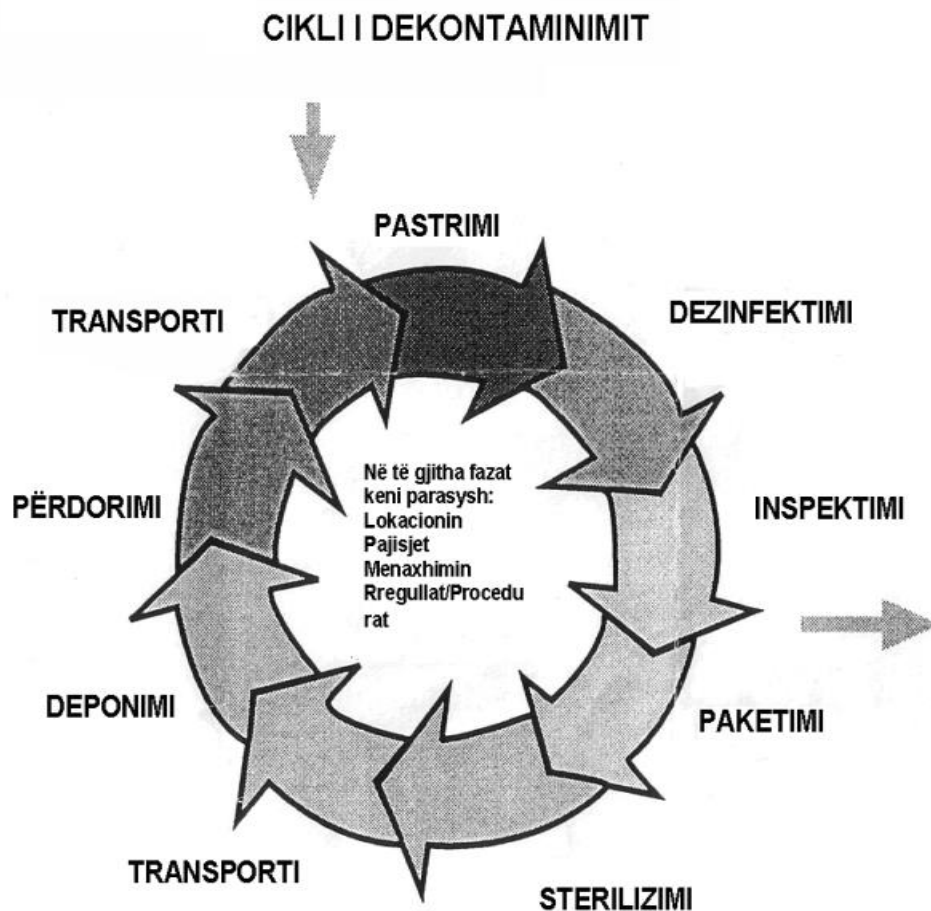


Figura Nr. 3 Cikli i dekontaminimit HSE Code of Practice for Decontamination of RIMD.

Version 1.0 (illustrated), 2007

Pastrimi manual

Të gjitha pajisjet, që duhen dezinfektuar apo sterilizuar, paraprakisht para pastrimit duhet të çmontohen. Për pastrim preferohet përdorimi i ujit të ftohtë, sepse ai e largon pjesën më të madhe të materialit proteinik (gjaku, këlbaza, etj). Përdorimi i ujit të ngrohtë apo dezinfektantit mund të shkaktojë koagulimin e materialit, i cili mandej shumë vështirë hiqet. Metoda më e thjeshtë dhe më e lirë është pastrimi i pajisjeve me furçë (brushë), e cila pozicionohet nën sipërfaqen e ujit për të evituar krijimin e aerosolëve. Pas përdorimit, furça duhet të dekontaminohet dhe të teret. Në fund pajisjet shpërlahen me ujë të ngrohtë dhe teren. Prej këtij momenti ato janë të gatshme për përdorim ose për dezinfektim e sterilizim. Personeli që punon me pajisjet e kontaminuar duhet të bart dorëza të cilësisë së mirë me qëllim të mbrojtjes në punë.

Përdorimi i ujit të nxehtë

Fig. Nr. 4 paraqet larësen e instrumenteve të metalta



Foto e marr në spitalin Rajonal Vushtrri

Një alternative për dezinfektimin e disa pajisjeve të ambientit është uji i nxehtë si dhe Temperatura dhe kohëzgjatja:

1. Pajisjet sanitare 80 °c 45-65 sekonda
2. Pajisjet e gatimit 80 °c 1 minutë

3. Rrobat 70 °c 25 minutë 95 °c 10 minuta

Materiet organike (serumi, gjaku, qelbi ose materiali fekal) ndikojnë në efikasitetin antimikrobik të të dy metodat. Sa më shumë që ka mikroorganizma të pranishëm, aq më kohë gjatë duhet të jetë dezinfektimi. Prandaj, pastrimi thelbësor para dezinfektimit ka rolin më të madh. Metodatat termike (nxehtësia)

Përkundrazi faktit se autoklavimi dhe sterilizimi me avull nuk janë të domosdoshëm për dezinfektim, nganjëherë mund të kenë përparësi për dekontaminimin e disa pajisjeve, siç është p.sh. spekulimi vaginal. Nxehtësia e lagësht në 70-100 °C Vlimi (100 °C) për së paku 5 minuta është metodë e thjeshtë dhe shumë e besueshme për inaktivimin e mikroorganizmave, përfshirë këtu edhe virusin e hepatitit B, virusin HIV dhe mikobakteret. Nëse bëhet me kujdes, kjo procedurë i përket dezinfektimit të nivelit të lartë. Paraprakisht, pajisjet duhet të pastrohen mirë, të vihen në kontejnerë dhe të mbushen me ujë. Uji nxehet deri sa ta mbërrijë pikën e vlimit. Dezinfektimi duhet të zgjasë 5 minuta prej momentit të mbërritjes së pikës së vlimit. Shtimi i tretjes 2% të bikarbonat natriumit e pengon korrodimin e instrumenteve dhe të pajisjeve të tjera. Nëse përdoren pincetatat e mëdha për nxjerrjen e materialit, ato duhet të zihen (apo autoklavohen) bashkë me dorëzat e tyre së paku një herë në ditë dhe të deponohen të terura. Bojleri (ena për vlim) duhet të zbrazet dhe të teret. Për instrumentet që mund të dëmtohen në temperaturë të vlimit, si alternativë është dezinfektimi në temperatura më të ulëta (p.sh. 80 °C në kohëzgjatje prej 5 minutash), me kusht që të ketë termometër për matjen e temperaturës. Dezinfektimi me ujë të nxehtë mund të bëhet në makina speciale të larjes p.sh. të rrobave, të enëve, të çarçafëve, të gypave për respiratorë, të qelqurave mikroskopike, dhe të instrumenteve kirurgjike para autoklavimit. Në këto makina procesi i pastrimit, dezinfektimit me ujë të nxehtë, dhe terjes janë të kombinuara në një procedurë shumë efektive, duke i përgatitur disa instrumente të gatshme për përdorim (p.sh. gypat për respirator) ose të sigurta për përpunim të mëtejshëm (p.sh. instrumentet kirurgjike). Shpëlarja dhe pastrimi fillestar i heqin shumicën e mikroorganizmave, prandaj edhe koha e nevojshme për dezinfektim mund të

shkurtohet, p.sh. 70°C për 3 minuta apo 80°C për 1 minutë. Makinat e përdorura për dezinfektim duhet të mirëmbahen dhe të kontrollohen rregullisht. Varësisht nga lloji i makinave dhe kompleksiteti i pajisjeve mund të realizohet dezinfektim i nivelit të ulët apo të lartë.

Metodat kimike të dezinfektimit

Para së të vendoset për përdorimin e dezinfektantëve, së pari duhet shikuar cila metodë është e mundshme dhe më praktike. Dezinfektantët kimikë përdoren kryesisht në dezinfektimin e pajisjeve që janë të ndjeshme ndaj nxehtësisë dhe në ato raste kur shfrytëzimi i pajisjeve për një përdorim nuk është ekonomik (kostefektiv).

Disa pajisje mjekësore (p.sh. bronkoskopët) kërkojnë dezinfektim të nivelit të lartë. Për këtë qëllim mund të përdoret një numër i kufizuar i dezinfektantëve (p.sh. glutaraldeidi 2%, peroksid hidrogjeni 6%, acidi peracetik 0.2-0.3%). Nëse kërkohet veprim sporocid i dezinfektantëve, atëherë pajisjet zhyten në glutaraldeid 2% në kohëzgjatje prej së paku 3 orësh. Dezinfektantët kimikë duhet të përgatiten të freskët dhe në përqëndrime korrekte sipas udhëzimeve të prodhuesit. Njëkohësisht, ato duhet të hidhen pas kohës së caktuar dhe pas serisë së caktuar të përdorimeve. Dezinfektantët duhet të deponohen në shishe të pastra me kapakë plastikë. Pas zbrazjes dhe para rimbushjes shishja duhet të pastrohet mirë. Shishet pjesërisht të zbrazëta nuk bën të mbushen, sepse ky veprim mund të shkaktojë kontaminim dhe shumëzim të mikroorganizmave rezistencë. Pas dezinfektimit, pajisjet duhet të shpërlahen mirë me ujë steril. Nëse nuk ka ujë steril, mund të përdoret uji i vluar dhe i ftohur. Pas shpëlarjes, pajisjet duhet të teren dhe të mbrohen mirë nga kontaminimi i serishëm.

Dezinfektimi i sipërfaqeve

Sipërfaqet e ndotura mund të pastrohen nga pluhuri dhe të dezinfektohen me agjensë përkatës kimikë. Në sipërfaqet e pastra, alkooli ka veprim të shpejtë baktericid dhe nuk ka nevojë për shpëlarje. Dyshemeja, sipërfaqet, lavabotë dhe drenazhet duhet të pastrohen me ujë dhe detergjent. Nuk ka nevojë për përdorimin rutinor të detergjentëve. Nëse ndodh derdhja e materialit të dyshimtë infektiv (p.sh. gjaku, këlbaza etj.), rekomandohet që para pastrimit të bëhet dezinfektimi. Pastrimi apo fshirja duhet bërë me dorëza duke përdorur 0.5-1% natrium hipoklorit (5.000- 10.000 ppm Cl₂)(zbardhues shtëpiak) apo me dezinficiensë të aktivitetit përkatës. Gjatë këtyre veprimeve gjithnjë duhet bartur dorëza. Nëse përnjëherë pastrohet ndonjë sipërfaqe e madhe me dezinficiensë të klorit, atëherë mund të lirohen sasi të mëdha të gazit klorik, i cili mund të jetë i rrezikshëm për personelin. Nëse materiali i derdhur largohet

menjëherë, atëherë nuk ka nevojë të bëhet dezinfektimi i tërë hapësirës, por mjafton vetëm pastrimi i mirë.

I. 7. a. Dezinfektimi i pajisjeve

Dezinfektimi është proces i zvogëlimit të numrit të mikroorganizmave patogjenë, por jo edhe i sporeve të baktereve, nga objektet e ndryshme apo nga lëkura, deri në atë shkallë që nuk e dëmton shëndetin. Dezinfektimi largon mikroorganizmat pa sterilizim të plotë për të ulur kështu transmetimin e mikroorganizmave ndërmjet pacientëve.

Teknika e dezinfektimit duhet:

- Të plotësojë kriteret për të vrarë mikroorganizmat
- Të ketë efekt detergjenti
- Të veprojë në mënyrë të pavarur nga numri i baktereve të pranishme, fortësia e ujit, dhe prania e sapunit dhe proteinave (të cilat frenojnë disa dezinfektantë).

Që të jone të pranueshëm në ambientin e spitalit duhet të jenë: të përdorshëm lehtë

- Jo gaz shpërthyes [2, 3, 7].
- Jo dëmtues për pajisjet, stafin ose pacientët
- Të mos kenë erë të papëlqyeshme

Efektiv në një kohë të shkurtër

Dezinfektimi i Endoskopit si një nga pajisjet invazive më të përdorshme Sterilizimi i Endoskopit si instrument invaziv minimal dhe pajisjeve të tjera aksesore të ngjashme është i nevojshëm por është i vështirë për arsye të strukturës së ndërlikuar të ndërtimit të këtyre instrumenteve.

Endoskopët janë pajisje mjekësore të cilat mund të jenë problematike për tu pastruar dhe dezinfektuar (kanalet e ngushtë, struktura e brendshme komplekse). Produktet dhe proceset e përdorura (dezinfektimi kimik ose termokimik) mund të mos jenë të sigurt aq sa metodat e sterilizimit. Për të ulur transmetimin e infeksioneve spitalore nëpërmjet endoskopit duhet të zbatohet një procedurë standarde sistematike.

Dezinfektimi i Endoskopit dhe hapat e pastrimit:

1. Menjëherë pas përdorimit duhet të pastrohet kanali i ajrit dhe ujit duke pompuar me forcë ajër dhe ujë ose detergjent që pompohet dhe thithet në kanalin e biopsisë dhe aspirimit për të larguar mbeturinat organike.

2. Të gjitha pjesët e ndashme (kapakët dhe valvulat) duhet të futen në një enë me solucion pastrues dhe pjesa e jashtme e endoskopit duhet të fshihet me kujdes.

3. Të gjitha pjesët e arritshme duhet të shpëlahen me ujë të rrjedhshëm ose solucion pastrues, të fshihen (duke përdorur një furçë sterile ose një përdorimëhe). Para zhytjes në solucion endoskopi duhet të testohet për ndonjë dëmtim.

Pas pastrimit mekanik endoskopi duhet të pastrohet dhe dezinfektohet qoftë mekanikisht qoftë automatikisht. Në të dyja rastet procesi i plotë përfshin disa hapa.

4. Pastrimi duke përdorur një detergjent pastrues të aprovuar (i cili nuk duhet të ripërdoret).

5. Shpëlarje (ujë i rrjedhshëm është i mjaftueshëm në këtë fazë).

6. Dezinfektimi duke përdorur një dezinfektantë të aprovuar të nivelit të lartë.

7. Shpëlarje: Niveli i pastërtisë bakteriale të ujit të përdorur do të varet nga përdorimi i mëtejshëm i endoskopit (ujë i kontrolluar bakteriologjikisht ose i sterilizuar).

8. Tharja: Nëse endoskopi do të përdoret përsëri kjo fazë përfshin vetëm tharjen me anë të futjes së ajrit për të larguar ujin e mbetur [1, 2, 3, 4, 7].

Në përdorimin e një dezinfektanti duhet të ndiqen dhe rekomandimet e përdoruesit.

Produkte dhe procese të ndryshme sigurojnë nivele të ndryshme dezinfektimi.

Desinfektantët janë klasifikuar si të fortë, - me nivel mesatar- ose me nivel të ulët dezinfektimi.

Dezinfektim me nivel të lartë (kritik)- Kjo shkatërron të gjithë mikroorganizmat me përjashtim të kontaminimit të lartë me spore bakteriale.

Dezinfektim mesatar (gjysëmkritik)- kjo inaktivizon mykobakterium tuberculosis, bakteret vegjetative, shumicën e viruseve dhe myqeve (fungi) por nuk vret sporet bakteriale.

Dezinfektim i nivelit të ulët (jo kritik)- Mund të vrasi shumicën e baktereve, disa viruse, dhe disa myqe por nuk është i aftë për të vrarë baktere rezistente si M. Tuberculosis, ose sporet bakteriale. Këto nivele të dezinfektimit arrihen duke përdorur produktet kimike të përshtatshme në mënyrën e duhur dhe për nivelin e duhur të dezinfektimit Dekontaminimi dhe Paketimi.

Paketimi në hapësirën për dekontaminim bëhet pranimi i materialit, pastrimi dhe sipas mundësive edhe dezinfektimi. Personeli duhet të mbajë doreza dhe përparëse plastike.

Preferohet që personeli po ashtu të jetë i pajisur edhe veshje të posaçme me mëngë rezistente ndaj lëngjeve, maska kirurgjikale dhe syze mbrojtëse.

Në këtë hapësirë bëhet grumbullimi dhe paketimi i materialeve të pastra por ende jo sterile.

Në këto materiale duhet të shënohet saktësisht përmbajtja, data e përpunimit dhe afati i skadencës. Materialet për paketim përfshijnë: Letrën e cila parandalon kontaminimin nëse është e padëmtuar, mban sterilitetin për një kohë të gjatë dhe mund të përdoret gjithashtu për të mbështjellë pajisjet e ndotura pas procedurës, disa lloj Plastikash; vetëm polietileni dhe polipropileni janë të përshtatshme për sterilizim me Oksid Etilenikutitë e ndryshme (kontenieret) mund të përdoren vetëm nëse janë të përbëra me materiale të caktuara për një procedurë të vetme trajtimi përnjë pacient të vetëm. Ato duhet të jenë të pajisura me filtër dhe valvulitit me anë të së cilës duhet të kontrollohen rregullisht. Sistemet e ambalazhimit për mjete sterile duhet të jenë në përshtatje me legjislacionin lokal dhe/ose normat e përcaktuara por megjithatë karakteristikat e përgjithshme janë: Të sigurojnë një izolim adekuat ,të sigurojnë një barrierë të mirë ndaj disa lëndëve specifike, të jenë rezistence ndaj kushteve fizike të procesit të sterilizimit, të sigurojnë një barrierë të mirë kundrejt lëngjeve, të lejojnë largimin e ajrit, të lejojnë largimin e mikrobeve, të jenë rezistence ndaj faktorëve fizike, ti rezistojnë grisjes dhe shpimeve, të mos ketë vrima, të mos ketë përbërës toksikë, të kenë përqindje të vogël garze, të kenë një përpjesëtim pozitiv të kostos me përfitimet. Të përdoren në përputhje me instruksionet e shkruara të prodhuesit, të jenë të datuar.

Inspektimi është vëzhgimi i rregullsisë së procedurës së kryer ndërsa paketimi nënkupton renditjen dhe grumbullimin e instrumenteve, pajisjeve dhe materialit tjetër të nevojshëm. Nevojitet shënimi i përmbajtjes, data e paketimit dhe skadimit të sterilizimit [1, 2].

I. 7. b. Sterilizimi

Sterilizimi është procedurë e shkatërrimit të gjithë mikroorganizmave të gjallë, përfshirë edhe sporet e baktereve. Për një objekt thuhet se është sterilizuar nëse e ka kaluar ecurinë e kontrolluar të sterilizimit. Sterilizimi mund të arrihet me metoda fizike dhe kimike Sterilizimi

kërkohej për produktet mjekësore që do të bien në kontakt me indet sterile të organizmit si: solucionet parenterale dhe barnat, si dhe për pajisjet e ndryshme kirurgjikale.

Shërbimi i sterilizimit duhet të udhëhiqet nga një person i kualifikuar. Tërë personeli duhet të trajnohet dhe të ketë edukim të vazhdueshëm në fushën e sterilizimit dhe të dezinfektimit. Për të gjitha procedurat duhet të jenë të shkruara protokollet e punës dhe po ashtu duhet të ketë program të kontrolluar për sigurimin e cilësisë.

Hapësira e shërbimit të sterilizimit qendror duhet të përbëhet nga 4 zona:

- Zonën për dekontaminim,
- Për paketim,
- Për sterilizim, dhe- Për menaxhimin dhe ruajtjen e tyre [1, 2, 3, 4].

I. 7. c. Metodat Kryesore të Sterilizimit

Sterilizimi Termal

- Sterilizim i Njomë: Ekspozim ndaj avujve të lagësht në temp. 121°C për 30 min ose 134°C për 13 minuta në autoklavë.



Figura .Nr. 5 Foto e autoklavat Spitali Rajonal Vushtrri 20 Nëntor 2016

Sterilizimi me avull është metoda më e shpeshtë dhe më e preferuara për sterilizimin e pajisjeve që depërtojnë lëkurën dhe mukozat, me kusht që nxehtësia dhe lagështia mos t'i

dëmtojnë ato pajisje. Sterilizimi me avull është metodë e sigurt, jo toksike, e lirë, sporocide, arrin shpejt nxehtësinë dhe depërton mirë në materiale

Tabela. Nr. 2 Përdorimi i autokllavit sipas gravitacionit

Tipi I Autokllavit	Paketimi	Temperatura	Koha e ekspozimit	Koha e Terjes
Gravitacional	I mbështjellë	121 °C	30 minuta	30 minuta
Gravitacional	I mbështjellë	132 °C	15 min	30-40 min
Paravakum	I mbështjellë	132 °C		
Gravitacional	“sterilizimi i shpejtë” (I pambështjellë)	132 °C	3-4 min	
Gravitacional	Mbeturina mikrobiologjike	121 °C	45 min	

- Sterilizim i Thatë: Ekspozim në 160°C për 120 minuta ose 170°C për 60 minuta; ky sterilizim konsiderohet më pak i sigurt se metoda e parë veçanërisht për disa pajisje me shumë tuba, gropa, etj..

Sterilizimi Kimik

- Oksidi i etilenit dhe formaldehida janë nxjerrë nga përdorimi për arsye të sigurisë në lidhje me emetimin e gazrave
- Acidi Paracetik përdoret gjerësisht në SHBA dhe disa shtete të tjera në proceset e sistemit automatik të sterilizimit

Duhet përkujdesur që gjithmonë pajisjet e sterilizuara të dalin jashtë departamentit sipas sistemit të rrotacionit: d.m.th. sipas datës së sterilizimit, duke filluar me pajisjet më të vjetra.

Kushtet e ruajtjes janë të rëndësishme për të ruajtur sterilizimin.

Përdoruesi duhet të kontrollojë integritetin e paketimit para përdorimit.

Parametrat e Kontrollit të Cilësisë për Procesin e Sterilizimit duhet të përfshijë të dhëna të tilla në lidhje me procesin si:-ngarkesa,përçindja,temperatura dhe kohëzgjatja,Testim i rregullt (të paktën çdo ditë) fizik dhe kimik,Testim biologjik (të paktën çdo javë), procesi i avullimit (bacili i sterrotermofilus), përpunim me oksid etileni (Bacillus subtilis v.niger).

Mirëmbajtja e rregullt duhet të dokumentohet. Për të gjitha llojet e sterilizimeve duhet të regjistrohen këto të dhëna: si, data e shërbimit, modeli dhe numri i serisë vendi, përshkrimi i pjesëve zëvendësuese, regjistrimet e testeve biologjike, Bowie-Dick test, nënshkrimi i kontrolluesit [2, 3, 7, 8].

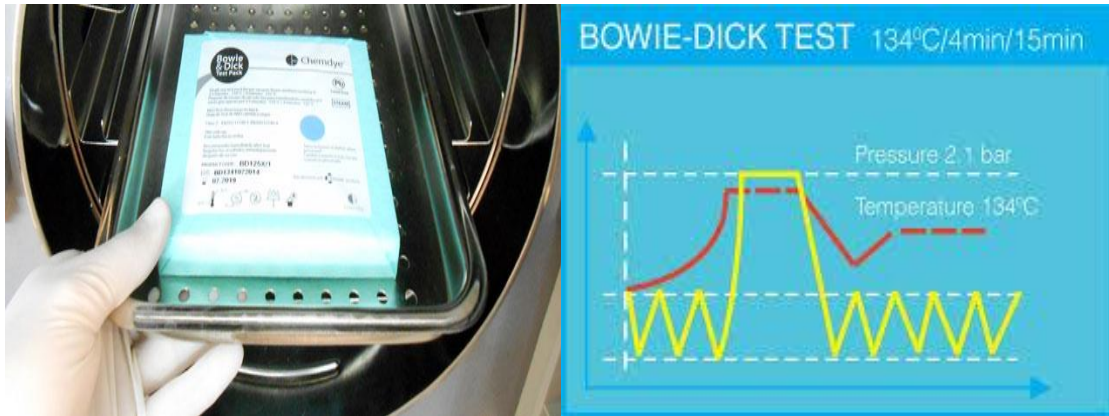


Figura. 6 Kontrolli i Sterilizimit metoda Bowie-Dick test

Ndryshimi i ngjyrës së indikatorëve kimikë, të cilët vendosen në sipërfaqen e jashtme të çdo paketime dëshmon se pakoja iu ka nënshtruar procesit të sterilizimit. Po ashtu, indikatorët kimikë duhet të vihen edhe në brendi të pakove për të vërtetuar depërtimin e avujve, si shtojcë e metodës fizike të kontrollit të sterilizimit. Para përdorimit të sterilizimit, preferohet që çdo ditë të bëhet testi i Bovi/Dikut në 134°C tek autoklavët me prevakum. Në disa vende, për vrojtimin e procesit të sterilizimit me nxehtësi dhe atij me avull përdoren indikatorët biologjikë të sporeve të *Geobacillus steraothermophilus*, kurse sporet e *Bacillus atrophaeus* përdoren për kontrollin e etilenoksidit. Indikatorët biologjikë vendosen në pajisje të veçantë për kontrollin e sterilizimit apo në pakot kontrolle që janë pjesë reprezentative e segmentit që sterilizohet .Transporti nënkupton transportin e instrumenteve, pajisjeve dhe materialit tjetër të nevojshëm ndërsa deponimi nënkupton sistemimin e instrumenteve, pajisjeve dhe materialit tjetër të nevojshëm. Deponimi duhet përkujdesur që gjithmonë pajisjet e sterilizuara të dalin jashtë departamentit sipas sistemit të rrotacionit: d.m.th. sipas datës së sterilizimit, duke filluar me pajisjet më të vjetra dhe gjithmonë duhet të jenë të terura. Përdorimi nënkupton përdorimin e instrumenteve, pajisjeve dhe materialit tjetër të nevojshëm. Zakonisht përdoret materiali i sterilizuar më parë nëse ai nuk është i desterilizuar, pra nuk është hapur dhe nuk i ka kaluar afati i përdorimit si material steril [2, 3].

Vërja në funksion e të gjitha i të gjitha këtyre e tapave të ciklit të dekontaminimit ka për qëllim reduktimin e mundësisë së paraqitjes dhe përhapjes së infeksioneve intrahospitalore.

I. 7. d. Mirëmbajtja e higjienës në spital

Personeli për mirëmbajtjen e higjienës në spital duhet të jetë i stërvitur dhe i mbikëqyrur. Në ecurinë e mirëmbajtjes së higjienës në spital, duhet doemos të vihet njëfarë rendi dhe rregulli. Planifikimi i mirëmbajtjes së higjienës në spital duhet të përcaktojë: Detyrat dhe përgjegjësitë e secilit punëtor veç e veç; Ecurinë e punës, përfshirë edhe mirëmbajtjen e pajisjeve specifike, materialin harxhues, pastrimin dhe deponimin e pajisjeve, ndërrimin e kokës së leckës për pastrim; Kërkesat për llojet e tretjeve për pastrim dhe shpeshtësinë e zëvendësimit të tyre; Përdorimin e pajisjeve personale mbrojtëse; Shpeshtësinë e pastrimit të dyshemeve, të mobileve, të mureve, të tualeteve, të instalimeve të fiksuara (makinat e akullit, gypat dhe rrjetet e ajrit); Shpeshtësinë e ndërrimit të perdeve. Në rrethana të caktuara mund të ketë nevojë për procedura speciale të pastrimit dhe mirëmbajtjes, si p.sh. gjatë epidemive të diarreve, Muret nuk grumbullojnë papastërti, prandaj edhe nuk kanë nevojë të pastrohen shpesh. Sipërfaqet horizontale siç janë dyshemetë dhe pajisjet e thjeshta, si mbajtëset për infuzione dhe kornizat e shtretërve të të sëmurëve, mirëmbahen duke i pastruar me ujë dhe detergjent. Mirëpo, disa pajisje dhe pjesë që preken më shpesh kanë nevojë për pastrim më të shpeshtë dhe më detal, përfshirë edhe nevojën për dezinfektim. Shembuj të tillë janë parrakët e shtretërve, dorëzat e dyerve dhe pjesët që mund të stërpiken.

I. 7. e. Roli Lavanderis se rrobave në spital dhe parandalimi i infeksionit

Manipulimi i kujdesshëm dhe përpunimi i rrobave dhe i pajisjeve të tjera të përlyera mund të parandalojë përhapjen e agjentëve infektivë. Sigurimi i ndërresave të reja dhe të pastra, po ashtu, ndikon në shtimin e komoditetit të sëmurëve.

Ecuria e klasifikimit

- Të evitohet kontaminimi i duarve me ndërresa të përlyera,
- Të vihen ndërresat e përlyera në qeskën e ndërresave në kontejner,
- Të mbyllen qeskat pas mbushjes me 3/4 e saj.

Nëse ka vetëm qeska nga pëlhura, punëtorët duhet të bartin dorëza dhe të manipulojnë me këto qeska me kujdes.

Qeskat me ndërresa të ndyra duhet të vihen në vend të sigurt derisa të transportohen në destinimin përfundimtar, Qeskat me ndërresa të përlyera duhet të dërgohen në një vend që është i dedikuar veçanërisht për klasifikimin e tyre para pastrimit, Personat që punojnë në klasifikimin e ndërresave të papastra duhet të aftësohen profesionalisht për procedurat e punës dhe për përdorimin e masave mbrojtëse kundër infektimit eventual, Personat që punojnë në klasifikim duhet të furnizohen me dorëza rezistence ndaj ujit dhe përparëse plastike apo mantele rezistencë ndaj ujit me qëllim të mbrojtjes në punë.

• Procesi i larjes

Cikli i parapastrimit prej 15 minutash i heq pjesët masive të ndyrësirave, Nëse lahen me ujë të ftohtë, duhet t'u shtohen kimikale si p.sh. zbardhues (në çdo litër të ujit duhet shtuar 2 ml zbardhues) me detergjent për ta lehtësuar dezinfektimin, Nëse nuk përdoret uji i ftohtë me detergjent dhe zbardhues, duhet bërë larja në temperaturë të lartë (>71°C) Gjatë shpëlarjes duhet shtuar ndonjë agjent acidik, me qëllim të uljes së alkalinitetit pas shpëlarjes. Kjo procedurë e zvogëlon iritimin e lëkurës dhe redukton më tutje numrin e baktereve të pranishme; Terja me ajër të nxehtë apo me rreze të diellit e redukton edhe më tej numrin baktereve të pranishme, sikurse edhe hekurosja me hekur të nxehtë, ndërresat e pastra duhet të deponohen dhe transportohen në atë mënyrë që të evitohet kontaminimi i kryqëzuar, Para se të dërgohen në shërbimin për sterilizim, ndërresat paraprakisht duhet të palohen (mbështillen). Tërë personeli duhet të vetëdijësohet për rrezikun që i kanoset punëtorëve të lavatores nëse

objektet e mprehta lihen nëpër ndërresat e përlyera. Personeli i lavatores duhet të jetë i vaksinuar ndaj sëmundjeve vaksinë preventabile.

I. 7. f. Menaxhimi i Mbetjeve Spitalore

Deponimi groposja dhe djegia janë mënyrat më të shpeshta të dispozimit të mbeturinave spitalore. Personeli që është përgjegjës për pastrimin e pajisjeve të kontaminuar duhet të ketë trajnim adekuat, të bartë veshmbathje adekuate mbrojtëse, siç janë dorëzat, përparëset, maskat, syzet mbrojtëse dhe duhet të jenë të vaksinuar, përfshirë edhe vaksinimin kundër hepatitit B.

Mbeturinat klinike, që janë potencialisht të kontaminuar, mund të ndahen në mbeturina laboratorike (përfshirë materialet anatomike dhe mikrobiologjike) gjaku me lëngjet trupore si dhe mbeturinat e tjera, siç janë fashat e kontaminuar dhe objektet e mprehta. Gjatë shpërthimit të epidemive të ndryshme, disa materiale duhet të kenë qasje dhe dispozim special. Mbeturinat klinike

kërkojnë dispozim special dhe mund të dallohen nga mbeturinat e tjera të përgjithshme, duke përdorur qeska plastike të ngjyrosura me ngjyra të veçanta.

Mbeturinat që japin barnat për kimioterapi, kërkojnë dispozim special - djegie në temperatura të larta. Mbeturinat e tjera të rrezikshme kimike (p.sh. formaldeidi apo hidrokسيد natriumi) kërkojnë metoda të tjera [1, 2, 8].

Menaxhimi i Sigurt i mjeteve të mprehta

Termi "të mprehta" përfshin objekte si aget, bisturitë, gërshërët, ampulat e thyera dhe objekte të tjera që mund të shkaktojnë laceracione apo shpime të lëkurës.

Mënyra e drejt e menaxhimit të mbeturinave spitalore sistemi i tre koshave

MBETJE TE ZAKONSHME	MBETJE INFEKTIVE SPITALORE		MBETJE TE MPREHTA
			
<p>LETER KARTON PLASTIK PAKETIME QELQ TUBA SERUMESH (TE PASTER) QESE SOLUCIONI SALIN MBETJE USHQIMORE LULE PELENA PRODUKTE SANITARE</p>	<p>TAMPONE, FASHO, PAMBUK TE NDOTUR ME GJAK TAMPONE, FASHO TE NDOTUR ME LENGJE TRUPORE QESËT E GJAKUT PER TRANSFUZION SISTEMET E KATETEREVE PJESE TRUPI TE PADALLUESHME (TEVOGLA)/MOSTRA INDESH TUBA DHE FILTRA DIALIZE PELENA NGA PACIENTET E INFEKTUAR SHIRINGA TE NDOTURA ME GJAK TUBA TE NDOTUR ME GJAK AMPULA PLASTIKE TE NDOTURA ME GJAK DOREZA KIRURGJIKALE TE PERDORURA/MASKA/PERPARESE</p>		<p>AGE BISTURI QELQE TE THYERA AMPULA GJILPERA QEPIE PLAGE KAPSE PER PLAGEN INSTRUMENTA TE TJERE TE MPREHTË</p>

Fig. 5 Sistemi i tre koshave Foto nga Spitali rajonal Durrës 2014

Të mprehtat janë përgjegjëse për një numër të lartë të dëmtimeve të stafit çdo vit në spital. Menaxhimi i sigurt i të mprehtave mund të ndihmojë në zvogëlimin e rrezikut të dëmtimeve, dhe për pasojë në marrjen e infeksioneve të tilla si viruset që transmetohen nëpërmjet gjakut si nga stafi ashtu edhe nga pacientët. Një numër i lartë i dëmtimeve nga të mprehtat ndodhin gjatë mbledhjes dhe asgjësimit të mbetjeve spitalore. Për këtë arsye, parandalimi i dëmtimeve nga të mprehtat është një pjesë e rëndësishme e praktikave rutinë, duke përfshirë menaxhimin dhe asgjësimin e të mprehtave në një mënyrë që do të parandalojë dëmtimin e përdoruesit dhe

të tjerëve. Tubat e IV-së nuk duhet të priten për tu hedhur pasi kjo do të krijonte një majë të mprehtë dhe një rrezik për daljen e mbetjeve të tubit. Është përgjegjësi e përdoruesit (në rastin tonë e infermierit) për të siguruar asgjësimin e sigurt të së mprehtës. Kontenierët e sendeve të mprehta duhet të jenë të disponueshëm në çdo kohë dhe në çdo zonë. Të mprehtat duhet të hidhen menjëherë pas përdorimit në kontenierin e tyre i cili duhet të ndodhet mundësisht afër vendit të përdorimit, Të mprehtave nuk duhet t'u vihet kapaku pas përdorimit, përpara hedhjes së tyre direkt në një kontenier të mprehtash:

- Asnjëherë mos përthyeri apo thyeni aget pas përdorimit
- Mos i ndani aget nga shiringat apo pajisjet e tjera; gjithmonë hidhini si një pajisje e vetme
- Tubat e serumit duhet të hidhen direkt në kontenierin e të mprehtave pa i prerë ato
- Bisturitë e skalpelit duhet të hiqen duke përdorur force pse
- Asnjëherë mos mbushet një kontenier i të mprehtave më shumë se 3/4
- Asnjëherë mos lini një të mprehtë të dalë nga kontenieri i të mprehtë

Roli i infermieres dhe njohurit e infermierore përfshijnë në fakt zbatimin e masave, veprimeve, aktiviteteve si dhe strategjive të planifikuara dhe të hartuara në bashkëpunim me pacientët.

Këto ndërhyrje infermierore duhet të jenë në përputhje me nevojat dhe reagimet e pacientëve. Në procesin e hulumtimit njohurit infermierore ishin fokusuar në veçanti në aktivitete që mundësojnë ndërprerjen e zinxhirit të infeksionit, menaxhimin e drejtë të mbeturinave spitalore, zbatimin e ciklit të dekontaminimit dhe larjen e drejtë të duarve [1, 2, 3, 7].

II. QËLLIMI I PUNIMIT

Qëllimi parësor i punimit është:

1. Hulumtimi i njohurive të infermiereve për infeksionet spitalore dhe parandalimin e tyre në Spitalin Sheikh Zayed në Vushtrri.
2. Hulumtimi i njohurive të infermiereve për masat e higjienës personale të personelit shëndetësor (larjen e duarve) në parandalimin e infeksioneve spitalore;
3. Hulumtimi i njohurive të infermiereve në zbatimin e ciklit të dekontaminimit;
4. Hulumtimi i njohurive të infermiereve për menaxhimin e mbeturinave infektive në parandalimin e infeksioneve spitalore.

Qëllimi dytësor i punimit është:

- Të zhvillojë një model të vlerësimit të ngritjes profesionale të stafit infermierior në parandalimin e infeksioneve spitalore;
- Edukim i vazhdueshëm profesional i infermiereve në parandalimin e infeksioneve spitalore.

II.1. HIPOTEZA

H1: Njohuritë e infermiereve për infeksionet spitalore dhe parandalimin e tyre në Spitalin Sheikh Zayed në Vushtrri, janë të qëndrueshme, pasi që përgatitjet dhe njohuritë e tyre ndihmojnë në uljen e infeksioneve spitalore.

III. MATERIALI DHE METODAT E HULUMTIMIT

Ky hulumtim paraqet një studim prospektiv të njohurive të infermiereve për infeksionet spitalore dhe parandalimin e tyre ne Spitalin Sheikh Zayed në Vushtrri të realizuar gjatë periudhës kohore nga 22. 09. 2016 deri me 29.09.2016. Në studim i kemi përfshirë të gjithë Infermieret e punësuar gjatë kësaj periudhe të cilët i kanë plotësuar kriteret për përzgjedhje ne forme të randomizur.

Në pjesën e parë, hyrje, janë të përshkruara të dhënat me te reja për infeksionet spitalore dhe parandalimin e tyre dhe gjithashtu fokusohet në rolin e kujdesit infermieror në njohjen dhe parandalimin e infeksioneve spitalore. Në pjesën e dytë prezantohet qëllimi parësor i hulumtimit e qe përfshin katër pika të hulumtimit të njohurive infermiere të hulumtimit si: njohuritë e tyre për infeksionet spitalore dhe parandalimin e tyre në Spitalin Sheikh Zayed ne Vushtrri, masat e higjienës personale të personelit shëndetësor (larjen e duarve) në parandalimin e infeksioneve spitalore, zbatimin e ciklit të dekontaminimit si dhe menaxhimin e mbeturinave infektive ne parandalimin e infeksioneve spitalore. Gjithashtu presentohet edhe qëllimi dytësor i punimit i cili konsiston ne zhvillimin e një modeli të vlerësimit të ngritjes profesionale të stafit infermieror ne parandalimin e infeksioneve spitalores dhe në edukimin e vazhdueshëm profesional të infermiereve në parandalimin e infeksioneve spitalore.

Pjesa tretë përmban materialin dhe metodat e punës, pjesa e katërt përmban rezultatet e punimit, ndërsa pjesa e pestë paraqet diskutimin, përfundimin dhe rekomandimet.

Të dhënat e fituara janë prezantuar përmes tabelave dhe grafikoneve.

III. 1. MOSTRA

Struktura e të anketuarve është e përbërë nga infermier/e të punësuar në Spitalin Sheikh Zayed në Vushtrri. Ne studim janë e përfshirë 40 Infermier/e të njësive të ndryshme të Spitalit Rajonal si:

1. Reparti i Mjekimit Intensiv
2. Reparti i Kirurgjisë
3. Reparti i Pediatriisë
4. Reparti i Gjinekologjisë
5. Reparti i Maternitetit

Grumbullimin e të dhënave lidhur me njohuritë e infermierëve për infeksionet spitalore dhe parandalimin e tyre e kemi bërë në bazë të anketës anonime te strukturuar enkas për këtë qëllim. Mënyra e përzgjedhjes se te anketuarve ka qenë e randomizuar, duke përfshirë çdo të dytin infermier/e në repartet e Spitalit ku është realizuar ky hulumtim dhe ku punojnë gjithsej 80 infermier/e.

Në anketë kemi përcaktuar gjithsej 29 pyetje, të grupuara në pese tërësi: I. Grupin e pyetjeve lidhur me të dhënat demografike të infermierëve (pyetjet 1-4), II. Grupin e pyetjeve lidhur me njohuritë e infermierëve për infeksionet spitalore dhe parandalimin e tyre ne Spitalin Sheikh Zayed ne Vushtrri (pyetjet 1-11), III. Grupin e pyetjeve për njohuritë e infermiereve për masat e higjienës personale të personelit shëndetësor (larjen e duarve) në parandalimin e infeksioneve spitalore (pyetjet 1-5), IV. Grupin e pyetjeve për njohuritë e infermiereve në zbatimin e ciklit të dekontaminimi (pyetjet 1-6) dhe V. Grupi i pyetjeve për njohuritë e infermiereve per menaxhimin e mbeturinave infektive ne parandalimin e infeksioneve spitalore (pyetjet 1-3).

Të ankoruarit u informuan lidhur me hulumtimin dhe informatat e nevojshme si dhe për aprovimin nga menaxhmenti i Spitalit Sheikh Zayed, ne Vushtrri. Pjesëmarrësit ne hulumtim gjithashtu u informuan lidhur me kohën e plotësimit dhe kthimit të pyetësorit gjate periudhës kohore nga 22.09.2016 deri me datën 29.09.2016.

Instrumenti me të cilin është menduar të studiohet kjo tematikë ka qenë pyetësori i strukturuar dhe i përbërë nga pyetjet e tipit të mbyllur, mënyra e përzgjedhjes e pjesëmarrësve në

hulumtimin tonë ka qenë e randomizar, të përfshirë kanë qen çdo i dyti infermier në Spitalin ku është realizuar ky hulumtim janë gjithsej 80 infermier ku nga kjo që i bie që ç'do i dyti infermier është përfshirë në hulumtim, pra gjithsej 40 Infermier te njësive të ndryshme.

Grumbullimi i të dhënave lidhur me njohuritë e infermiereve për infeksionet spitalore dhe parandalimin e tyre, është bërë përmes anketës anonime dhe të dhënat janë përdorur vetëm për këtë punim. Programi për përpunimin e statistikave është përdorur programi Aplikativ Excel

IV. Rezultatet Punimit

Në hulumtim janë anketuar 40 Infermier/e të pesë njësive të spitalit Rajonal Sheikh Zayed në Vushtrri:

1. Reparti i Mjekimit Intensiv
2. Reparti i Kirurgjisë
3. Reparti i Pediatriisë
4. Reparti i Gjinekologjisë
5. Reparti i Maternitetit

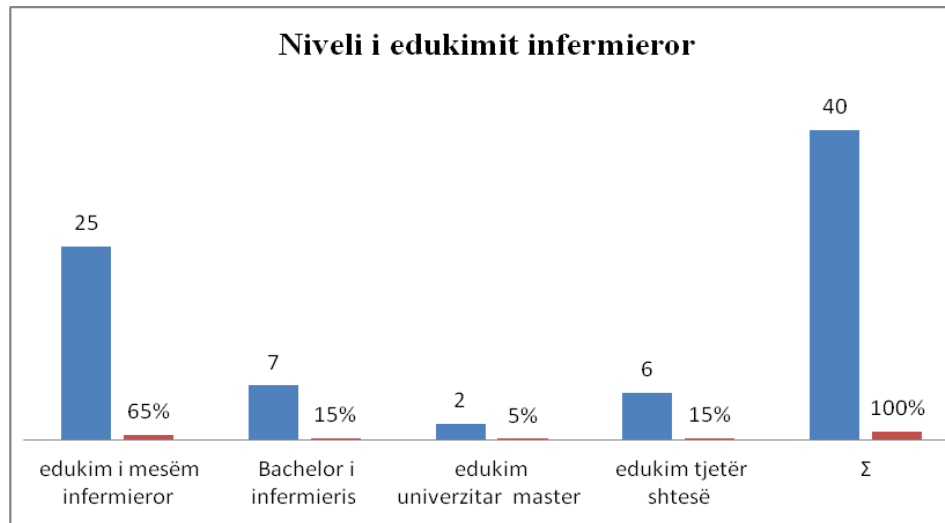
Të dhënat demografike

Nga 40 infermier/e te anketuar, 39 (94%) i takuan gjinisë femërore dhe vetëm një i anketuar ishte i gjinisë mashkullore (Tabela 1).

Gjinia e të anketuarve	F	%
Femër	39	94%
Mashkull	1	6%
Σ	40	100%

Tabela 1. Prezantimi i strukturës gjinore te të anketuarve

Nga 40 (100%) pjesëmarrës në hulumtim, 25 (65%) kanë pasur nivel të mesëm të edukimit infermieror kurse 7 (15%), prej tyre kanë pasur nivel të lartë të edukimit infermieror. Vetëm 2 (5%) të tjerë kishin nivel univerzitar të edukimit ndërsa 6 (15%) kanë pasur edhe edukim tjetër shtesë (Grafikoni 1).



Grafikoni 1. Presentimi i nivelit të edukimit të infermiereve

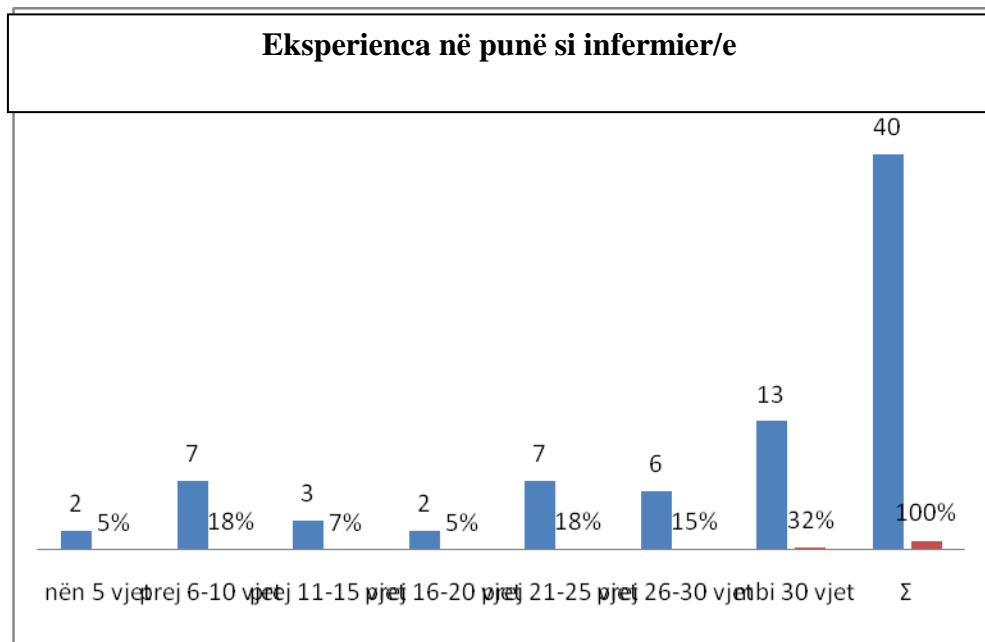
Nga 40 (100%) pjesëmarrës në hulumtim, nuk kishte pjesëmarrës nën moshën 28 vjeçe. Nga ky numër i përgjithshëm i subjekteve, 6 (15%) prej tyre i takuan grup-moshës nga 28-36 vjeçe, ndërsa numri më i madh i subjekteve, 25 (62%), i takuan grup-moshës nga 37-45 vjeçe. Numri më i vogël i subjekteve, 9 (23%) kanë pasur moshën mbi 40 vjet (Tabela 2).

Mosha e të anketuarve	F	%
Nen 18 vjet	0	0%
Prej 19-27 vjet	0	0%
28-36 vjet	6	15%
37- 45 vjet	25	62%
Mbi 40 vjet	9	23%
Σ	40	100%

Tabela 2. Prezantimi i moshës të anketuarve

Në grafikunin numër 2 është prezentuar përvoja e punës së infermiereve të anketuar. Prej 40 të anketuarve, 2 (5%) prej tyre kanë pasur përvoja pune infermiere nën 5 vite kurse 7 (18%) prej tyre kanë pasur përvoja pune nga 6-10 vite. Vetëm 3 (7%) prej tyre kishin përvojë pune nga 11-15 vite kurse 2 (5%) prej tyre kishin përvojë pune nga 16-20 vite. Nga numri i përgjithshëm i subjekteve, 7 (18%) kanë pasur përvojë pune nga 21-25 vite ndërsa 6 (15%)

kanë pasur përvojë pune nga 26-30 vite. Numri më i madh i subjekteve, 13 infermiere (32%) kanë pasur përvojë pune mbi 30 vite.

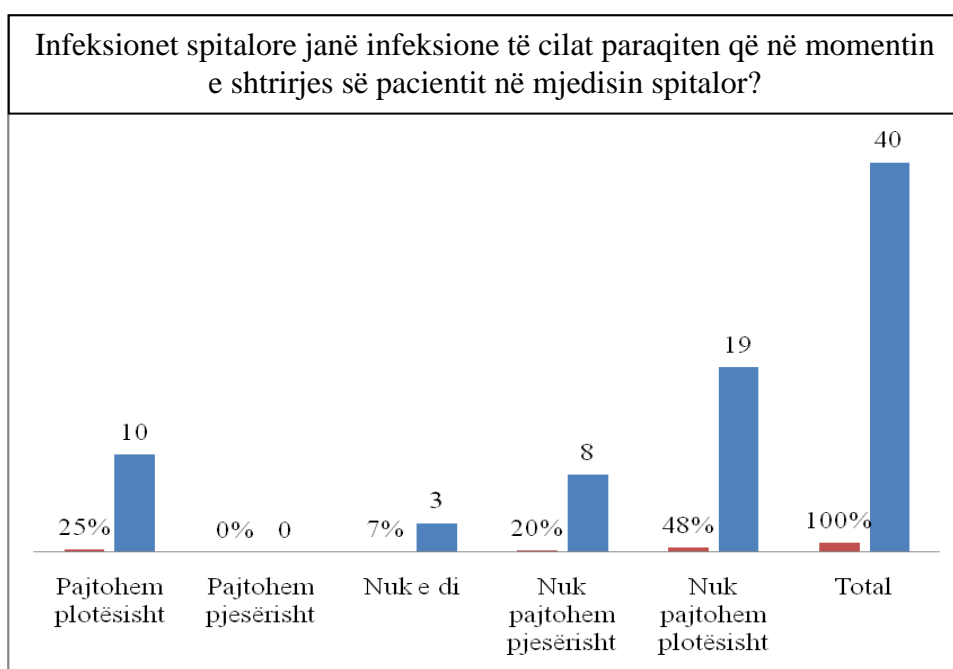


Grafikoni 2. Eksperienca në punë si infermier /e

1. Hulimtimi i njohurive te infermiereve për infeksionet spitalore dhe parandalimin e tyre në Spitalin Sheikh Zayed në Vushtrri.

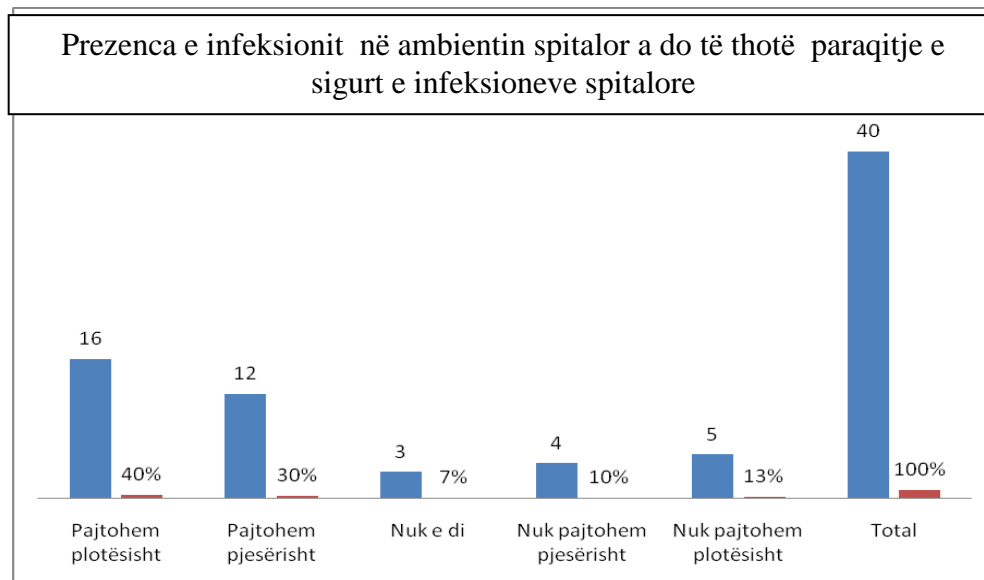
Ne grafikonin numër 3 janë presentuar njohuritë e te anketuarve nëse infeksionet spitalore paraqiten që në momentin e shtrirjes së pacientit në mjedisin spitalor.

Nga 40 (100%) pjesëmarrëse në hulumtim, 10 (25%) e tyre janë pajtuar plotësisht se infeksionet spitalore paraqiten që në momentin e shtrirjes së pacientëve në mjedisin spitalor.



Grafikoni 3. Parandalimi i infeksioneve spitalore

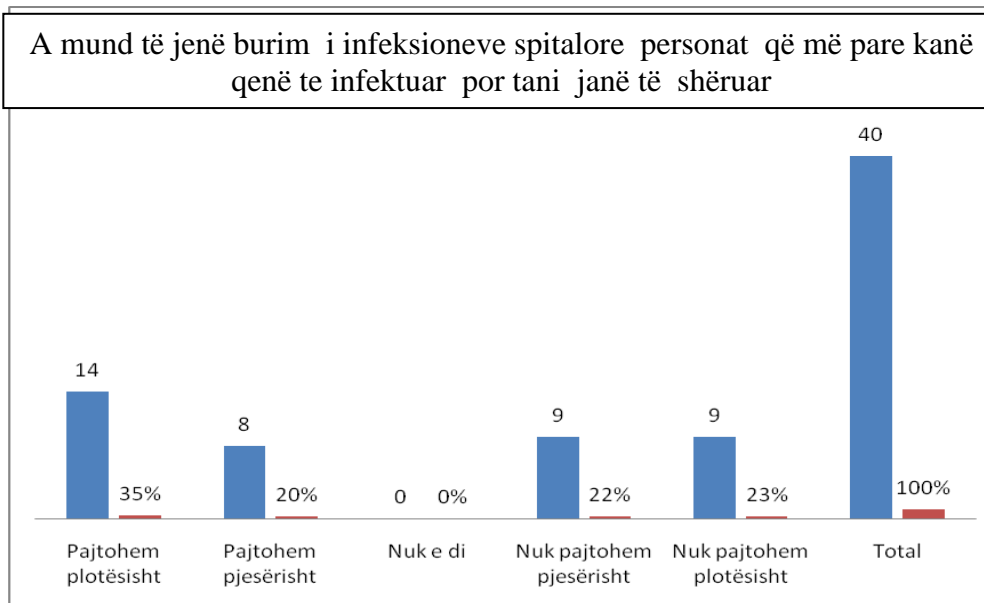
Nga numri i përgjithshëm i tyre, 16 (40%) te anketuar kanë qenë të bindur plotësisht se prezenca e shkaktarëve të infeksionit në ambientin spitalor do të thotë paraqitje e sigurt e infeksionit spitalor, ndërsa 5 (13%), nuk janë pajtuar plotësisht me këtë konstatim (Grafikoni 4).



Grafikoni 4. Prezanton ambientin spitalor

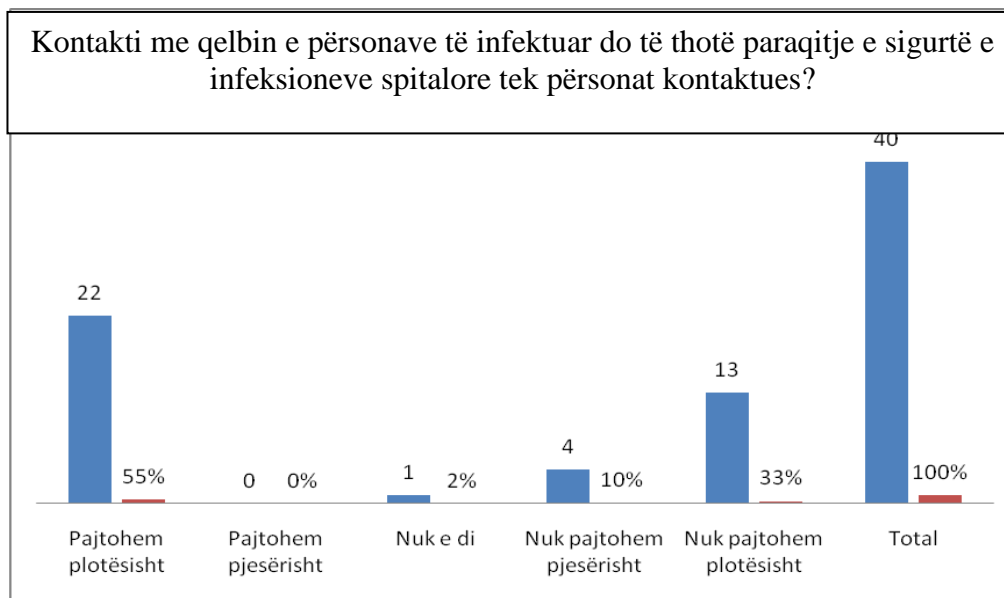
Ne grafikunin numër 5 janë paraqitur njohuritë e të anketuarve nëse burim i infeksioneve spitalore mund të jenë edhe personat që më parë kanë qenë të infektuar por tani më janë të shëruar.

Po ashtu në këtë pyetje 14 (35%) te anketuar ishin pajtuar plotësisht se personat që më parë kanë qenë të infektuar por tani më janë shëruar mund të jenë burim i infeksionit, kurse 9 ose 23% e subjekteve nuk pajtohen me konstatimin e këtij burimi të infeksionit.



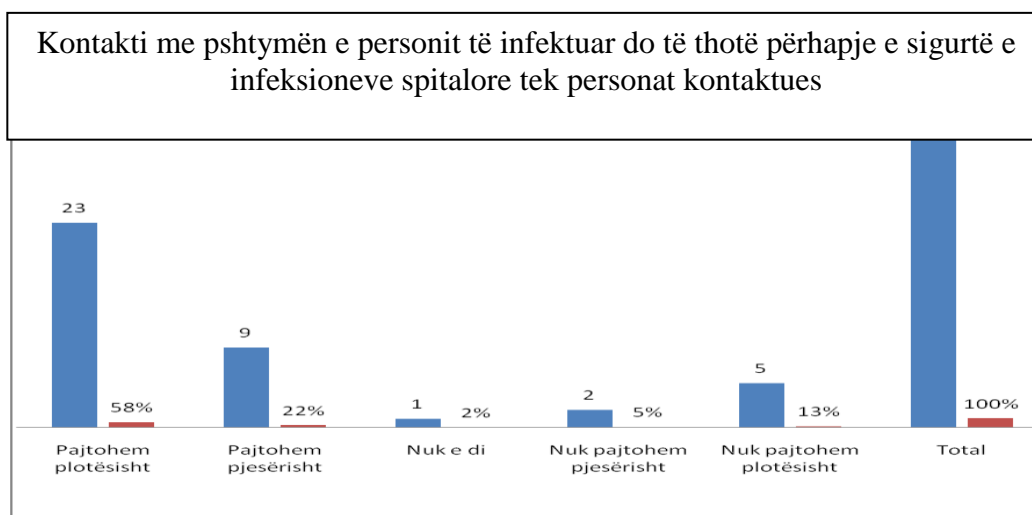
Grafikoni 5. Paraqet burimin e infeksioneve spitalore

Kontakti me qelbin e personave të infektuar do të thotë paraqitje e sigurtë e infeksioneve spitalore tek personat kontaktues. Një numër i te anketuarve, 22 (55%) janë pajtuar se kontakti me qelbin do të thotë paraqitje e sigurtë e infeksioneve spitalore, ndërsa rreth 13(33%) nuk janë pajtuar se është e sigurve kjo forme e paraqitjes se infeksionit (Grafikoni 6).



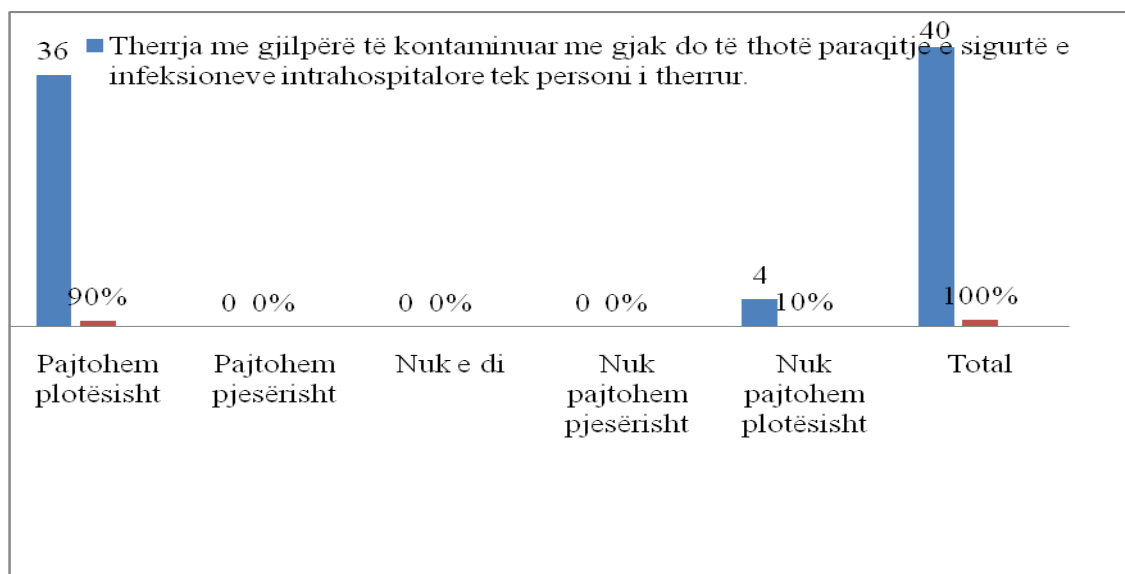
Grafikoni 6. Personat kontaktues me infeksion

Grafikon 7. paraqet një pajtueshmëri ku 23 (58%) te anketuar kanë menduar se këto infeksione mund të paraqiten edhe nga kontakti i drejtpërdrejtë me pështymën e të sëmurit, por këtë mendim e kanë shprehur me ndryshe 7 apo 18% sepse nuk janë pajtuar me këtë .



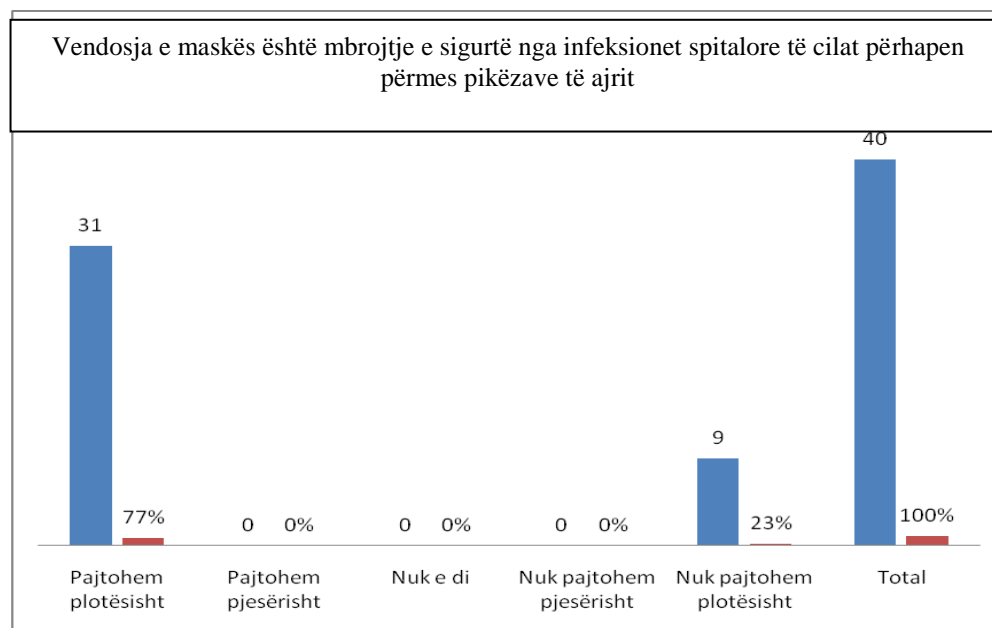
Grafikon 7. Paraqet kontaktin me pështymën e personit të infektuar

Grafikoni 8. Numri më i madh, 36 (90%) i pjesmarrësve ne hulumtim janë pajtuar se therja me gjilpërën e ndotur me gjak do të thotë paraqitje e sigurtë e infeksioneve spitalore, ndersa 4 ose 10% mendojnë të kundërtën dhe nuk pajtohen plotësisht me këtë konstatim.



Grafikoni 8. Paraqet therjen me gjilpërën e personit të infektuar

Nga numri i përgjithshëm i të anketuarve, 31 (77%) te anketuarve kanë qenë të bindur plotësisht se prezenca e shkaktarëve të infeksionit në ambientin spitalor do të thotë paraqitje e sigurtë e infeksionit spitalor, ndërsa 9 (23%), nuk janë pajtuar plotësisht me këtë konstatim.



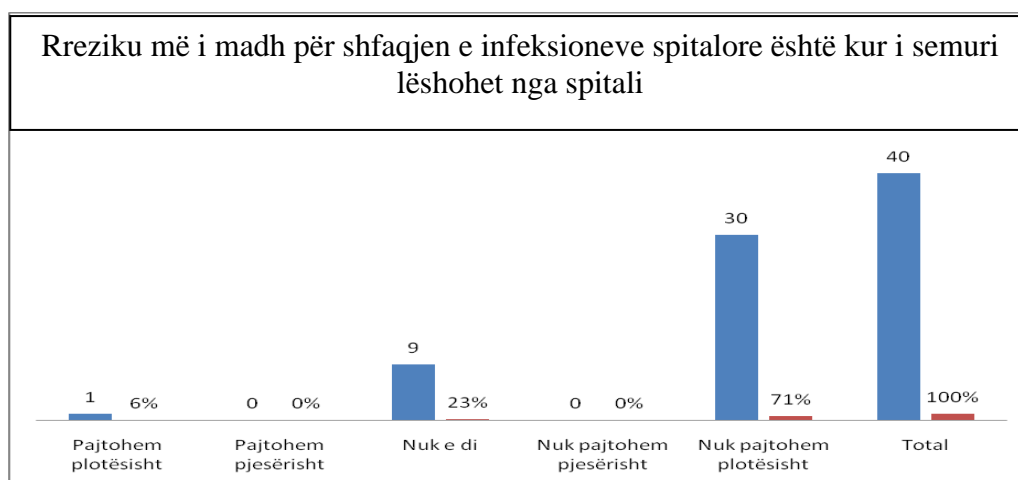
Grafikoni 9. Paraqet maskën si mase mbrojtje në parandalimin e infeksioneve spitalore

Grafikoni 10 Paraqet përgjigjet e infermiereve ku 30 apo 71% pajtohen plotësisht se infeksionet shkaktohen nga mosrespektimi i rregullave në spitale dhe nga mos respektimi i higjienës gjatë përkujdesjes infermiere, ndërsa me këtë konstatim nuk pajtohen 9 apo 23%, ndërsa vetëm 1(6%) është përgjigjur se nuk ka njohuri për këtë.



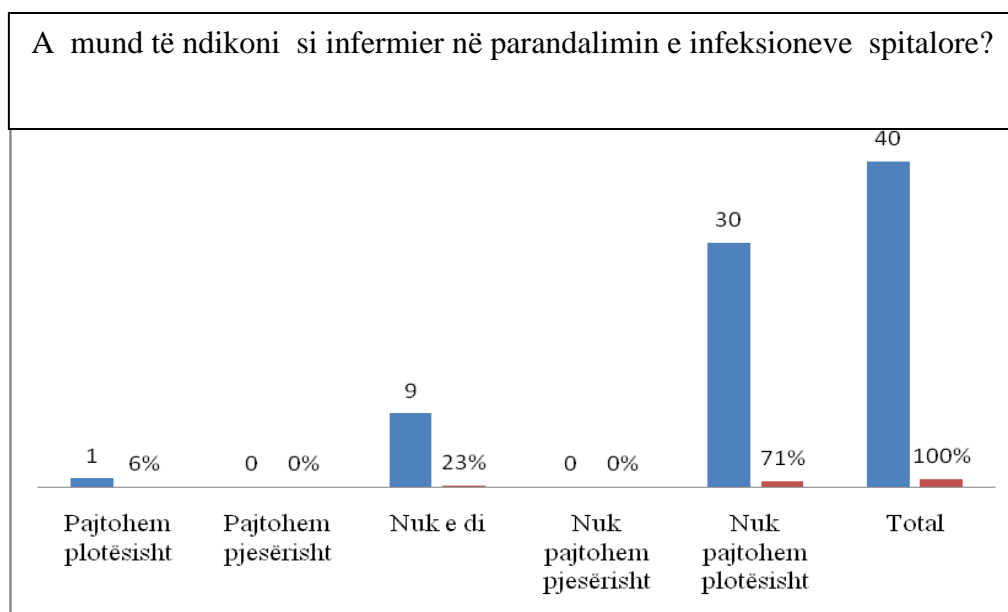
Grafikoni 10. Prezanton respektimin e rregullave në spitale gjatë përkujdesjes infermiere

Numri më i madh 30 (71%) e subjekteve nuk janë pajtuar se rreziku më i madh për shfaqjen e infeksionit spitalor është kur i sëmuri lëshohet nga spitali, ndërsa 9 (23%) te anketuar nuk janë pajtuar me këtë dhe vetëm një i anketuar është pajtuar plotësisht.



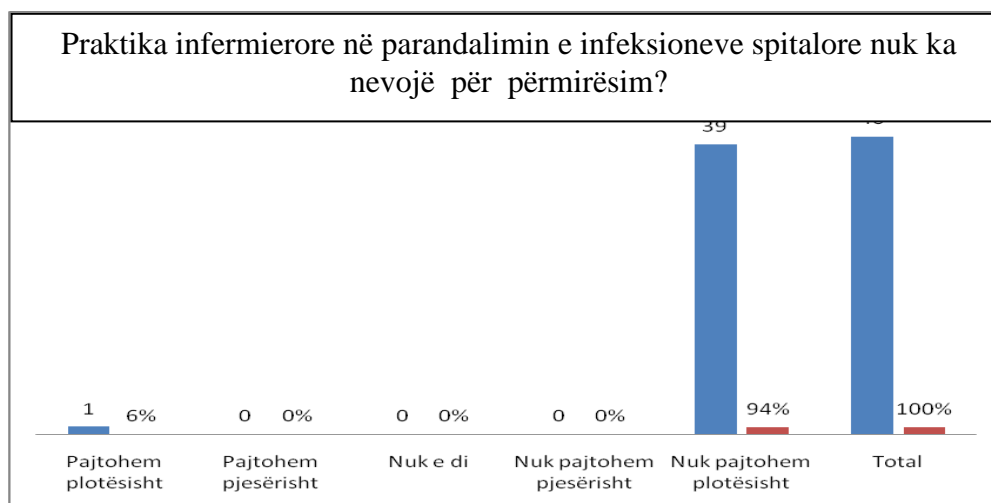
Grafikoni 11. Prezanton kur i sëmuri lëshohet nga spitali dhe shfaqjen e infeksionit

Prej 40 (100%) pjesëmarrësve në hulumtim, 30 (71%) nuk pajtohen plotësisht se ata mund të ndikojnë në parandalimin e infeksioneve spitalore, ndërsa 9 (23%) te anketuar nuk e dinë nëse mund të ndikojnë në parandalimin e infeksioneve spitalore.



Grafikoni 12. Prezanton rolin infermieror në parandalimin e infeksioneve spitalore

Ne pyetjen tone se praktika infermierore në parandalimin e infeksioneve spitalore nuk ka nevojë për përmirësim, shumica e te anketuarve, 39 (94%), nuk pajtohen me këtë të dhënë, ndërsa vetëm një i anketuar pajtohet plotësisht.



Grafikoni 13. Praktika infermierore në parandalimin e infeksioneve spitalore

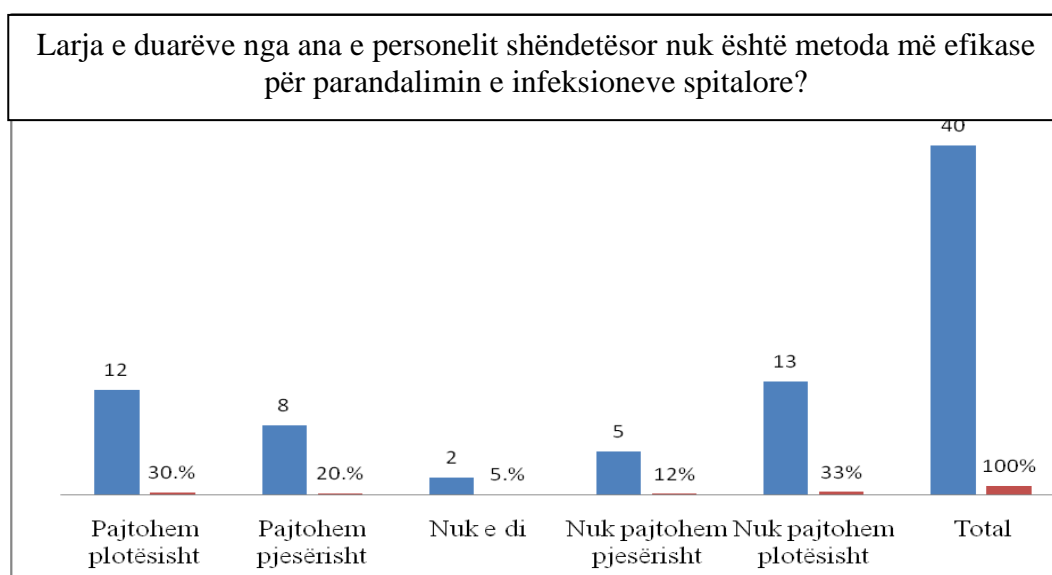
I. Njohurit infermierore për infeksionet spitalore dhe parandalimin e tyre

Tabela A. Rezultatet e pyetjeve prej 05-14 në përqindje (%) dhe frekuenca (F)

Nr.	Njohurit infermierore në parandalimin e infeksioneve spitalore	Pajtohem plotësisht		Pajtohem pjesërisht		Nuk e di		Nuk pajtohem pjesërisht		Nuk pajtohem plotësisht	
		%	F	%	F	%	F	%	F	%	F
05	Infeksionet spitalore janë infeksione të cilat paraqiten që në momentin e shtrirjes së pacientit në mjedisin spitalor.	25%	10	0%	0	7%	3	20%	8	48%	19
06	Prezenca e shkaktarëve të infeksionit në ambientin spitalor do të thotë paraqitje e sigurtë e infeksioneve spitalore.	40%	16	30%	12	7%	3	10%	4	13%	5
07	Burim i infeksioneve spitalore mund të jenë edhe personat që më parë kanë qenë të infektuar por tani më janë të shëruar.	35%	14	20%	8	0%	0	22%	9	23%	9
08	Kontakti me qelbin e personave të infektuar do të thotë paraqitje e sigurtë e infeksioneve spitalore tek personat kontaktues.	59%	22	0%	0	2%	1	10%	4	33%	13
09	Kontakti me pëshptomën e personit të infektuar do të thotë përhapje e sigurtë e infeksioneve spitalore tek personat kontaktues.	58%	23	22%	9	2%	1	5%	2	13%	5
10	Therja me gjilpëra të kontaminuar me gjak do të thotë paraqitje e sigurte e infeksioneve spitalore tek personi i therur.	90%	36	0%	0	0%	0	0%	0	10%	4
11	Vendosja e maskës është mbrojtje e sigurtë nga infeksionet intrahospitalore të cilat përhapen përmes pikëzave të ajrit .	77%	31	0%	0	0%	0	0%	0	23%	9
12	Infeksionet spitalore shkaktohen nga mos respektimi i rregullave në spitale dhe nga mos respektimi i higjienës gjatë dhënies së kujdeseve nga ana e infermiereve.	71%	30	0%	0	6%	1	0%	0	23%	9
13	Rreziku më i madh për shfaqjen e infeksioneve spitalore është kur i sëmuri lëshohet nga spitali.	6%	1	0%	0	23%	9	0%	0	71%	30
14	Unë si infermier nuk mund të ndikoj në parandalimi infeksioneve-brendaspitalore.	6%	1	0%	0	23%	9	0%	0	71%	30
15	Praktika infermierore në parandalimin e infeksioneve spitalore nuk ka nevojë për përmirësim	6%	1	0%	0	0%	0	0%	0	94%	39

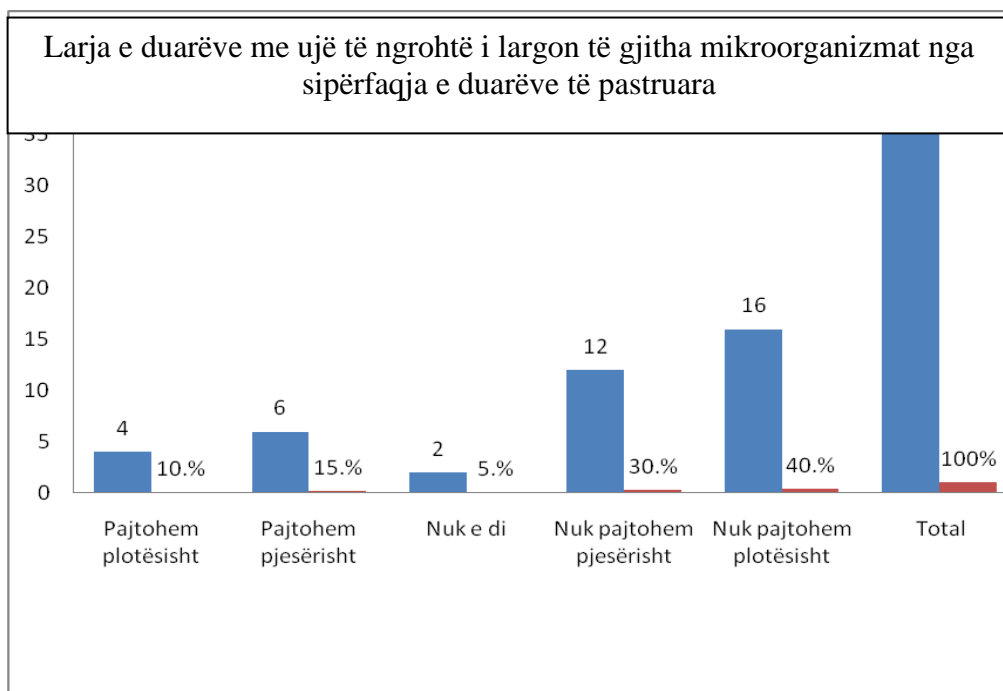
II. Njohurit infermierore për masat e higjienës personale të personelit shëndetësor (larjen e duarve) në parandalimin e infeksioneve spitalore

Nga 40 (100%) pjesëmarrës në hulumtim vetëm 13 (33%) prej tyre kanë qenë të bindur plotësisht se larja e duarve është metoda më efiçase për parandalimin e infeksioneve spitalore, ndërsa 12 (30%) pajtohen plotësisht se larja e duarve nuk është mënyra më efiçase për parandalimin e infeksioneve spitalore.



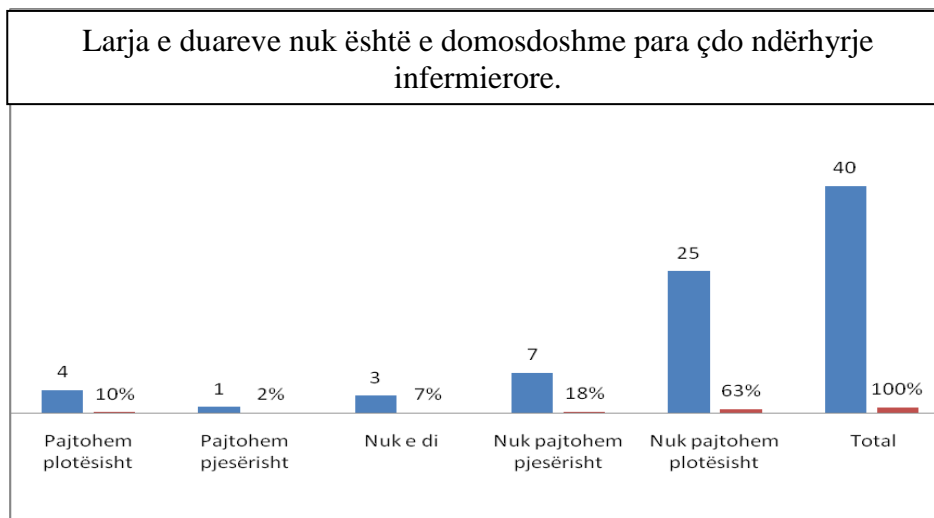
Grafikoni 14. Larja e duarve në parandalimin e infeksioneve spitalore

Nga numri i përgjithshëm i të anketuarve, 4 (10%) të anketuar janë pajtuar plotësisht se larja e duarve me ujë të ngrohtë i largon të gjitha mikroorganizmat nga duart e pastruara, ndërsa 28 (70%) nuk pajtohen se larja e duarve me ujë të ngrohtë mund të largon të gjithë mikroorganizmat nga sipërfaqja e duarve.



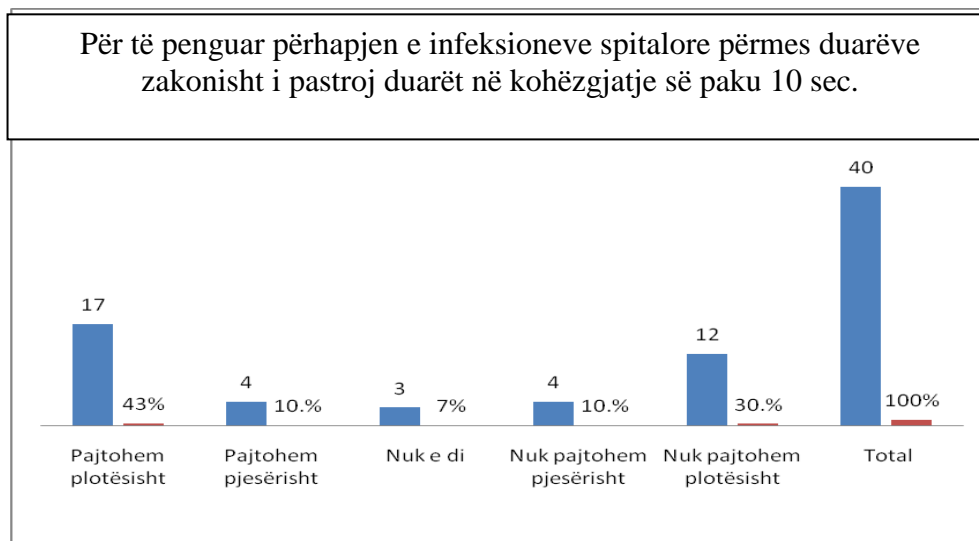
Grafikoni 15. Larja e duarve me ujë të ngruhtë

Ne pyetjen se larja e duarve nuk është e domosdoshme para çdo ndërhyrje infermiore, 25 te anketuar ose 63% nuk janë pajtuar plotësisht, ndërsa 4 te anketuar ose 10% janë pajtuar me këtë konstatim.



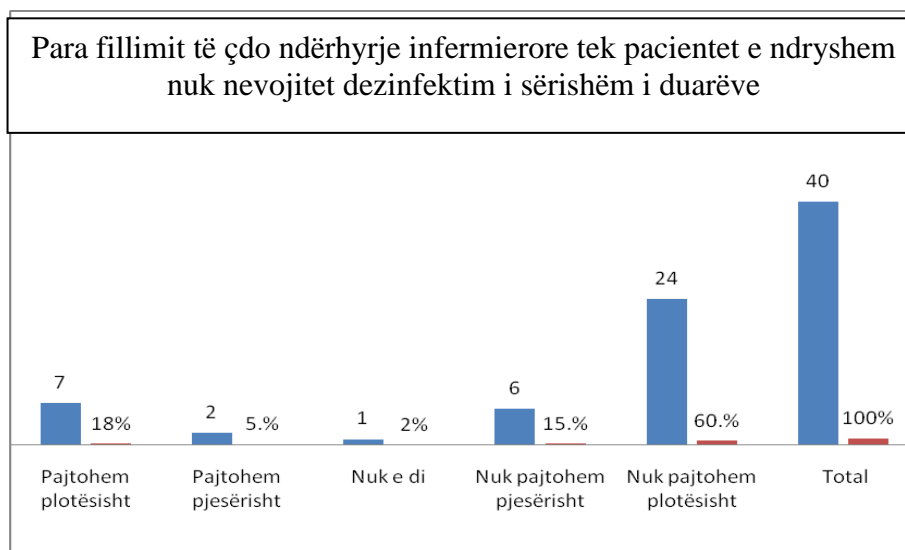
Grafikoni 16. Paraqet larjen e duarve dhe ndërhyrjet infermiore

Se larja e duarve është e nevojshme të zgjasë së paku 10 sec, janë pajtuar plotësisht 17 (43%) të anketuar në hulumtim , 12 (30%) nuk janë pajtuar, ndërsa 4 (7%) prej tyre nuk e dinin.



Grafikoni 17. Paraqet kohëzgjatjen e larjes së duarve

Numri më i madh i të anketuarve 24 ose 60% nuk janë pajtuar plotësisht se para çdo ndërhyrje infermierore është i nevojshëm dezinfektimi i serishëm i duarve, ndërsa vetëm 7 (18%) janë pajtuar plotësisht me këtë konstatim.



Grafikoni 18. Prezanton dezinfektimin e duarve si domosdoshmëri

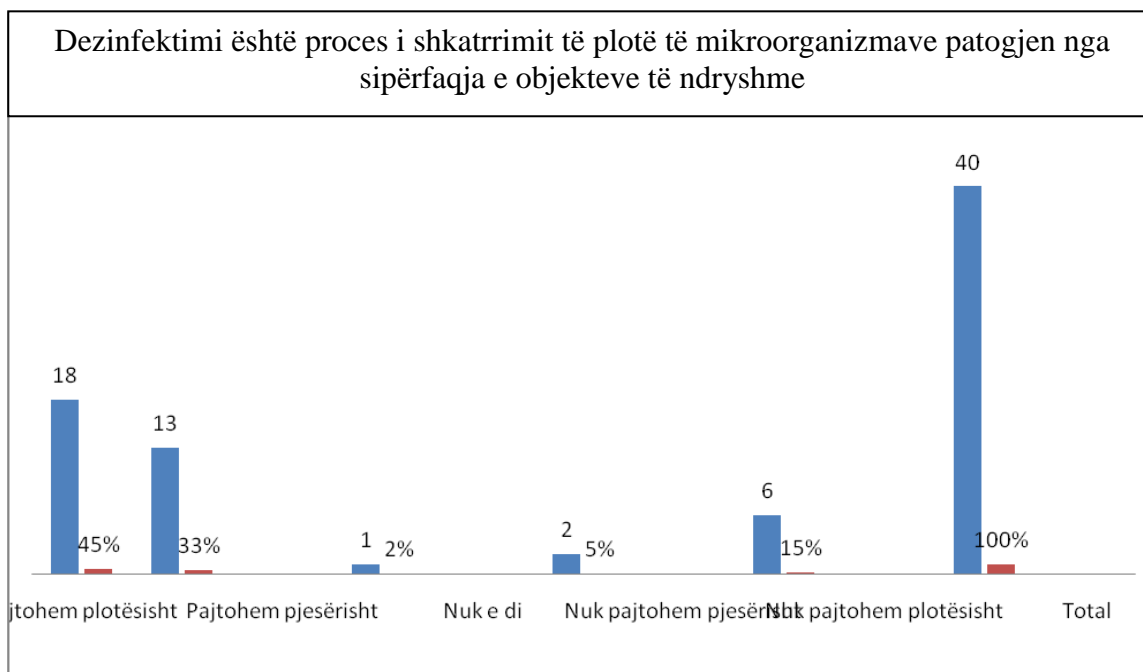
**II. Njohurit infermierore për masat e higjienës personale të personelit shëndetësor
(larjen e duarve) në parandalimin e infeksioneve spitalore**

Tabela B. Rezultatet e pyetjeve prej 16-19 në përqindje (%) dhe frekuence (F)

Nr.	Njohurit infermierore për masat e higjienës personale (larjen e duarve) në parandalimin e infeksioneve spitalore	Pajtohem plotësisht		Pajtohem pjesërisht		Nuk e di		Nuk pajtohem pjesërisht		Nuk pajtohem plotësisht	
		%	F	%	F	%	F	%	F	%	F
16	Larja e duarve nga ana e personelit shëndetësor nuk është metoda më efektive për parandalimin e infeksioneve spitalore.	30%	12	20%	8	5%	2	12%	5	33%	13
17	Larja e duarve me ujë të ngrohtë i largon të gjitha mikroorganizmat nga sipërfaqja e duarve të pastruara.	10%	4	15%	6	5%	2	30%	12	40%	16
18	Larja e duarve nuk është e domosdoshme para çdo ndërhyrje infermierore.	10%	4	2%	1	7%	3	18%	7	63%	25
19	Për të penguar përhapjen e infeksioneve intrahospitalore përmes duarve zakonisht i pastroj duart në kohëzgjatje së paku 10 sec.	43%	17	10%	4	7%	3	10%	4	30%	12

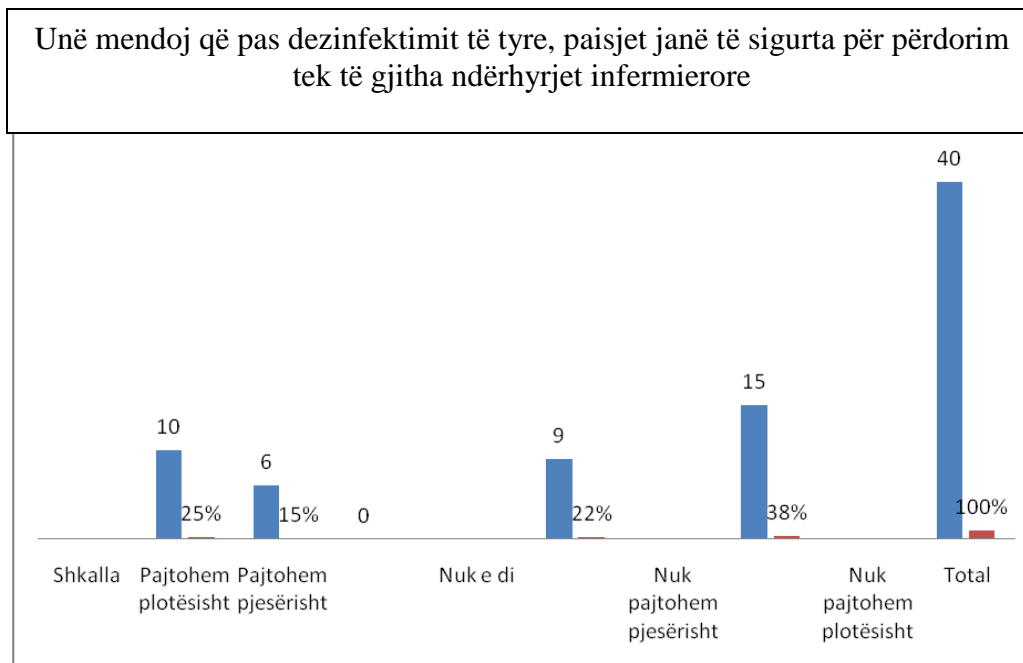
III.Njohurit infermierore në zbatimin e ciklit të dekontaminimit

Nga numri total i te anketuarve, 45% ose 18 te anketuar pajtohen se dezinfektimi i shkatërron plotësisht të gjitha mikroorganizmat, ndërsa 13 (33%) pajtohen pjesërisht me këtë konstatim.



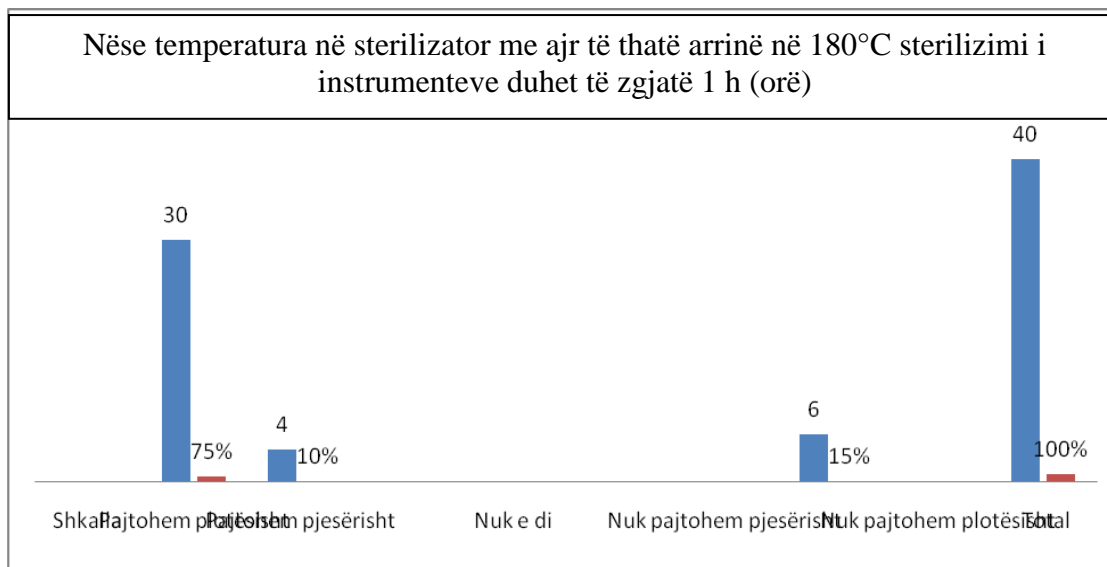
Grafikoni 19. Prezanton dezinfektimin si proces

Nga numri i përgjithshëm i të anketuarve, 10 (25%) prej tyre plotësisht janë pajtuar se pajisjet e dezinfektuara mund të përdoren tek të gjitha ndërhyrjet infermierore, ndërsa 15 (38%) nuk pajtohen me këtë konstatim.



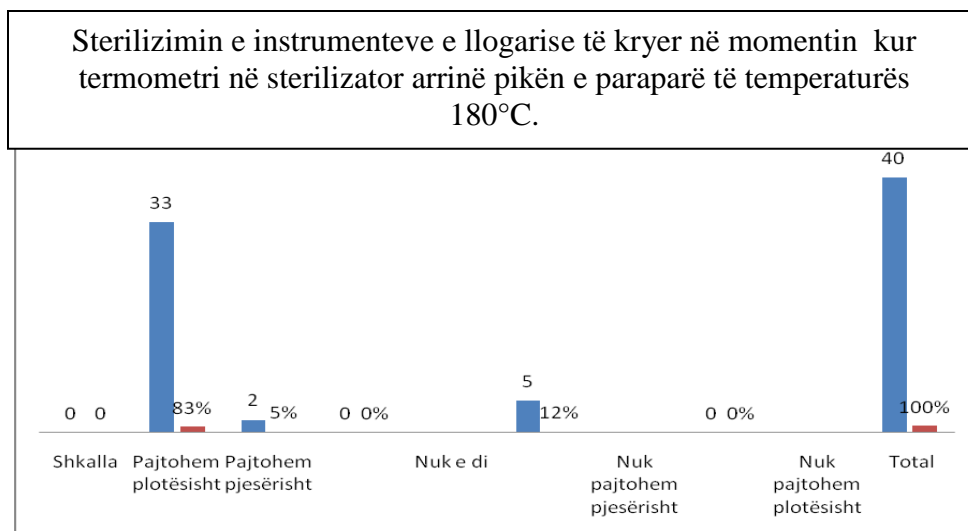
Grafikoni 20. Prezanton dezinfektimin e paisjeve

Me pyetjen lidhur me temperaturat e sterilizimit me ajër të thatë, numri më i madh i pjesëmarrësve në hulumtim, 30 (75%), janë pajtuar se sterilizimi me ajër të thatë duhet të zgjat 1h në 180 C kurse 6 (15%) nuk pajtohen plotësisht me këtë.



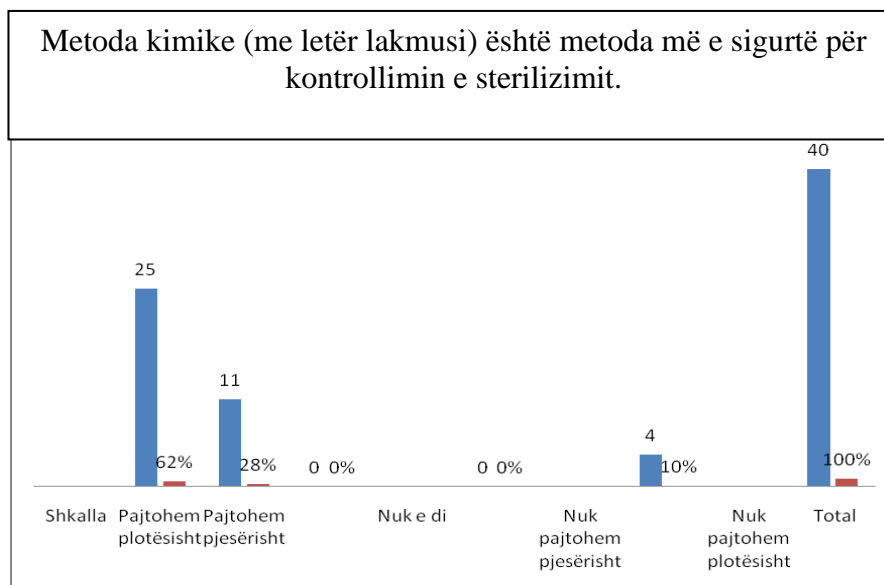
Grafikoni 21. Prezanton sterilizimin me ajër të thatë dhe kohëzgjatjen e tij

Nga numri total i te anketuarve, 33 ose 83% e të anketuarve e llogarisin të kryer sterilizimin pikërisht në momentin kur temperatura në sterilizator me ajër të thatë arrijnë në 180C, ndërsa 5 ose (12%), nuk dinë fare për këtë procedure.



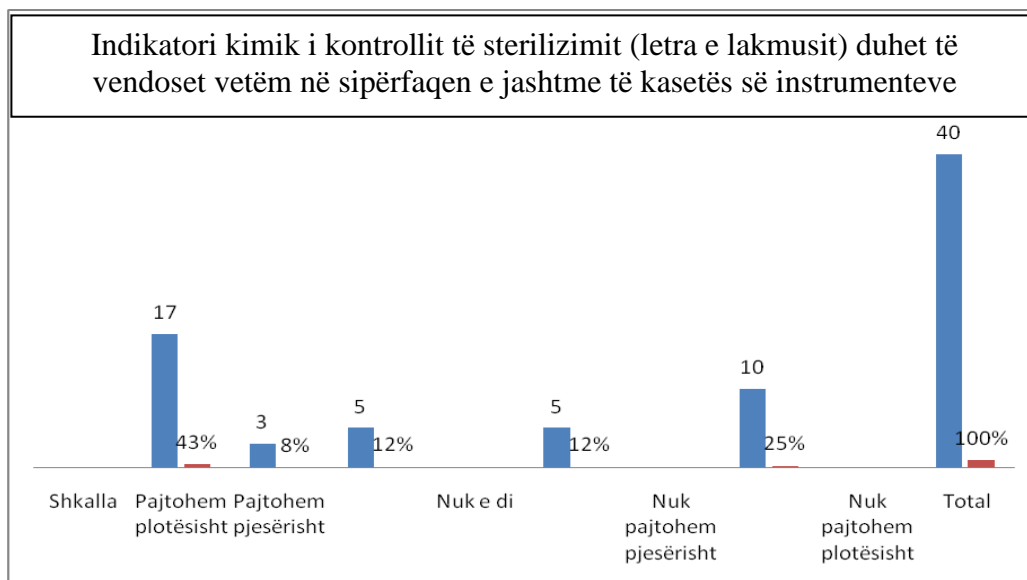
Grafikoni 22. Sterilizimi sipas temperaturës dhe kohëzgjatjes

Të anketuarit pjesëmarrës ne hulumtim në pyetjen e radhës rreth metodave të kontrollit të sterilizimit ne përqindje me të madhe, 62% ose 25 të anketuarve janë pajtuar plotësisht se metoda kimike është metoda më e sigurtë për kontrollimin e sterilizimit, ndërsa 4 ose 10% nuk janë pajtuar.



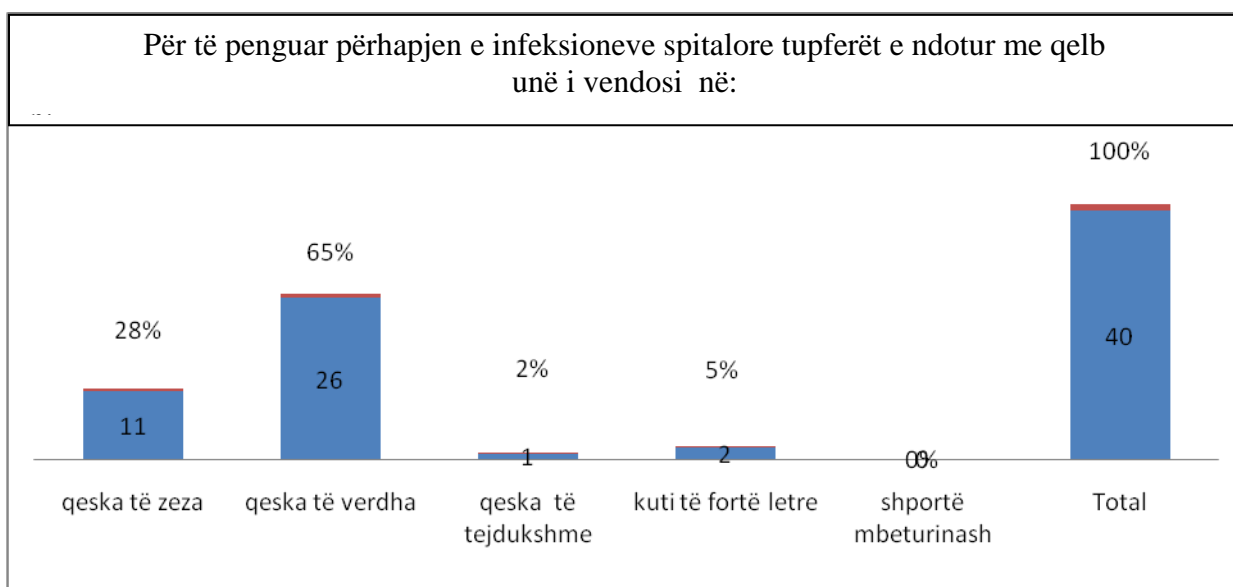
Grafikoni 23. Prezanton metodën e kontrollit të sterilizimit

17 te anketuar ose 43% kanë menduar se letra e lakmusit duhet të vendoset vetëm në sipërfaqen e jashtme të kasetës me gazë dhe tufëra, ndërsa 10 (25%) nuk pajtohen me këtë konstatim.



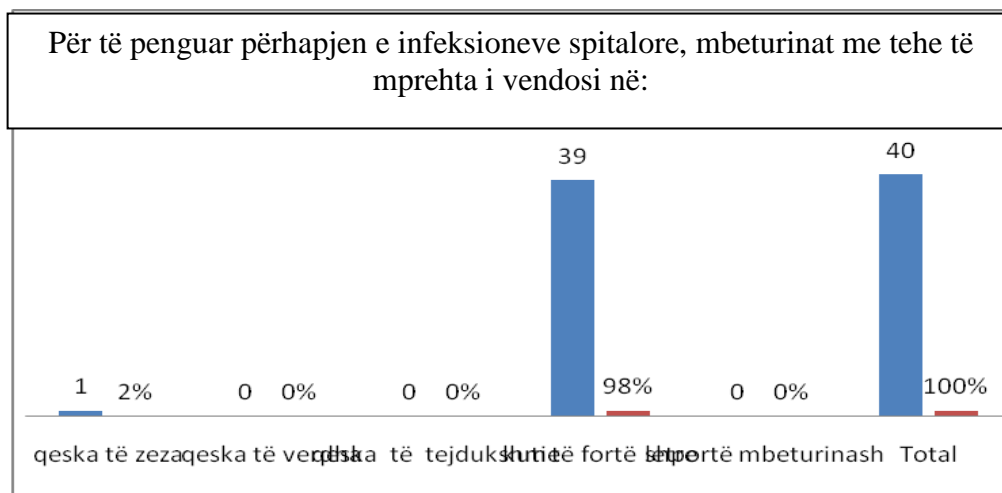
Grafikoni 24. Prezanton indikatorin kimik te kontrollit të sterilizimit

Vetëm 26 ose 65% e të anketuarve i kanë vendosur gazat e ndotura me gjak në qeska të verdha kurse të tjerët në qeska tjera (të zeza apo të tejdukshme) apo kuti letre.



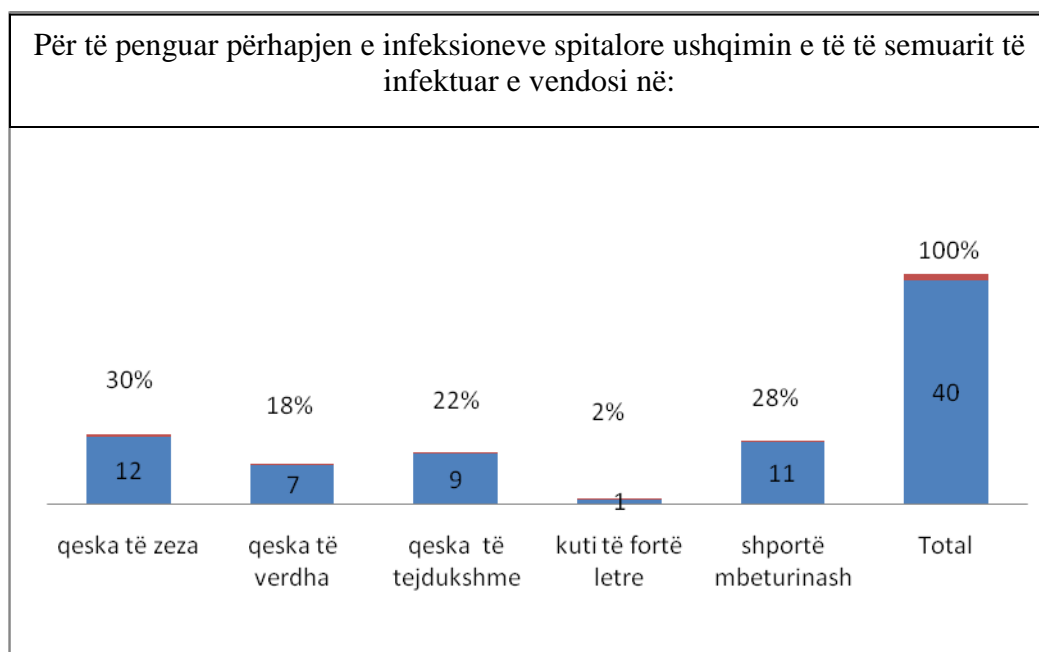
Grafikoni 25. Njohurit infermiore ne menaxhimin e mbeturinave spitalore

Shumica e të anketuarve, 39 (98%), mbeturinat me tehe të mbrehtë i kanë vendos në kuti të fortë letre, ndërsa vetëm një i anketuar është shprehur se i vendos në qeske të zeze gjërat e prehtë.



Grafikoni 26. Prezanton menaxhimin e mbeturinave me tehe te mprehta

Ushqimin e mbetur të sëmurit të infektuar, 11 ose 28% e të anketuarve e kanë vendosur në shportë mbeturinash, ndërsa 9 (2%) e kanë vendosur në qeska të tejdukshme ushqimin e të sëmurëve të infektuar. Ndryshe prej tyre, 7 të anketuar ose 18%, këto mbeturina i hedhin në qese të verdha.



Grafikoni 27. Paraqet ushqimin e të sëmurave të infektuar

III. Njohurit infermiore në zbatimin e ciklit të dekontaminimit

Tabela C. Rezultatet e pyetjeve prej 21-24 në përqindje(%) dhe frekuencë (F)

Nr.	Njohurit infermiore në zbatimin e ciklit të dekontaminimit	Pajtohem plotësisht		Pajtohem pjesërisht		Nuk e di		Nuk pajtohem pjesërisht		Nuk pajtohem plotësisht	
		%	F	%	F	%	F	%	F	%	F
21	Dezinfektimi është proces i shkatërrimit të plotë të mikroorganizmave patogjen nga sipërfaqja e objekteve të ndryshme.	45%	18	33%	13	2%	1	5%	2	15%	6
22	Unë mendoj që pas dezinfektimit të tyre, pajisjet janë të sigurta për përdorim tek të gjitha ndërhyrjet infermiore.	25%	10	15%	6	0%	0	22%	9	38%	15
23	Nëse temperatura në sterilizator me ajr të thatë arrin në 180°C sterilizimi i instrumenteve duhet të zgjatë 1 h (orë).	75%	30	10%	4	0%	0	0%	0	15%	6
24	Sterilizimin e instrumenteve e llogarisë të kryer në momentin kur termometri në sterilizator arrin pikën e paraparë të temperaturës 180°C.	83%	33	5%	2	0%	0	12%	5	0%	0
25	Metoda kimike (me letër lakmushi) është metoda më e sigurt për kontrollimin e sterilizimit.	62%	25	28%	11	0%	0	0%	0	10%	4
26	Indikatori kimik i kontrollit të sterilizimit (letra e lakmushit) duhet të vendoset vetëm në sipërfaqen e jashtme të kasetës së instrumenteve.	43%	17	8%	3	12%	5	12%	5	25%	10

V. Diskutimet

Mënyra më optimale dhe e sigurt e njohurive infermierore vazhdimisht mbetet një sfidë dhe çështje kontroverse mes profesionistëve shëndetësor.

Akumulimi i njohurive mbi aspekte të ndryshme të infermierisë përbën një "trup të dijes", që infermieret dhe të tjerët mund të tërheqë mbi të dhe kontribuojnë për të. Shfrytëzuar kërkime për të mbështetur praktika është qëllimi i fundit i hulumtimit infermieror.

Arsye pse jemi përcaktua me hulumtua këtë temë ka të beje me shumë ndërhyrje, aplikime, procedura infermierore përben një sërë veprime që mund të kontribuojnë si në parandalim ashtu edhe në paraqitjen e infeksioneve spitalore. Një tematikë e tillë rreth hulumtimit të njohurive infermierore në parandalimin e infeksioneve spitalore nuk është hulumtuar më parë në spitalin tonë, dhe kjo na pamundëson realizimin e krahasimeve të gjetjeve tona të hulumtimit me të dhëna paraprake në Spitalin e Vushtrrisë, por sipas krahasimit me literaturën e përdorur.

VI. Përfundimi

Ky studim prospektiv analizoi njohuritë e stafit infermieror për Infeksionet Spitalore dhe parandalimin e tyre në spitalin rajonal Sheikh Zayed në Vushtrri.

Në hulumtim morën pjesë 40 Infermier/e të njësive të ndryshme të Spitalit Rajonal në Vushtrri: Repartin e Mjekimit Intenziv, Repartin e Kirurgjisë, Repartin e Pediatriisë, Repartin e Gjinekologjisë dhe Repartin e Maternitetit.

Nga 40 infermier/e të anketuar, 39 (94%) i takuan gjinisë femërore dhe vetëm një i anketuar ishte i gjinisë mashkullore. Moshë me e presentuar në punim ishte ajo nga 37 deri 45 vjeçe (62%).

Shumica e pjesëmarrësve në hulumtim kanë pasur nivel të mesëm të edukimit infermieror, 25 pjesëmarrës ose 65%, 7 pjesëmarrës (15%) kanë pasur nivel të lartë të edukimit infermieror dhe vetëm 3 (5%) kishin nivel universitar të edukimit.

Numri më i madh i pjesëmarrësve në hulumtim patën përvojë pune mbi 30 vite (32%).

Qëllimi parësor i punimit ishte:

- të hulumtoj njohuritë e infermiereve për infeksionet spitalore dhe parandalimin e tyre;
- të hulumtoj njohuritë e infermiereve për masat e higjienës personale të personelit shëndetësor (larjen e duarve) në parandalimin e infeksioneve spitalore;
- të hulumtoj njohuritë e infermiereve në zbatimin e ciklit të dekontaminimit;
- të hulumtoj njohuritë e infermiereve për menaxhimin e mbeturinave infektive në parandalimin e infeksioneve spitalore.

Njohuritë e infermiereve të Spitalit Rajonal në Vushtrri për infeksionet spitalore dhe parandalimin e tyre rezultuan si më poshtë:

Pjesa më e madhe e të anketuarve pati njohuri për epidemiologjinë e infeksioneve spitalore dhe dhënë përgjigje pozitive për kohën e paraqitjes së tyre (68%). Mbi 70% të pjesëmarrësve në punim ishin të mendimit se prezenca e infeksionit në ambientin spitalor do të thotë paraqitje e sigurtë e infeksioneve spitalore ndërsa vetëm 55% patën njohuri të mjaftueshme për rrugët e përhapjes së infeksioneve spitalore.

Rreth 90% të infermiereve patën njohuri të mjaftueshme për rrezikun nga sëmundjet profesionale të personelit shëndetësor.

Mbi 77% të të anketuarve patën njohuri të mjaftueshme për masat dhe parandalimin e infeksioneve spitalore. Për rolin e infermiereve në parandalimin e infeksioneve spitalore patën njohuri mbi 70% të të anketuarve, ndërsa 94% mendojnë se praktika infermiere në parandalimin e infeksioneve spitalore ka nevojë për përmirësim.

Sa i përket njohurive të infermiereve për masat e higjienës personale të personelit rezultatet ishin si me poshtë:

Për rolin e higjienës së duarve si mase kryesore në parandalimin e përhapjes së infeksioneve spitalore patën njohuri vetëm 45% të stafit infermior. Për metodat e pastrimit të duarve patën njohuri rreth 50% të stafit infermior, ndërsa për domosdoshmërinë e larjes së duarve para çdo ndërhyrje infermiere pozitivisht u përgjigjen 80% të anketuarve. Për shkallet e dekontaminimit të duarve patën njohuri 70% e të anketuarve. Njohurit infermiere në zbatimin e ciklit të dekontaminimit ishin të kënaqshme:

Për metodat e dezinfektimit patën njohuri rreth 78% e të anketuarve, ndërsa për metodën e sterilizimit patën njohuri 85% e të anketuarve. Njohuritë e infermiereve për menaxhimin e mbeturinave infektive në parandalimin e infeksioneve spitalore ishin të kënaqshme. Në analizën rreth klasifikimit të mbeturinave 36 (90%) të anketuarve në hulumtim i klasifikojnë, menaxhojnë drejtë mbeturinat spitalore, ndërsa 4 (10%) nuk e zbatojnë drejt menaxhimin e mbeturinave spitalore.

Edhe pse studimi tregon se të anketuarit kanë njohuri rreth disa masave (ndërhyrjeve) infermiere në parandalim të infeksioneve spitalore, po ashtu tregon edhe për disa intervenime të cilat do duhej të përmirësohen.

Mënyra me e mirë në parandalimin e infeksioneve spitalore ka të bëjë me model të vlerësimit të ngritjes profesionale të stafit infermior. Bazuar në gjetjet e punimit dhe qëllimeve kryesore, dhe shqyrtimi e literaturës, mund të vijmë në përfundim se infeksionet spitalore përbëjnë kërcënim serioz për shëndetësinë moderne, si në vendet e industrializuara, ashtu edhe tek vendet në zhvillim siç është Kosova. Punimi paraqet një pasqyre reale të njohurive infermiere në spitalin Sheikh Zayed Vushtrri. Njohuritë e infermiereve për infeksionet spitalore dhe parandalimin e tyre në Spitalin Sheikh Zayed në Vushtrri, janë të qëndrueshme, pasi që përgatitjet dhe njohuritë e tyre ndihmojnë në uljen e infeksioneve spitalore.

Ajo që duhet potencuar në rezultatet e punimit siç kemi paraqitur edhe në diskutim shumë praktika infermierore duhet avancuar, prandaj hulumtimi ynë ishte shume i qëlluar në mënyrë që të vërehen lëshimet e mundshme që mund të ndodhin gjatë praktikave dhe punës së infermierëve.

Roli i infermierisë në parandalimin e infeksione spitalore është unik pasi që shumë ndërhyrje dhe aplikime, procedura si higjiena e duarve, dezinfektimi, sterilizimi, menaxhimi i mbeturinave, e që kanë lidhje shumë të ngushtë edhe me ofrimin e kujdesit të pacientet dhe ambienti spitalor, përbejnë sfidën kryesore në parandalimin e infeksioneve në përgjithësi.

Dhe në fund përmes këtij hulumtimi mund të sugjerojmë jo vetëm për spitalin ku është kryer hulumtimi por edhe për spitale tjera që të mendojnë më shumë për studim apo hulumtime, sepse infermiera ka të bëjë me një spektër të gjer veprimesh, sjellje, aplikime, edukime, këshilla etj.

VII. Rekomandimet

Rekomandimet për parandalimin e infeksioneve spitalore :

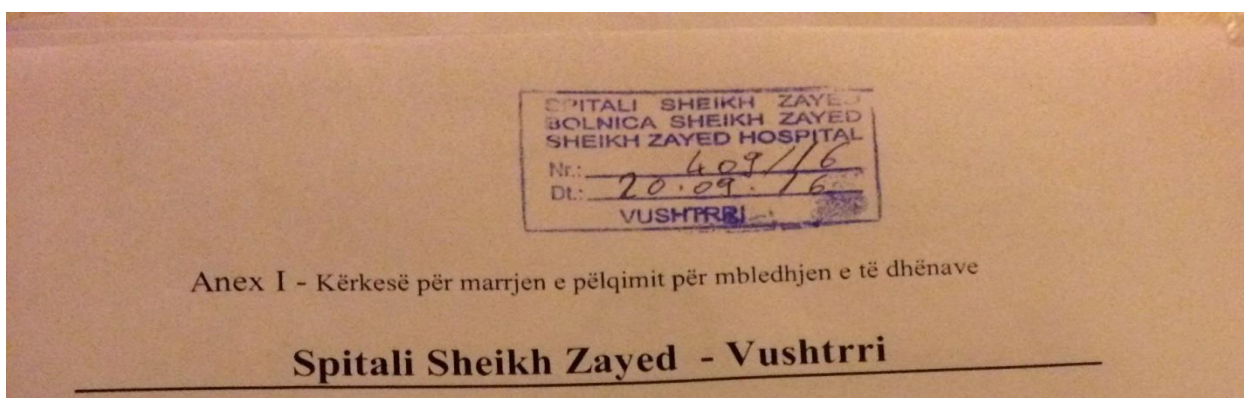
1. Krijimi e një sistemi funksional për mbikëqyrje me të cilin identifikohen problemet nëpër departamente të ndryshme të spitalit.
2. Të mbikëqyret zbatimi me përpikëri i planit dhe programit për parandalimin dhe kontrollin e infeksioneve spitalore.
3. Trajnime në vazhdimësi rreth masave të parandalimit të infeksioneve spitalore, larges se duarve dhe përdorimi i dorëzave .
4. Zbatimi i ciklit të dekontaminimit në veçanti (pastrim, dezinfektim, sterilizimin).
5. Imunizimi i stafit mjekësor dhe zbatimi i masave tjera mbrojtëse nga ana e të gjithë profesionistëve si p.sh: vendosjen e maskës, dorëzave, kapelës, syzeve në mënyrë që të mbrojnë veten e tyre dhe pacientët nga infeksionet (gjatë procedurave gjatë të cilave vijnë në kontakt me gjakun, sekrecionet, qelbin dhe të vjellurat e pacientëve).
6. Në të njëjtën kohë përmes këtij hulumtimi mund të rekomandojmë jo vetëm për spitalin ku është kryer hulumtimi por edhe për spitale tjera që të mendojnë më shumë për studim apo hulumtime edhe në fusha tjera infermierore sepse infermiera ka të bëjë me një spektër të gjërë veprimesh, sjellje, aplikime, edukime, këshilla etj.

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ANEX I



ANEX II- Pyetëtori

Pyetëtori

Të nderuar kolegë – Infermier/e

Unë Mexhit Mustafa student i “Fakultetit të Shkencave Mjekësore Rezonanca”, dhe pjesë e studimit tim është realizimi i një hulumtimi që do duhej të bëhej në spitalin tonë.

Tema e hulumtimit tim është “**Njohurit infermierore në parandalimin e infeksioneve spitalore**”.

Për plotësimin e këtij pyetësori është marrë pëlqimi nga ana e stafit menaxhues të spitalit Sheikh Zayed.

Pyetësori është plotësisht anonim, nuk nevojitet të shkruani emrin tuaj në të ose të identifikoni vetën tuaj.

Pyetësorin do ta merrni nga ne si hulumtues të kësaj teme me datën 22.09.2016 dhe do të keni kohë ta plotësoni deri me datën 29.09.2016 ose edhe më herët nëse e keni plotësuar.

Ju lutem përgjigjuni personalisht në pyetjet duke vendosur tikun (√) vetëm në njërën nga përgjegjjet e mundura. Pasi ta plotësoni ju lutem ta ktheni në kohën e duhur tek ne, dhe ta vendosni personalisht në kutinë e vendosur për mbledhjen e pyetësorëve.

Ju lutem për çdo paqartësi të na kontaktoni në adresën e poshtë shënuar.

Mexhit Mustafa

mexhiti_77@hotmail.com

Tel: +3744 235-503

IV. Të dhënat demografike

Ju lutem rrumbullakosni vetëm njërën nga zgjidhjet e poshtë shënuara:

1. Gjinia

a) Mashkull

b) Femër

2. Cila është mosha juaj ?

- a) nën 18 vjeç
- b) prej 19- 27 vjeç
- c) prej 28-36 vjeç
- d) prej 37- 45 vjeç
- e) mbi 45 vjeç

3.Cili është niveli i shkollimit – edukimi tuaj infermierore?

- a) edukim i mesëm infermieror
- b) edukim i lartë infermieror
- c) edukim universitar infermieror
- d) edukim tjetër shtesë

4.Sa është përvoja e juaj profesionale infermierore?

- a) Më pak se 2 vjet
- b) 2-4 vjet
- c) 5-10 vjet
- d) 11 – 15 vjet
- e) 16 – 20 vjet
- ë) Më e madhe se 21 vjet

V. Njohurit infermierore per infeksionet spitalore dhe parandalimin e tyre

Nr.	Njohurit infermierore në parandalimin e infeksioneve spitalore	Pajtohem plotësisht	Pajtohem pjesërisht	Nuk e di	Nuk pajtohem pjesërisht	Nuk pajtohem plotësisht
05	Infeksionet spitalore janë infeksione të cilat paraqiten që në momentin e shtrirjes së pacientit në mjedisin spitalor					

06	Prezenca e shkaktarëve të infeksionit në ambientin spitalor do të thotë paraqitje e sigurt e infeksioneve spitalore.					
07	Burim i infeksioneve spitalore mund të jenë edhe personat që më parë kanë qenë të infektuar por tani më janë të shëruar.					
08	Kontakti me qelbin e personave të infektuar do të thotë paraqitje e sigurt e infeksioneve spitalore tek personat kontaktues.					
09	Kontakti me pështymën e personit të infektuar do të thotë përhapje e sigurt e infeksioneve spitalore tek personat kontaktues.					
10	Therja me gjilpëra te kontaminuam me gjak do te thotë paraqitje e sigurt e infeksioneve spitalore tek personi i therur.					
11	Vendosja e maskës është mbrojtje e sigurt nga infeksionet intrahospitalore të cilat përhapen përmes pikëzave të ajrit .					
12	Infeksionet spitalore shkaktohen nga mos respektimi i rregullave në spitale dhe nga mos respektimi i higjienës gjatë dhënies së kujdeseve nga ana e infermiereve .					
13	Rreziku më i madh për shfaqjen e infeksioneve spitalore është në kur i sëmuri lëshohet nga spitali.					
14	Ju si infermier nuk mund të ndikoj në parandalimi infeksioneve-brendaspitalore.					
15	Praktika infermiere në parandalimin e infeksiooneve spitalore nuk ka nevojë për përmirësim					

Ju lutem, rumbullakosni vetëm një rën nga zgjidhjet e poshtë shënuara:

**VI. Njohurit infermiere për masat e higjienës personale të personelit shëndetësor
(larjen e duarve) në parandalimin e infeksioneve spitalore**

Nr.	Njohurit infermiore për masat e higjienës personale (larjen e duarve) në parandalimin e infeksioneve spitalore	Pajtohem plotësisht	Pajtohem pjesërisht	Nuk e di	Nuk pajtohem pjesërisht	Nuk pajtohem plotësisht
16	Larja e duarve nga ana e personelit shëndetësor nuk është metoda më efektive për parandalimin e infeksioneve spitalore					
17	Larja e duarve me ujë të ngrohtë i largon të gjitha mikroorganizmat nga sipërfaqja e duarve të pastruara.					
18	Larja e duarve nuk është e domosdoshme para çdo ndërhyrje infermiore.					
19	Për të penguar përhapjen e infeksioneve intrahospitalore përmes duarve zakonisht i pastroj duarët në kohëzgjatje së paku 10 sec.					
20	Para fillimit të çdo ndërhyrje infermiore tek pacientet e ndryshëm nuk nevojitet dezinfektim i serishëm i duarve					

VII. Njohurit infermiore në zbatimin e ciklit të dekontaminimit

Nr.	Njohurit infermiore në zbatimin e ciklit të dekontaminimit	Pajtohem plotësisht	Nuk pajtohem plotësisht	Nuk e di	Nuk pajtohem pjesërisht	Nuk pajtohem plotësisht

21	Dezinfektimi është proces i shkatërrimit të plotë të mikroorganizmave patogjen nga sipërfaqja e objekteve të ndryshme.					
22	Unë mendoj që pas dezinfektimit të tyre, pajisjet janë të sigurta për përdorim tek të gjitha ndërhyrjet infermierore.					
23	Nëse temperatura në sterilizator me ajr të thatë arrin në 180°C sterilizimi i instrumenteve duhet të zgjatë 1 h (orë).					
24	Sterilizimin e instrumenteve e llogaris të kryer në momentin kur termometri në sterilizator arrin pikën e paraparë të temperaturës 180°C.					
25	Metoda kimike (me letër lakmusi) është metoda më e sigurt për kontrollimin e sterilizimit.					
26	Indikatori kimik i kontrollit të sterilizimit (letra e lakmusit) duhet të vendoset vetëm në sipërfaqen e jashtme të kasetës së instrumenteve.					

Ju lutem, jepni opinionin tuaj rreth konstatimeve në vijim duke rumbullakuar në opsionin më të përafërt ndaj përgjigjes tuaj

VIII. Njohurit infermierore për menaxhimin e mbeturinave infektive në parandalimin e infeksioneve spitalore

Ju lutem, rumbullakosni vetëm një të një nga zgjidhjet e poshtë shënuara:

27. Për të penguar përhapjen e infeksioneve spitalore tupferët e ndotur me qelb unë i vendosi në:

- a) qeska të zeza
- b) qeska të verdha
- c) qeska të tejdukshme
- d) kuti të fortë letre
- e) shportë mbeturinash

28. Për të penguar përhapjen e infeksioneve spitalore, mbeturinat me tehe të mprehta i vendosi në:

- a) qeska të zeza
- b) qeska të verdha
- c) qeska të tejdukshme
- d) kuti të fortë letre
- e) shportë mbeturinash

29. Për të penguar përhapjen e infeksioneve spitalore ushqimin e mbetur të të smuarit të infektuar e vendosi në:

- a) qeska të zeza
- b) qeska të verdha
- c) qeska të tejdukshme
- d) kuti të fortë letre
- e) shportë mbeturinash

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ORIGINAL PAPER

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Knowledge of Nurses in Prevention of Hospital Infection in Hospital "Sheikh Zayed" Vushtrri

Mexhit Mustafa¹, Aferdita Lahu¹

¹College of Medical Sciences "Rezonanca", Str. Glloku te Shetgjet – Veternik 10000 Prishtinë, Kosovo, and, Teaching clinic of Nursing of Hospital "Sheikh Zayed" Vushtrri

Corresponding author: Aferdita Lahu, Msc. Teaching of Nursing "College of Medical Sciences "Rezonanca", and, Klinikal Nursing of Hospital "Sheikh Zayed" Vushtrri. E-mail: mexhitmustafa857@gmail.com. ORCID ID:<http://www.orcid.org/0000-0000-0000-0000>.

ABSTRACT

Introduction: Hospital infections are the major challenge of modern health care, and they are indicative of the quality of the services provided, a hospital or other health institution. Hospital infections also belong to gastric infections, which are the result of the mistakes of health workers during various diagnostic and therapeutic procedures. **Aim:** This study deals with the research of knowledge, experiences, and the work of nurses and their interventions in the prevention of hospital infections in the Vushtrri regional hospital center.

Material and Methods: We used dry Sterilization (exposure at 160 ° C for 120 minutes or 170 ° C for 60 minutes) which is considered less safe than the first method especially for some devices with many tubes, pits, etc. Ethylene oxide and formaldehyde are released from use for safety reasons with the emission of gases. **Results:** From the research conducted we found that about 70% of nursing staff have satisfactory knowledge of hospital infections and the role of nursing care in preventing hospital infections, while only 55% have sufficient knowledge about the ways of spreading hospital infections. Over 77% of respondents have sufficient knowledge about the measures and prevention of hospital infections. **Conclusion:** The reason why we have been researching this topic has to do with many interventions, applications, nursing procedures is a series of actions that can contribute both to prevention and to the appearance of hospital infections. For their definition around the world, definitions of hospital infections are used according to the Center for Disease Control (CDC) from Atlanta. **Conclusion:** The reason why we have been researching this topic has to do with many interventions, applications, nursing procedures is a series of actions that can contribute both to preven-

tion and to the appearance of hospital infections.

Keywords: Nurses, patients, illnesses, spatulatory infections, medical care.

1. INTRODUCTION

Hospital Infections are an major problem that greatly reduces the quality of health care, and of life hospitalized patients (1-10). And, because of intensive therapy, and the duration of hospital stay has a very high cost, which is difficult to afford; when meanwhile, evidence suggests that this problem is avoidable or possible minimized (11-20).

Hospital infections or otherwise called nosocomial infections can are defined as: " An infection that a patient receives while in hospital for other reasons or conditions. " So, this infection was not present at the time of hospitalization hospital. Hospital infections include hospital-acquired infections that can also appear after the patient leaves the hospital, as (20) well as those affecting the medical staff who works in hospital.

The purpose of infection control is to prevent and minimize infections hospitalized patients and health care workers who suffer from e medical care. In this research, the best methods are described important to achieve two goals:

- a] . prevent or minimize the rate of hospital infections, and
- b]. protecting health personnel and visitors from the potential risks of Infection (20,21).

Also, this scientific research is needed to serve as a professional-scientific-practical tool for all subjects that provide health care to established infection prevention team's hospital in health institutions. The information in this

scientific research will come to you also assisting administrators, infection control personnel and health workers.

2. AIM

The aim of this study was to explore what are deals with the research of knowledge, experiences, and the work of nurses and their interventions in the prevention of hospital infections in the Vushtrri regional hospital center.

3. MATERIAL AND METHODS

This research presents a prospective study of nursing knowledge on hospital infections and their prevention. The structure of the respondents is comprised of 40 Nurses of different units of the Regional Hospital in Vushtrri (5). In hospital we use several types of sterilizations (Table 1):

Thermal sterilization

Fluff Sterilization: Exposure to humid temp. 121 ° C for 30 min or 134 ° C for 13 minutes in autoclave. Steam Sterilization is the most common and preferred method of sterilizing equipment penetrating the skin and mucosa, provided that heat and humidity do not damage those devices. Steam

Type of Autoklave	Packing	Temperature	Exposure time	Drying time
Gravitacional	I rolled	121 °C	30 minutes	30 minutes
Gravitacional	I rolled	132 °C	15 minutes	30-40 minutes
Paravakum	I rolled	132 °C		
Gravitacional	"Rapidsterilization" (nonferrous)	132 °C	3-4 minutes	
Gravitacional	Microbiological Waste	121 °C	45 minutes	

Table 1. Use of autoclaves by gravity

sterilization is a safe, non-toxic, free, sporocidal method, quickly achieves heat and penetrates well into the material.

Dry Sterilization

Exposure at 160 ° C for 120 minutes or 170 ° C for 60 minutes; this sterilization is considered less safe than the first method especially for some devices with many tubes, pits, etc.

Ethylene oxide and formaldehyde are released from use for safety reasons with the emission of gases.

Paracetamol is widely used in the US and some other countries in system processes.

Automatic sterilization.

Care should always be taken to ensure that sterilized devices go out of the department under the curve system: d.m.th. according to the date of sterilization, starting with older equipment.

- Storage conditions are important to maintain sterilization.
- The user must check the integrity of the packaging before use.

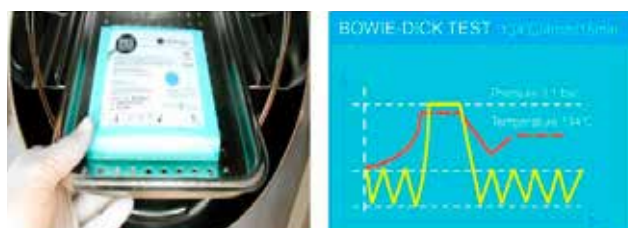


Figure 2. Check Steroid Bowie-Dick Test Method

The Quality Controls for the Sterilization Process (QCSP) should include such data about the process as: Loading, percentage, temperature and duration, Physical and Chemical Testing (at least every day), Biological Testing (at least every week), the evaporation process (sterrothermophilus bacilli), processing with ethylene oxide (Bacillus subtilis v. niger). Regular maintenance should be documented. For all types of sterilization, the following data should be recorded: how, date of service, model and country serial number, description of replacement parts, biological testing records, Bowie-Dick test, signing of the controller

4. RESULTS

The research included 40 nurses of different units of the Regional Hospital in Vushtrri: Intensive Care Unit, Surgery Unit, Pediatric Unit, Gynecology Unit and Maternity Unit. Results of our investigation are presented at Tables 2-4.

In the research of hospital nurses' knowledge of hospital infections, personal hygiene measures of health personnel (hand washing), implementation of the decontamination cycle and management of infectious waste in preventing hospital infections at Sheikh Zayed Hospital in Vushtrri (1). Of the 40 nurses surveyed dominated female gender (94%), age group 37 to 45 (62%), secondary nursing education (65%) and work experience over 30 years (32%) (2). From the research conducted we found that about 70% of nursing staff have satisfactory knowledge of hospital infections (19-20) and the role of nursing care in preventing hospital infections, while only 55% have sufficient knowledge about the ways of spreading hospital infections (2-4).

Over 77% of respondents have sufficient knowledge

Nr.	Nursing knowledge in preventing hospital infections	I completely agree		I agree in part		I do not know		I do not agree in part		I completely disagree	
		%	F	%	F	%	F	%	F	%	F
05	Hospital infections are infections that occur at the time of the patient's extension to the hospital environment.	25%	10	0	0	7	3	20	8	48	19
06	The presence of the cause of the infection in the hospital environment means a safe occurrence of hospital infections.	40%	16	30	12	7%	3	10	4	13	5
07	The source of hospital infections may also be people who have previously been infected but are now recovering.	35%	14	20	8	0%	0	22	9	23	9
08	Contact with the pus of infected persons means a safe introduction of hospital infections to contact persons.	59%	22	0%	0	2%	1	10	4	33	13
09	Contact with the spit of the infected person means the safe spread of hospital infections to the contact persons.	58%	23	22	9	2%	1	5	2	13	5
10	Slapping with blood-contaminated needles means safe introduction of hospital infections to the slaughtered person.	90%	36	0%	0	0%	0	0	0	10	4
11	Placement of the mask is safe protection from intrahospital infections that spread through the air drops.	77%	31	0%	0	0%	0	0	0	23	9
12	Hospital infections are caused by non-compliance with hospitals' rules and by non-compliance with nursing care.	71%	30	0%	0	6%	1	0	0	23	9
13	The greatest risk for the occurrence of hospital infections is when the patient is released from the hospital.	6%	1	0%	0	23	9	0	0	71	30
14	I as a nurse can not influence the prevention of internal-infection.	6%	1	0%	0	23	9	0	0	71	30
15	Nursing practice in preventing hospital infections does not need improvement	6%	1	0%	0	0%	0	0	0	94	39

Table 2. Results of questions from; 05-15 in percent (%) and frequency (F).

Nr.	Nursing knowledge on personal hygiene measures, (hand washing), in preventing hospital infections	Icompletely agree		I agree in part		I donot know		I do not agree in part		Icompletely disagree	
		%	F	%	F	%	F	%	F	%	F
16	Hand washing by health personnel is not the most effective method for preventing hospital infections.	30%	12	20%	8	5%	2	12%	5	33%	13
17	Washing hands with warm water removes all the microorganisms from the surface of the cleaned hands.	10%	4	15%	6	5%	2	30%	12	40%	16
18	Hand washing is not necessary before any nursing interventions.	10%	4	2%	1	7%	3	18%	7	63%	25
19	To prevent the spread of intra-hospital infections through hands, I usually clean my hands for at least 10 sec.	43%	17	10%	4	7%	3	10%	4	30%	12

Table 3. Results of questions from; 16-19 percent (%) and frequency (F).

Nr.	Nursing knowledge in the implementation of the decontamination cycle	Icompletly agree		I agree in part		I do not know		I do not agree in part		Icompletly disagree	
		%	F	%	F	%	F	%	F	%	F
21	Disinfection is a process of complete destruction of pathogenic microorganisms from the surface of different objects.	45%	18	33%	13	2%	1	5%	2	15%	6
22	I think that after their disinfection, the equipment is safe for use in all nursing interventions.	25%	10	15%	6	0%	0	22%	9	38%	15
23	If the temperature in the dry air sterilizer reaches 180 ° C the sterilization of the instruments should last 1 hour (hours).	75%	30	10%	4	0%	0	0%	0	15%	6
24	Sterilization of the counting instruments performed at the moment when the thermometer in the sterilizer reaches the prescribed temperature point of 180 ° C.	83%	33	5%	2	0%	0	12%	5	0%	0
25	Chemical method (with reagent paper) is the safest method for sterilization control.	62%	25	28%	11	0%	0	0%	0	10%	4
26	The chemical sterilization check indicator (litmus paper) should only be placed on the outer surface of the instrument cassette.	43%	17	8%	3	12%	5	12%	5	23%	10

Table 4. Results of questions from 21-24 in percent (%) and frequency (F).

about the measures and prevention of hospital infections. The role of nurses in the prevention of hospital infections is knowledgeable by over 70% of respondents, while 94% think that nursing practice in preventing hospital infections needs improvement (3-5).

The role of nurses in the prevention of hospital infections is knowledgeable by over 70% of respondents, while 94% think that nursing practice in preventing hospital infections needs improvement (9-10).

For the role of hand hygiene as a major measure in preventing the spread of hospital infections (17) only 45% of the nursing staff have knowledge, whereas only 75% of the respondents know the techniques and length of the handwashing (11-12).

About 75% of the research participants have sufficient knowledge of the decontamination cycle with a special emphasis on sterilization and disinfection as a key element in preventing hospital infections (3-15), while 90% of re-

spondents classify and manage in-hospital waste (9-13).

5. DISCUSSION

A topic such as nursing research on preventing hospital infections has not been previously investigated in our hospital, and this prevents us from comparing our findings with preliminary data at the Vushtrri / Vucitrn Hospital but by comparing literature used (2-5). What should be noted is that many nursing practices need to be advanced, so our research was very good in order to see the possible failures that may occur during internships and nursing work (4-6).

Nurses' knowledge of hospital infections and their prevention are consistent, as their preparations and knowledge help reduce hospital infections (3-8). Although the survey shows that respondents are aware of some nursing measures (interventions) in preventing hospital infections (16) also, shows some interventions that need to be improved (13). Nursing knowledge of hospital infections and their prevention. This study shows that respondents are aware of some nursing interventions to prevent hospital infections and also indicate some interventions that need to be improved.

As the best way to prevent hospital infections, it is about a model of assessing the professional development of nursing staff. Therefore, based on the findings of the main work and goals, and the literature review, we can conclude that hospital infections pose a serious threat to modern health, both in industrialized and developing countries, such as Kosovo.

The paper presents a realistic overview of nursing knowledge at Sheikh Zayed Vushtrri Hospital, where nurses' knowledge of hospital infections and their prevention at Sheikh Zayed Hospital in Vushtrri is consistent, as their preparation and knowledge help in reducing hospital infections.

What should be emphasized that the many nursing practices need to be advanced, so our research was very deliberate in order to detect potential omissions that may occur during nursing practices and work.

The role of nursing in preventing hospital infections is unique, as many interventions, applications, or procedures such as: hand hygiene, disinfection, sterilization, waste management, all of which are closely linked to patient care and the hospital environment, constitute the main challenge in preventing infections in general.

Through this research, we can suggest not only the hospital where the research was conducted, but also other hospitals to think more about study or professional research, because, nursing has to do with a wide range of actions, behaviors, applications, education, advice, and many other professional practices. The frequency and types of these infections depend on many factors:

Type of hospital, patients' immunological status, hospital hygiene, staff training, doctrines on antibiotic use for preventive and curative purposes, etc.

The onset of nosocomial infections complicates the course of primary disease (increases morbidity and mortality), prolongs treatment time and increases cost.

Causes of intrahospital infections can be almost all microorganisms (bacteria, viruses, mosquitoes) (20-21).

The types of bacteria causing the infections over time

have changed, depending on the type of antibiotics applied and aggressive diagnostic and therapeutic procedures that cause mucosal and skin lesions (integrity damage). These bacteria develop resistance to antibiotics and disinfectants. The most common causes are: *Escherichia coli*, *Staphylococcus aureus* (especially methicillin resistant species), *Enterococcus sp.*, *Pseudomonas aeruginosa*, *Acinetobacter spp.*, *Klebsiella pneumoniae* (18-19). *Proteus mirabilis*, etc.

This research has been researched to assist hospitals and especially services where infections hospitals appear more frequently to implement preventive measures in their work and better corrections, and based on scientific evidence. It will help that hospitals to meet safety and quality standards requirements of patients and staff designed by the Health Centre Hospital (18-19) and approved by the Ministry of Health, for the purpose of hospital accreditation (20-21). The research of this paper was done with respect essential recommendations of the national strategy of the SSSC of the Republic of Kosovo (18-19).

The most optimal and safe way of nursing knowledge constantly remains a challenging and controversial issue among health professionals. Accumulation of knowledge on various aspects of nursing is a "body of knowledge" that nurses and others can draw on and contribute to it. Exploiting research to support practice is the ultimate goal of nursing research.

The reason why we have been researching this topic has to do with many interventions, applications, nursing procedures is a series of actions that can contribute both to prevention and to the appearance of hospital infections. Such a topic on nursing research on preventing hospital infections has not been previously investigated in our hospital, and this prevents us from comparing our findings with preliminary data at the Vushtrri Hospital but by comparing literature used.

6. CONCLUSION

For the role of hand hygiene as a major measure in preventing the spread of hospital infections, only 45% of the nursing staff have knowledge, whereas only 75% of the respondents know the techniques and length of the handwashing.

About 75% of the research participants have sufficient knowledge of the decontamination cycle with a special emphasis on sterilization and disinfection as a key element in preventing hospital infections, while 90% of respondents classify and manage in-hospital waste.

Key recommendations regarding personal hygiene.

a) Selection of protective equipment shall be based on transmission risk assessment the patient's microorganisms, and the risk of medical personnel being contaminated from blood, body fluids and patient secretions (Grade D).

b) For any invasive procedure and for any procedure thought to be at risk.

Exposed to blood, bodily fluids, secretions or contaminated work tools must be worn gloves (Grade D).

c) Gloves should be disposable, they should be worn immediately before a situation where they will have contact with the patient and be removed immediately after contact

with the patient is completed (Grade D).

d) Gloves should be disposed of where medical waste is disposed of and hands should be cleaned after removing gloves (Grade D).

e) No gloves or polythene-containing gloves should be used (Grade D).

f) Where there is a risk that the clothing may be exposed to blood, bodily fluids or secretions should be worn with disposable plastic protective aprons.

After using them should be disposed of at the medical waste site (Grade D).

g) Where there is a risk that the face may be sprinkled with blood, body fluids or secretions face masks and eye protection should be used (Grade D).

- **Author's contribution:** M. A. P, M. B. and S. G. gave substantial contributions to the conception or design of the work in acquisition, analysis, or interpretation of data for the work. A. B and S. P. had a part in article preparing for drafting or revising it critically for important intellectual content, and A. P gave final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
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Research Article

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Information System Management in Health Institutions of Kosovo: Case Study

Afrim Loku

*University of Applied Sciences in Ferizaj
Rruga e Universitetit, Ferizaj, Kosovo;
Corresponding Author*

Nadire (Shehu) Loku

*College "Rezonanca"
Prishtine, Kosovo*

Lindita Loku

*University "Mother Teresa",
Skopje, North Macedonia*

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Abstract

Introduction: The main point in this paper is dedicated to the role and importance of the information system in public institutions with emphasis on health system: system preparation, establishment, implementation, and its application. Objective: The aim of this study is to increase the awareness and importance of health information system in health institutions and management and, to present a concept model of health information system to health institutions in Kosovo. Results: Presented Health Information System model shows the benefits and the role if implemented. The main aspects this concept takes in consideration is overall network, strategic flexibility and cost-reducing. Conclusion: This study highlights the benefits possible when new electronic health information system is fully integrated in health system of Kosovo. This case study illustrates the importance of developing new health information system that meets the actual challenges of health system, improve the system quality, usage and care quality.

Keywords: Health Information System, Electronic Health Information System, Healthcare

1. Introduction

Overall Social and Economic Development has raised the need for continuous information availability, in order to make successful managerial decisions, because everything is transitioning very fast. The fact that the continuous progress and growth of medical sciences, the diversity and harmonization of health activity, the high cost and investment in health, have conditioned a large amount of information in all areas of health, however they should definitely be in a fair and unique way to systematize, exploit and evolve. Developed countries in the world have long established a unique health information system, using all its benefits, while in our country this part of the work in the framework of health activity is left in the background. Although reforms have been greatly promoted and efforts have been made to restructure the health system in Kosovo, we still have no progress when it comes to the unique health information system (HIS). There is no coordination of a unique working methodology, system compatibility accounting, tools, compatible software support, unique communication system in setting unique health policy indicators on the basis of which are followed and orientations of the health status of a population fall. Health decision makers should know that a good information system, in future health activity, among other things, ensures quality in health care, rational presentation, efficient health care, reduces municipal costs, improves prevention, cure and provides evidence evolutionary work based on scientific facts. A unique program would support the health information system (HIS) which would connect all institutions in one center in order to provide and assist health care from the ministry to the municipal health level, regional health information insurance, primary health care and secondary, drug supply institutions, health - educational institutions, etc. This system would provide health information for all decision makers, as well as for the population and would actively involve them in the implementation (undertaking) of health protection measures, research of health phenomena in the population, improving efficiency and the labor economy in health, planning, programming and decision making in health activity, as well as scientific research.

1.1 Development and the Understanding of Health Information System (HIS): Promoting Health Information Technology in California – Reference System

Information processes are essentially conditional, starting from obtaining, processing, storage and the use of data. Information is presented as a social need and necessity for successful governance with objects or systems of different nature. Today an increasing number of enterprises have realized that without complete, accurate and relevant information there is no successful governance. Therefore, before giving a definition of information systems we will give some definitions of the notion of information. Information is neither a material nor energy, but in the system it conveys the material

and the energy. The material, is needed to transport and store it, while energy is needed to collect, process and transfer it. However, information is not subject to the laws of material and energy, because it is not spent on exploitation and is not reduced by sales. I.Turk says: "Information is governed and purposefully oriented to an address. (Ivan Turk: Building a business information system).

In this strategy the term of "Health Information Technology" refers to electronics systems to health care professionals and increasingly, patients who use it to store, exchange and analyze health information. Additionally, Health Information Technology is a mechanism of technologies and processes which help electronic generation, storage and transmission of medical information. This platform electronically stores all individual data about health history, enables communication between employees and health care units as well as electronic processing of prescriptions or laboratory test results.

According to the Legislative Analyst Office (2007) the federal system (Figure 1) is described as a process of the sharing of medical information by each participating health units, e.g., hospitals, regional hospitals, and local health units, laboratory, and stores the data pertaining to its patients on its separate computer systems. Further, health units are then connected by a computer network that allows its users to search for health data on each of the other systems through using patient indexing software and record locator software. However, this system allows each participating health facility to maintain different computer programs on its location as long as those programs are connected and interactive with each other.

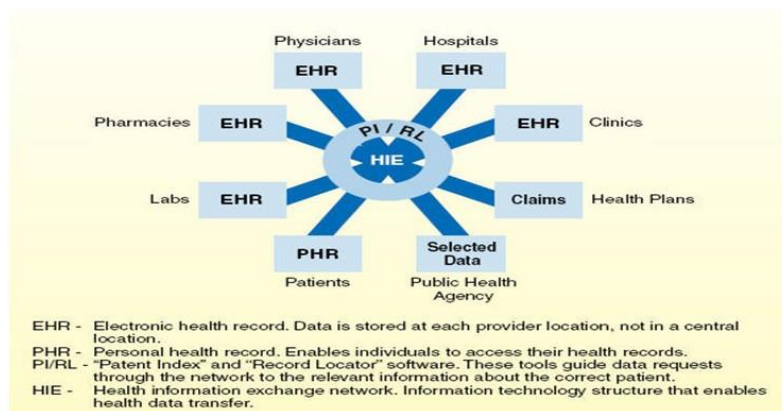


Figure 1: Regional Health Information Organization, Federated System Example. Legislative Analyst's Office (2007). A State Policy Approach: Promoting Health Information Technology in California. Regional Health Information Organization, Federated System Example. [Accessed 10/01/2019, https://lao.ca.gov/2007/health_info_tech/health_info_tech_021307.aspx]

2. Methods

2.1 *The current situation of the functioning of the Information System in Health Institutions of Kosovo*

The organization and functioning of the Information System in health institutions so far has been non-functional and as such has not been able to successfully perform information tasks.

It takes time to implement and fully integrate the health information system. Developments that have occurred in terms of the advancement of information technology, especially in the last decade, have made the information system in all institutions change from the concept of functionality. The health information system continues to be organized in a more classical form which means a chain that starts from primary health care, ambulances, health stations, health homes, secondary and tertiary health care, rehabilitation (hospitals), all of which are referred to Kosovo Institute of Public Health. Which was responsible for the health information system where it provided reports (quarterly, semi-annual, annual, statistical yearbook). The problem of applying a new system were primary derived by the rising health costs, health care lagging in information technology and the premises of technological advancement.

However, many of the proposed projects were for implementation during basic phases of establishing a health information system, where in some cases of health institutions it was necessary to develop the information system in low-cost and simultaneously with its separate systems in laboratories and pharmacies. Some of the key activities of the health system were not properly supported, such as the durability and ease of printing the material. Inefficient projects and implementation has left users dissatisfied all these years. Introducing key features of the system takes precedence over managerial reporting, thereby reducing immediate decision-making benefits. Failure to respond quickly to various malfunctions results in some Clinics / Hospitals computers not working for several days. Restricted access causes some of the employees / staff not to be able to log in to the system, which also leads to nonsense. The need to change the evaluation component to that of external control before and during the design is necessary, as the system had to be implemented as soon as possible in all clinics. The same system currently does not perform the efficient function, therefore the definition for another more advanced form of HIS (health information system), is necessary in order to advance the health services, proposing a more advanced program for all clinics, for what needs to contract experts for the implementation of this project. In the two clinics we monitored where the system was installed, a marked change was observed in the field of the analyzed variables of quantitative outcomes (mean time of outpatients, length of stay, number of drugs prescribed per patient, improved participation collection, case price and number of deliveries from other centers).

The functioning of the health information system which has mainly covered a part of the health services with a limited access as can be seen in figure 1:

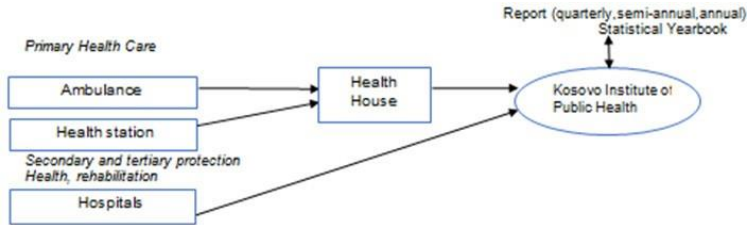


Figure 2: Data collection by units

From what we have encountered as part of the data for HIS, Primary Health Care is mainly concerned with: health services, staff, morbidity (diagnoses), number and type of health services, type of health insurance, medical leave.

While in Hospitals the following data have been collected: such as health services, staff, morbidity, mortality, health capacity, type of health services. In addition to those mentioned above, data were collected from other sectors such as: occupational medicine, public health, immunization, air pollution, water and food and demographic data such as water supply and sewage network, etc. While data on the use of / Expenditures of drugs in the health system have not been collected (although there has been a tendency).

The role of the Kosovo Institute of Public Health (KIPH) in HIS in the previous period was: Collection, inclusion control and quality control of data;

- Data processing;
- Reporting towards higher levels;
- Publication of the Statistical Yearbook for population health;
- Analysis and proposal for problem solving (based on the requests and needs of the executive bodies - Ministry of Health, Health Insurance, etc.)
- For professional and scientific needs (specializations, dissertations, symposia, congresses).

2.2 Health Information System Development and Perspective in Kosovo

All information systems should be based on a clear information strategy, identifying information priorities and needs at each level of the organization. Based on this the system should best suit the identified information needs of the services. Many systems are implemented in which case after implementation they are not used properly, because they do not provide the specific information that a particular service need. Different management structures, cultures, competencies and organizational priorities

should be taken into account when choosing the appropriate information systems. It is the managerial responsibility of the Ministry of Health, to lead this process and to engage the key parts of the services: hospitals, family medicine, etc. It is the managerial responsibility of each part of the services to determine the information needed for efficient management, and it is also the responsibility of the hospitals / clinics to determine the information needed to effectively manage the diagnoses, care and treatment of their patients. Possession of information, administration and circulation of information should always be clearly defined and this is the responsibility of the management of the Hospitals and the ministry. Managerial responsibility for accuracy, ease and adaptability should be included in the job description of the corresponding individuals, as is the provision of accurate information. In order to enable the management of Kosovo health institutions to make reasonable decisions on service planning, it is necessary to create a new information system. For the benefit of the people of Kosovo, for which it is necessary to have an accurate and standard system of high quality, for data collection on the needs of health institutions. All Clinics must place special forms according to the services performed and the same to be registered in the computer. Comply with the content of the forms that must be submitted for the registration of data from diagnostics and activities in operating rooms and ensure that the necessary training and technical support for all are followed.

2.3 Reference system

Major improvements will not be for a short time, and will not be achieved only by the UCCK (University Clinical Center of Kosovo). Some strategies can be taken at the level of UCCK, others at the level of the Ministry. The Ministry of Health in cooperation with UCCK, should limit the number of patients that will be checked within a day and in cooperation with UCCK should start implementing the standard system of pre-arranged visits. The Ministry, in cooperation with other actors, should continue to train staff in primary and secondary care and provide incentives for their stay in peripheral areas by limiting the number of referrals from other health centers and regional hospitals sent to the UCCK. Competent institutions in this process should also consider including some form of potential strategy that should include rewards for general practice to encourage them to act as true advocates. Distinguishing different types of referrals, specialist consultations; for example, referral for opinion, referral for relevant services and referral for taking total responsibility for patient treatment. This should be part of improving communication between low referral and high referral. Reinforcement of referral guidelines (e.g. adjusting numbers and imposing penalties for over-referrals). This will indicate which patients should be referred (appropriate referrals) and further research into the reasons why health homes are referring high number of patients. A public information campaign will encourage a change in the attitude of staff and patients. The Institute of Public Health, with the support of local health directorates,

could lead these types of campaigns. As part of the information campaign, improving communication between the UCCK and other regional hospitals would have a mean, as well as improving communication between the UCCK and its patients. Therefore, encouraging a better information system would increase the possibility of efficiency of services not only in activities with patients but also in all other follow-up services through which the referral system would be improved. Since the most appropriate referral models will come from clear improvements in primary and secondary services in Kosovo. In Figure 3 you can see the collection of data which will be used for the preparation of various reports and analyzes by all health institutions.

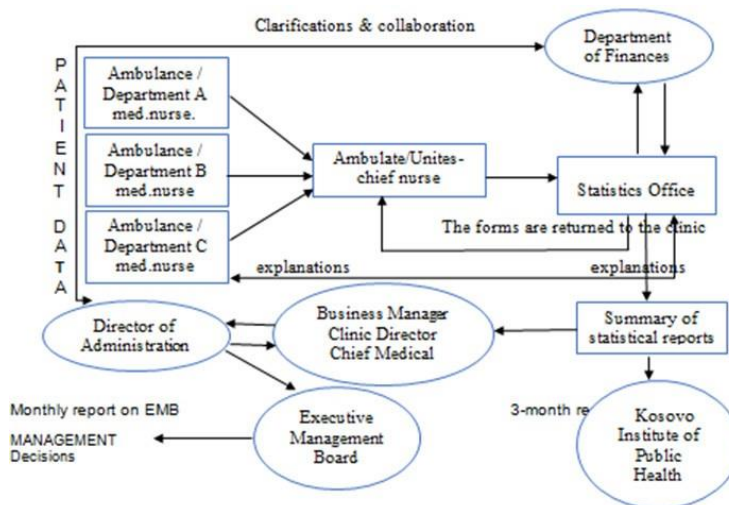


Figure 3: Data collection on patient activity and reporting flow

3. Results

3.1 The concept of new HIS Model in health institutions of Kosovo

Further progress needs to be made by all related parties to advance the capacity and structure of the health care system in relation to the construction of the Electronic Health Information Network. Further progress needs to be made by all related actors to advance the capacity and structure of the health care system in relation to the construction of the Electronic Health Information Network:

- Strengthen the legal infrastructure and human resource training by explaining the current stages of the Health Information System.
- Improving the referral system from Regional Hospitals to UCCK through Telecommunications training and continuing professional development programs.

- Monitoring and testing the technical performance of the infrastructure.
- Integration of the National Telemedicine Center in the Health Information System network in the field of health economics.

The reflection of the strategies of the Ministry of Health, for the reforms and the improvement of the quality of services in the field of health care, of the existing and feasible results in the future. It should be based on the performance of informing the health indicators in order to maximize the sustainability and continuity of investments in health information system, through regular reporting and monitoring of the current situation and directions of development of human resource skills in the future. In order to optimize the risk / benefit relationships in all stages and areas of management for integration and definition of a system architecture we must also consider:

- Decentralization of authority towards the municipal level,
- Sectorial cooperation and intersectorial cooperation
- Community participation in health services and,
- Budget

In Figure 4, the scheme of functioning and organization is presented eliminating the raised dilemmas that; as well as by whom all data will be collected.

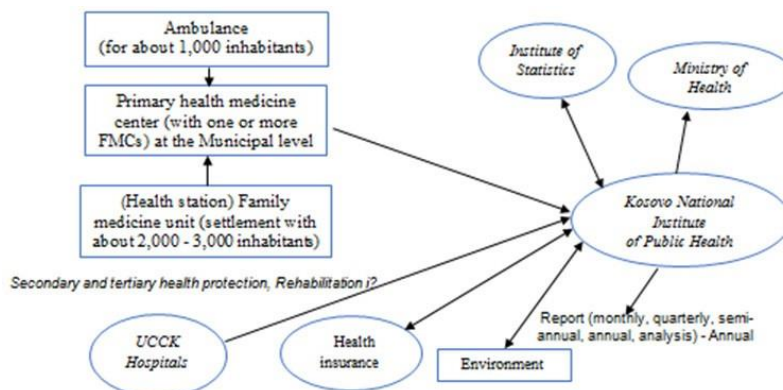


Figure 4: Organizational chart which shows which data will be collected, who, how, when will be collected, and to whom are they dedicated?

The minimum statistical data to be collected by HIS according to the above scheme would be the answer to the following questions: What to collect and how, and where the data comes from, how it is analyzed and who did it, how it is derived, and from who was used, who collects the data and by whom they are collected, what data they collect, in what form they are, were they used at the local level, when the data was collected, how often it is reported and how much is reacted based on them, where the data was sent are they first analyzed at the municipal level before moving to higher levels, why is

the data collected, why the collector needs to justify himself; At the request of the director or decision maker at the highest level, how the data / information is transformed into a concrete health action.

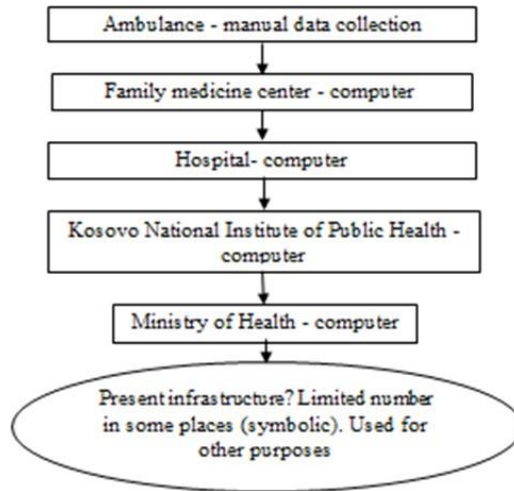


Figure 5: Development of skills and knowledge of the decision-making staff at different levels for the collection, flow, interpretation and utilization of information.

Currently a small number of persons have managerial expertise at the municipal level. This makes it difficult to identify and determine the needs of people locally for the type and other elements of information they need to have. It also limits and disables the analysis and practical use of information. This conditions a dependence of expertise from outside the institutions.

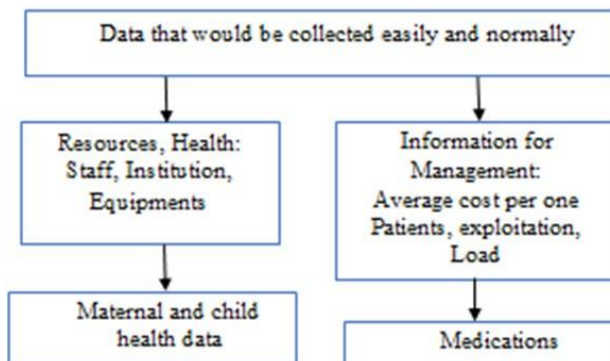


Figure 6: Data collection / information scenarios

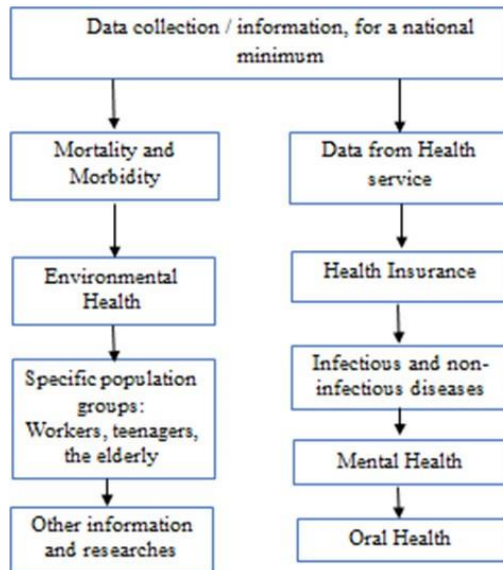


Figure 7: Data collection - Proposal for the minimum at the National level

4. Discussion

4.1 Importance of developing new HIS models

Based on the information system and the research done, different alternatives for investments or new reforms can be presented, whether they are in the information system or in projects related to developments in other areas. By selection a group of possible alternatives is obtained where the purpose of this selection is to remove in the first step from further consideration of all alternatives that are not in line with the intended goals and are unlikely to be successful. Pre-investment analysis is the first step in investment management, because it serves to determine investment ideas. This means that no investment is presented by itself but that a preparation of them must be made first. The identification of investment projects starts from possible investment ideas, where the ideas usually have their source in development policy and investment policy. We will have good ideas only if we have well defined development strategy. If there is no development strategy then there can be no question of successful investment projects. The future is uncertain and mistakes are possible. This stems from the fact that when deciding on investments the data on the past, mostly serve for decision making, the effects of which are expected in the future. Therefore, to be successful in investing we need to think about what we expect to happen. We know what happened, but the past may or may not be repeated. However, the future is uncertain so it is reasonable to evaluate the efficiency of investment placement.

Evaluating the efficiency of investment placement means a set of activities which aim to examine its reasonableness. This means that proposing an application and making investment decisions also includes economic evaluation where costs are compared to profit (effects). "Expenditures can be estimated accurately, but the effects are difficult to measure." Once such assessments are made, economic efficiency can also be calculated. For example, the payback period can be reviewed. The evaluation of the efficiency of the investment project is a specification of data from the entire investment study. For this purpose, the expression is made through indicators which express the efficiency and effectiveness of the project. The evaluation of the efficiency of the investment project aims to derive the efficiency indicators on which the investor relies during the final decision. This system should include: Services, Network Design, Networking, Networking in Detail, Backbone - Object Connection, Networking Applications, Services, Help Desk (HIS Rapid Interventions), Workshop (Office within SISH where quick interventions are made - replacement of computer parts, etc.), increase of capacities as needed (new computers to be connected to the system: physically and software), technical support for internet (for internet use for those who do not have experience). Standardization of the information system and compilation of the action line (Internet & Intranet use Policy), Preparation of a detailed plan for the new Information System in general, Evaluation of HIS requirements for a unique database software for the future:

E.g.: Patient registration, Billing, Warehousing, IT equipment, Doctors, organization of on-demand trainings, connection with other hospitals (internal services: FTP, access to documentation, servers - intranets, etc.). The computer network that would be installed in each institution consists of a certain number of computers and other network equipment (printers, scanners, etc.).

The network is used for electronic mail (e-mail), data transfer, as well as for eventual access to another computer via the Internet in another location or for interactive follow-up of events (telemedicine, hospital board meetings via multimedia web camera, high-screen). Today's development of IT (information technology) of various companies and organizations includes the security of sensitive data of companies. The use of the network will also develop the use of new software applications and communication technology as well as data storage. The importance of the Intranet is considered a priority in all world organizations and companies. Applying and using the rules of international telecommunications organizations (TIA / EIA, IEEE, BNI) increases work efficiency.

Computer networking is essential in integrating information technology and digital information into the use of computer systems.

4.2 Benefiting from network

Benefits in communication in general, Coordination and internal communication,

shortening of time and potential up to 80% affects productivity and shortens the time for:

- 40% of working time in document processing
- 40% of working time for communication

4.3 Strategic flexibility

Energetic growth of dialogue and access to information and allocation of knowledge resources.

- Productivity (Randell R, et al. 2007; Chaudhry B, et al. 2006; Balas et al. 2004;)
- Easy, fast and flexible communication, dialogues: One-on-one, much-more-more. (van der Kam WJ, et al. 2000; Georgiou A, et al. 2007)
- Productive cooperation (Bryan C, et al. 2008)

Possibility to use various media without limit of geographical borders. Possibility of limiting internet access and intranet data according to importance and hierarchy, use of the Internet for teaching in different professions, development, distribution and application of intellectual capital as well as sufficient free and paid training resources.

4.4 Cost Reducing

In addition to reducing expenses for meetings, traveling and telephone conversation time, reducing printing and distribution costs, due to the development of the information system. We will also have many benefits that consist of fairer budget planning for medium-term periods of interest to Health Institutions by reducing unnecessary costs, but not only that. Starting from the current moment of contemporary social and economic development, currently the Kosovar society and all institutions are at the stage when they are making efforts to organize the management information system, and its role is very necessary for the organization and the function (Niès J, et al, 2006) of health institutions, in other words in rationalizing and increasing its efficiency (Jackson TL. et al. 2006; Urquhart C, et.al) , both by reducing costs (BalasEA, et al, 1996; Kramer et al. 2003; Dorr D et al. 2007) and decision-making time that we witnessed during the pandemic "Covid-19".

5. Conclusion and Recommendations

The role and importance of the information system is related to the processing and delivery of information to their places of use, thus enabling the monitoring of the process of preparation, receipt and implementation of decisions. This is where the importance and necessity of applying the information system lies. Overall Social and Economic Development has raised the need for continuous information availability to make successful managerial decisions because change is happening very fast. The fact of

advancement and continuous growth of medical sciences, diversity and harmonization of health activity, high cost and storage price, have conditioned a large amount of information in all health subjects, however they definitely need to be rightly and uniquely systematized, exploited, and evolved. By health information system we mean the mechanism of collection, processing, analysis and receipt of information necessary for the organization of health protection, scientific and medical research. To achieve this, mechanisms, methods, people and machines are needed, which together form the system through which information is processed. Today, this elaboration represents not only a suitable ground for decision-making, but it can be rightly said that he who has information, also has knowledge and managerial power. Developed countries in the world have long established a unique health information system, using all its benefits, while in our country this part of the work in the framework of health activity is left in the background. In the future, the importance of the information system in health institutions will be very prominent, even for current and future development trends, and it will be a limiting factor of development in the modern economy. Based on the current development trends and the need of businesses for their most successful development, the affirmation of the need for organization and functioning of the information system is very necessary both at the national level and at the level of health institutions, especially in UCCK (University Clinical Center of Kosovo). Both the organization and functioning of the health information system within the UCCK and its future development requires first acquaintance with the organizational structure of functional units within the UCCK and of course their follow-up with the very important part of the health management system. Each system in its functioning has its own difficulties and advantages in organization and functioning. Therefore, the future model will aim to minimize the difficulties in the functioning of the system and to adapt it to the highest possible extent to the needs of the management, presenting their products as essential for an effective and active health system. Mistakes that may occur during the implementation of the H.I.S. may be repeated until the unique nature of health information system is understood and a regular assessment is established for all, from the outset. Implementation weaknesses often result in failed aspirations by dedicated information technology personnel, health managers, and other professionals. Most demoralizing, however, may be the loss of initial investment. Many countries have gone through difficult and often depressing phases in terms of building the Health Information System including the UK which has poured in millions and applied various disciplinary measures to build S.I.S.H. Most authors see the definition of information system as an organized whole of its elements and a formalized part of the communication system of a particular organizational unit, which consists of units and machines that generate, or use information and establish communicative relationships with purpose of realization of information processes. Therefore, we can conclude that the information system is a functional link in the process of collecting, processing, transmitting, using and storing data and information through electronic data processing systems.

The purpose of the information system is to process, store and transmit the right information in the right place, and the best information system is the one that performs this function as quickly and inexpensive as possible.

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Internal factors affecting the performance of Small and Medium Enterprises in Kosovo

Assoc. Prof. Afrim Loku

Vice Rector for Budget, Finance and Infrastructure – University of Applied Sciences in Ferizaj, Kosovo

afrim.loku@ushaf.net

Dr. Nadire Loku

Lecturer – UBT College, Pristina, Kosovo

nadire.loku@ubt-uni.net

Abstract. This study aims to provide an understanding of the importance of the SME sector in the socio-economic development of Kosovo and examine in particular the influence of internal factors on them. A cross-sectional survey was conducted with Kosovo SMEs through an anonymous online questionnaire. This study confirms that all internal factors - managerial skills and competencies, business experience, access to finance and technological capabilities, depending on a period, have a significant impact on the performance of SMEs. While access to finance is considered as a major challenge, technology is considered to help improve business performance. However, results revealed that poor managerial skills and competencies, despite having a significant impact on the performance of SMEs, they were also related to access in finance and technological skills, making it difficult to run a business. Training and empowerment of SME managers in key business areas can be an opportunity to improve business performance.

Keywords. internal environment, SME performance, managerial competencies and skills, access to finance, technology.

1. Introduction

The main problem that prompted this study was the inadequacy and scarcity of empirical and up-to-date knowledge about the SME sector in Kosovo, which consequently made it difficult for entrepreneurs and researchers to implement and adapt practices and theories. This research addresses in particular the impact of internal factors in the SME sector. Considering that most of the studies by national and international actors did not include the micro-enterprise sector, which constitutes more than 93.1% of the SME sector, as a research subject we also included the micro-enterprise sector along with small and medium enterprises of Kosovo, which are vital to the country's economy, and the impact of factors such as managerial skills and competencies, access to finance and technological capabilities in the performance of their enterprises.

1.1. Small and medium enterprises – concepts and relevance

The most common determination that distinguishes large and small enterprises is the number of employees [1]. According to Bolton Report [2], describe two main approaches that summarize all definitions: quantitative and qualitative approach [3]. The majority of nations, policymakers,

academics, statistical agencies, and international institutions mainly applying quantitative criteria in defining SMEs. In Kosovo the classification of SMEs is defined by law on foreign investment which entered into force in 2014. This law defines SMEs by the sole criterion of the size of the number of employees, which is in line with the definition of the EU.

Table 1. Definition of SMEs in Republic of Kosovo

	EU definition	Kosovo definition
Micro	< 10 employees, turnover or balance sheet total \leq EUR 2 m	< 10 employees
Small	< 50 employees, turnover or balance sheet total \leq EUR 10 m	< 50 employees
Medium	< 250 employees, turnover or balance sheet total \leq EUR 43 m	< 250 employees

Source: [50]

SMEs play a special role in the economy of Kosovo, accounting for 99.8% of all enterprises in 2016. They generate 81% (56.2% in EU) of the total value added and 76.2% (66.5%) of the total employment in the economy of Kosovo, making a significant deviation comparing to the EU average in the respective accounts (EC, 2019). In addition, the SME sector in Kosovo achieved strong growth rates in terms of value added with 88.5% (11.7% in EU) and employment 27% (6.6% in EU), substantially exceeding the average achievement by EU SMEs [4].

However, their contribution to export is only around 5-6% [4]. The largest value generated by Kosovo SMEs comes from medium-sized enterprises (47% of total value added) representing only 1% of the business population, which employs one-fifth of the total business sector employment, however the value added created by micro-enterprises (13.2% of total value added) remains very low compared to other economies in the region [5].

Services are largest and most important sector of Kosovo's economy contributing significantly to GDP (72.63% of the GDP) and job creation (85.3% of the employment in Kosovo), driven by construction, real estate, and retail [6]. Although the contribution of SMEs is undeniable for the economy of Kosovo, SME sector is characterized by traditional and non-innovative enterprises and often suffers from a lack of skills and training related to business activities; access to finance, poor competitiveness, technological capabilities, etc.

1.2. Literature Review

SME sector has always raised the interest of academics to adapt and create applied models and economic theories to achieve the best performance due to their socio-economic importance, specific business characteristics and behavior [7][8][9].

Despite the large role for the economic and social development, growth of small and medium enterprises (SMEs) continues to be challenged by a various number of factors. Based on many empirical studies, it has been pointed out that most of the SMEs do not survive more than 5 years. Considering this conclusion, it is very important to increase attention to the factors that influence such results.

The main characteristics of SMEs tend to derive from their limitation such as personalized management (owner/manager), constraints on resources (management/organization, human

resources, finance) and technological capabilities, limited market impact, and greater flexibility to the external environment [7].

Although the authors have paid great attention to identifying the factors that affect the growth of SMEs, however, they have not yet been clearly defined. But, in an extensive study with 1386 fast-growing small businesses identified four groups of factors that affect the rapid growth of businesses such as entrepreneur's personality, business strategy, resources and capabilities of the business entity and the external environment [7]. Considering that most of the identified factors that affecting SME performance were internal, this study focused only on identifying and influencing internal environment in Kosovo SMEs.

According to [7], "managing the internal environment is usually connected to the degree of performance achievement of a business entity". Considering that studies examining only the impact of internal factors as a whole on the performance of businesses are rare, it increased the interest and attention to perform one.

Internal environmental factors are considered as factors within a business that affect the business and are more controllable by the business entity [10]. Internal factors can influence the performance of a business both positively and negatively. Main internal factors affecting performance can be grouped into three categories - human resources, finance and technology. However, we will focus on the internal factors that challenge SMEs. Therefore, in the literature review we have included factors such as managerial skills and competencies, access to finance, and technological capabilities.

1.2.1. Managerial competency and skills. Managerial competencies which consist of a set of skills (human, financial and information), knowledge and attributes that enable an individual or small business owner to perform a specific task or activity within a function [11], are considered as crucial factors that contribute to survival and performance of SMEs. Recently, changes in the global market have increased attention to the impact of competencies and skills on the performance of SMEs. Many authors have found a positive relationship between managerial competencies and the performance of SMEs [12][13][14]. However, a number of studies have examined the relationship between managerial competence and small business failure [15][16][17], and identified that the lack of relevant skills or expertise (supervision of financial, marketing, HR) in basic business management are the major contributors to small business performance and failure [18][19]. In order to ensure a superior performance, a firm or owner must possess appropriate skills and abilities before running the businesses [20]. In Kosovo there is an insufficiency of studies in relation to factors such as managerial competencies and skills. Given the high importance we discussed of managerial competencies and skills in the performance of small and medium firms, it is of great importance to examine the impact of these factors on Kosovo SMEs.

1.2.2. Access to finance. Access of SME to finance is considered as main barrier faced by SME in the region [21], impacting growth and development of business entities. Given the characteristics of the sector, the authors find that young firms of small size face greater obstacles when they seek to obtain financial resources [22]. However, it is noted that mostly of the SMEs rely on internal finance, such as existing cash, contribution from the owners, family and relatives, which is often insufficient for SMEs to survive and growth [23]. Kosovo as a developing economy with different market forces which are hindering the competitiveness of local businesses, the necessity of investment has been created in order to grow their businesses. As a result, the demand for external financing has been stimulated, especially for micro-enterprises. Credit is the main instrument of SMEs for funding. The most apparent private sector financing gaps in Kosovo include those for loans to micro-enterprises and for equity finance [24], which MFI struggle to meet the growing demand due to limited funding sources. However,

as a result banking sector have decided to stop serving the microenterprise segment, which is hindering the growth and survival of 93,1% of SMEs. While small and medium loan finance availability is sufficient, high collateral requirements present a major barrier for access to finance by SMEs [24]. The lack of sophistication in areas such as business planning, financial record keeping, and growth management has created a barrier to responding low collateral and equity finance requirements, which have consequently limited credit.

1.2.3. Technological capabilities. Technology has become a crucial and inseparable mechanism for all kinds of organizations. Through it, organizations improve the performance and overall effectiveness of products, services and systems by enabling businesses to expand quickly and efficiently. Technological advancement in the modern era presents a great challenge for the SME sector. The role of information technology for the SME sector is being considered critical in relation to performance enhancement, competitive advantage and firm survival [25][26]. Given its importance, we conclude that technology is no longer something of a luxury, but a necessity for firms. However, the opportunity to adapt to technological developments is small, based on the fact that most of the SME sector in Kosovo consists of traditional and non-innovative enterprises. Despite the obstacles faced by these businesses, surprisingly, the technology sector is booming, contributing to economic growth in terms of employment of young men and women as well as increasing the level of exports. About 78% of firms in the technology sector export their services [27]. Based on these data, we notice that the domestic market has been left behind and overshadowed by the focus on exports, because they do not see it as a profitable market. This disconnect does not promote the local market and makes it impossible to capitalize on the benefits of technology. Consequently, skilled workforce, knowledge and financial means are main barriers for digital transformation of SME firms in Kosovo [28]. Also, in many literatures the positive relationship between IT capabilities and firm performance has been demonstrated [29]. IT capabilities are defined as the ability of the firm to use and mobilize IT resources along with other firm resources in order to improve the core performance mechanisms of the firm [30]. This section examined how IT capabilities affect the performance outcomes of SMEs in a dynamic environment.

2. Research methodology

This paper seeks to investigate the internal environment factors affecting performance of Small and Medium Enterprises in Kosovo. The purpose of this study is to examine the internal environment toward the performance of SMEs, considering the importance level of sector for the economy of Kosovo. Registered SMEs in Kosovo Business Registration Agency operating in three local governments, namely: Pristina, Ferizaj, Gjilan as shown on the Ministry of Trade and Industry, represents our target population. They are categorized in manufacturing, service and agricultural SMEs. To achieve objectives of the study, it is employed a cross-sectional study which the collected information are analyzed from each subject at a specific point in time. Based on general scientific guidelines for sample size developed by Krejcie & Morgan (1970) as cited in [31], the recommended sample size for this study was 379.

2.1. Questionnaire and data collected

In order to collect the data, it is developed a structured questionnaire mostly with yes/no and Likert Scale type of question. The questionnaire is composed in two sections. First section contains details of socio-demographic profile of the respondents. The second section of questionnaire contains the internal environment factors: managerial competencies and skills, access to finance and technological capabilities. Questionnaires were administered between

10th January 2020 and 10th March 2020 on SMEs from selected local governments through online survey using emails. Random sampling technique employed to determine the eligible owners/managers to be questioned.

2.2. Reliability and validity

Reliability in quantitative research in the process of measuring a phenomenon concerns with repeatability, stability, and consistency of the results over the time. Based on a minimum internal consistency coefficient [32][33], Cronbach Alpha Coefficient in the actual study is above .70 which shows that results are reliable. However, for a test to be reliable, it also needs to be valid [34].

Validity in quantitative research explains how well the collected data covers the actual area of investigation [35]. It is also meaning a “measure of what is intended to be measured” [36]. To improve our survey results, we used pre-testing method of Schindler & Cooper [37]. To validate the appropriateness of the questionnaire, pre-tests were performed with a group of relevant experts and as a result no major concerns were experienced or observed, thus paving the way for data to be collected as planned.

2.3. Data analysis

After taking the online results, data collected then imported to SPSS 25.0 version for analysis. The Demographic details are descriptive and are presented in tables and graphics. For continuous variables we used means (standard deviation), for categorical variables we used percentage. In order to find the significant factors relating the business performance its employed Chi-squared test of association. The majority of statistical tests were conducted A two-sided test its used employed at the significance level of 0.05 to conduct statistical tests. Considering that p-values in this study were reported to be less than 0.05, results are considered statistically significant.

3. Results and discussion

The presentation of results comprises in four parts. First, we summarize the socio-demographic profile of the respondents, showing the gender, job position and the level of education completed by owners/managers of relevant SMEs, and other company profile details. In the second part we present managerial competency and skills to further analyse the company practices in the context of operational activities. We also present results of challenges and impact in accessing finance in the context of business growth. The last part of results elaborates the impact of technological capabilities toward improving business performance of surveyed SMEs. Questionnaire was emailed to 379 SME owner/managers through online survey, resulting in 94 returns. The response rate was 24.8%.

Table 2. Socio-Demographic profile of the Respondent

Category	N	Percentage
Gender		
Male	71	75.53
Female	23	24.47
Job Position		
Owner	58	61.70
Manager	21	22.34
Both	11	11.70

Other	4	4.25
Highest education completed		
Diploma	34	36.17
Degree	41	43.61
Master	4	4.25
Doctorate	2	2.12
Other	13	13.82

Majority of the participants (owners/managers) were males, which shows the dominance of SME ownership by males. According to Riinvest Institute [38] over 80 percent of women are inactive in the labour market, while only ten percent of the country's active businesses are owned by women. Our findings show an increase rate of women in business activities mostly oriented towards services and trade, particularly beauty and hairdressing. The average age of the participants was found to be 32 year, thus, reflecting the average working population of Kosovo according to 2011 census which is 30,2 years. A well-trained labour force could be a comparative advantage and a key resource for economic growth [39].

More than a half (50%) of participants had tertiary educational preparation, while the rest had vocational or secondary education. Many authors and scholars have found a positive impact between education of SME owners and performance of SMEs [40][41][42][43]. However, owner/managers need to seek knowledge in relate with their businesses, on the contrary education may not assist performance [44]. But still, higher education preparation of owner/managers is considered to provide a significant performance advantage for SMEs.

Table 3. Background information of the participated SMEs

Category	N	Percentage
Legal status of your business		
Individual Business	31	32.97
General partnership	7	7.44
Limited liability company	53	56.38
Joint stock company	1	1.06
Foreign company	2	2.12
Sector of your business		
Agriculture	3	3.1
Services	67	71.27
Manufacturing	24	25.5
How many people you employ?		
Less than 10	79	85.1
11-49	11	11.7
50-249	2	2.12
Over 250	1	1.06
How long has your enterprise been in this business?		
Less than 3 years	32	34.04
3-5 years	40	42.55
5-10 years	7	7.44

10-15 years	10	10.63
Over 15 years	5	5.31
Business performance in the past year in comparison to the previous year		
Improved	47	50.00
Worsened	36	38.29
Unchanged	11	11.70
Running an SME over the past year in comparison to the previous year		
More difficult	67	81.27
Easier	0	0.00
Unchanged	27	18.73

All of the surveyed SMEs are registered business with a legal status. Given the fact that mostly of SME owners perform many roles our majority of the participants were both manager and owner. Regarding the legal status, recently in Kosovo it is seem more appropriate for SME owner/managers to choose the legal form of Limited Liability Company (56%), while the Individual Business form was option for about 1/3 of participants. However, Kosovo still continue to have large informal sector which is estimated at around 30% of GDP [45], and as a result creates unfair competition and weakens labor rights. Although, informal SMEs can be a significant source of job creation, it is considered that the formalization of SMEs is often associated with better performance [46]

With a low level of industry as a developing country, Kosovo as the most important sector have services and industry, a fact that was emphasized in the survey results. The structural stretch of surveyed SMEs was mostly service-oriented (71%) and manufacturing oriented (25%), while agricultural stands at 3%. More than two-third of surveyed SMEs employ 1-10 people, which is in line with the Kosovo Government [47] definition criteria for MSMEs.

The majority of surveyed SMEs are in business for less than five years, indicating that Kosovo SMEs remain in the embryonic phase, which is 3-5 years since start-up, lacking growth. An empirical study conducted in 956 Croatian food industry businesses found a negative impact between firm age and business performance, stating that their accumulated knowledge in all crucial aspects of the business become overcome with their inertia, inflexibility and osseous by accumulated rules, routines and organizational structure [48]. Respondents were asked to indicate whether their SME performance is improved, worsened or unchanged in the past year in comparison to the previous year. As Table 2 summarize, 50% of participants indicated that their performance has improved, whilst more than 80% of them stated that running an SME was more difficult in the past year in comparison to the previous year.

3.1. Managerial competencies and skills

Regarding the experience of owner/managers in the relevant type of business, majority of them indicate that they have relevant experience (63%), while more than a half (30%) of them lack prior experience in managing SME.

Table 4. Summary of accessing managerial skills of the participated SMEs

Category	Yes	No
Owner/management having the necessary experience and training		
Does management/owner have prior experience in managing this type of business?	61	33
Does management/owner have prior experience in small business management?	65	29
Does management/owner have formal training in business management?	31	69
Does management/owner have formal training in financial management and planning?	29	71
Does management/owner have formal training in marketing?	37	63
Does management/owner have formal training in bookkeeping and accounting?	29	71
Management/owner having the necessary skills to undertake the following		
Prepare financial statements	27	73
Prepare trading budgets	26	74
Prepare cash flow forecasts	27	73
Analyze financial statements	25	75
Do you outsource any of the business functions?	69	31
If yes to outsourcing, then which of the following functions is outsourced?		
Accounting	73	27
Human Resources	4	96
IT	38	62
Marketing	12	88

More than two-third of SME owner/managers are not engaged in formal training regarding business management activities and marketing, showing more difficultness level in running their businesses. The lack of training in business functions of SME owner/managers through empirical studies it's proved to have a negative impact in the business performance and growth [49]. In Kosovo, a majority of SME owners lack financial and planning skills which indicates directly on the performance indicators like effectiveness and goal achievement, firm growth, employment growth in long-term periods. As it stated above, most of the participants were found to have lack of skills in analyzing and preparing financial statements and cash flow forecasts. This situation is driven by the lack of training in relevant fields of SME owner/managers and low monthly fees of accountants' firms. Influenced by the current situation, more than 70% of surveyed SME owner/managers are forced to take services from external sources. The survey response reflects that majority (76%) of the SMEs outsource business functions, with accountancy and IT services being the most outsourced functions.

3.2. Access to finance

In Kosovo, access to SME finance remains a major obstacle to the survival and growth of this sector. This is confirmed by two-thirds (74%) of the surveyed owners / managers, emphasizing that access to finance is a great challenge to grow their business. With the increasing need for investment and development which was driven by the formalization of the micro-enterprise sector in particular which represents 97% of the entire private sector, created the continuing

need for external financing (41%), mostly on loans, relatives and other sources of financing. Banks in Kosovo have restricted access to finance for the micro-enterprise sector through requirements for high collateral, business turnover and age, indicating difficulties (55%) in accessing external finance for surveyed businesses. The sector of new micro-enterprises has more difficulties in accessing finance, which as a result of lack of skills in the field of business planning, financial record keeping, growth management and low awareness have created a barrier to borrow with lower collateral requirements. This indicates a blockade of more than 93% of the total enterprises in private sector by banks in Kosovo, challenging the microfinance sector with limited funding sources to meet the growing demand of micro-enterprises.

Table 5. Summary of accessing finance of the participated SMEs

Category	N	Percentage
Access to finance is a major challenge that affects the growth of my business		
Strongly disagree	7	7.44
Disagree	16	17.02
Agree	26	27.65
Strongly agree	45	47.87
What financing methods you use to finance your business?		
Existing cash	56	59.57
Bank loans	21	22.34
Relatives	13	13.82
Other	4	4.25
Does your business experience difficulties in accessing external finance?		
Yes	52	55.32
No	42	44.68

3.3. Technological capabilities

The majority of respondent's state that they are interested and willing to invest in the technology of their firms. More than 90% of respondents point out that technology helps them improve performance. As awareness grows, they have realized that investments in technology contribute to increased performance and competition against other firms especially against the informal sector which has the main advantage of price. However, most respondents noted the difficulties they face during the digital transformation of their firms. The main barriers that challenged the surveyed businesses were more of a financial nature and lack of expertise and knowledge in the field of IT. Based on this approach, we note that the surveyed enterprises want to embrace the integration of technology but have not yet developed any concrete strategy or action plan on how to do it.

4. Conclusions, Limitations and Recommendations for Further Studies

The contribution of this research can be seen in two contexts, practical and scientific, with a dynamic approach which covers only the impact of internal factors as a whole, and their impact on the performance of SMEs.

The impact of factors such as managerial skills and competencies requires a further and detailed study especially for the micro enterprise sector in the terms of growth and development, considering their significant impact on performance of the SMEs, which is found to be also related to other factors such as access to finance and technological skills.

The factors examined in the study were grouped into three groups - managerial skills and competencies, access to finance and technological capabilities.

This study confirms that all internal factors - managerial skills and competencies, access to finance and technological capabilities, depending on a period, have a significant impact on the performance of SMEs.

Lack of training and experience in relevant important business activities was a negative indicator of business performance, making it difficult to run a business.

Access to finance is considered a major challenge for businesses, especially for micro-enterprises which in the absence of skills in the field of business planning, financial record keeping, and growth management created barriers to respond to the requirements and procedures of financial institutions.

Based on results, the majority of respondents considered technological capabilities as the main indicator for improving performance. Struggling to meet their customer expectations, they see technology as their savior. However, the lack of IT skills and expertise, the lack of concrete action plan and strategy, and the associated costs limited the utilization of its technological capabilities.

The study has its own limitations. The first is poor response rate of the selected SME (24.8%), which could be due to time factor to complete the survey, and by the inadequacy of respondents to complete an online survey. The second limitation in this study was that the survey was conducted only in three regions of Kosovo and does not represent the sample of all Kosovo SMEs. Another limitation of this study is that performance appraisal is only analyzed within the framework of internal factors, excluding product innovation, organizational features of autonomy, market roles, and type/importance of goals. Examining the impact of these factors would strengthen the research results.

However, performance measurement can be completed by adding the influence of external factors in Kosovo SME sector, a study which would be necessary in future academic research.

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Risk assessment of workplaces for electrical energy production

Naim Baftiu¹, Afrim Loku², Nadire Loku-Shehu³

¹University "Ukshin Hoti" Prizren/Kosovo, ²University of applied sciences in Ferizaj/Kosovo,

³Kolege AAB-Pristina/Kosovo

naim.baftiu@uni-prizren.com¹, afrimloku@yahoo.com², nadire.loku@universum-ks.org³

Abstract. In this paper is presented the assessment of the workplace hazards for power plant-PP "Kosova" A. The main purpose of this paper is to evaluate the work places based on the realized measurements of the conditions for safety and protection at work, classification and categorization of jobs with increased risk as well as measures for improving working conditions and preventing endangered and injured workers from injurious. Measurements of working conditions and assessment of workplace and workplace risk have been carried out by exploring, identifying, recording and analyzing any workplace in a riskiness case. With Application Software, all workplaces in which the measurements were performed are processed.

Keywords. measurements, country, risk, software

1.0 Introduction

In the Electricity Generation Department PP "Kosovo A", there are generally jobs that are not directly related to the technological process and jobs that are related to the technological process, ie hazardous jobs, as well as countries Work with specific or high risk conditions. The risk assessment methodology is based on the valid documentation of the Republic of Kosovo and on the realized measurements of harmful physico-chemical and microclimatic conditions at the workplace PP "Kosova" A.

1.1 Description of the technological and work process

The Kosovo A power plant consists of five working blocks known as A1, A2, A3, A4 and A5. The technological process for the production of electricity is quite complex, from the transport of coal which is realized with conveyor belts to the final product. During the technological process of manufacturing in this company, there are factors that affect the health and working skills of the employee. The factors in the HUMAN-MACHINE report are related and depend on the effect of the work and thus on the totality of production. Company requirements for realization of planned production can be achieved if, inter alia, care is taken to eliminate negative factors in safety in Work and health of the worker and works are carried out in a safe and secure environment with "Zero" injuries and incidents. Competitors of the company "Kosova" A, with responsibility, make continuous efforts for protection and safety at work throughout the organization, To create a safe environment, while respecting the legal obligations regarding the safety and health of workers. This company possesses personnel engaged in safety at work, with the focus on maximizing awareness to preserve the health of all workers directly and indirectly with management control. These efforts should be intensive and consistent to enhance safety awareness and have an impact on eliminating the risk of injury or occupational disease at work. Physical-chemical injurious and micro-climatic conditions for working conditions are carried out at a time when The facilities have been in operation. The results of these measurements are given in the Measurement Report submitted in August 2014. For each work



1.2 Basic notes

Observations regarding the state of protection and security are based on evidence from the relevant sector of the company, where the state of the work safety is reported in a country-specific report on cases of workplace disasters, occupational diseases and other official reports. It should also be noted even workplaces, that is, jobs for which special security measures should be applied, longer working than full working hours, shortened time jobs due to difficult working conditions, riskiness in any work, especially from falls, demolitions, slips, electricity, chemical-biological substances, fires, explosions, noise, dust, vibrations, radiation, micro-climate, air flow etc. The work is carried out in three shifts, The first shift starts at 7:00 and ends at 3:00 p.m. There are 845 employees in this company.

1.3 Analysis of workplaces for electricity production

- Systematic description of all aspects related to the workplace to assess what could have caused injury or injury to employees during the production of electricity
- Classification and placement of works which damage the health of the worker in the production of energy
- Measures to improve the safety and health of employees so that the risk and damage as a whole are eliminated or reduced to the lowest possible level.

2.0 Risk assessment methodology

The method used for risk assessment at workplaces in TPP "Kosovo A" is the matrix based on the potential risk factors and calculated according to formula.

Where are :

R – Risk

L –The probability of the occurrence of the risk,

F- Frequently

D – Damage that may cause the identified risk

E – Exposure, the number of exposed a risk given at the same time .

The frequency of exposure to risk	Exposure factor value
Once a year	0.5
Monthly	1.0
Weekly	1.5
Once a day	2.5
Every day	4.0
Uninterrupted	5.0

Table.1. The likelihood of the occurrence of the risk.

The injury likely	The value of the likelihood factor
Almost incredible, it can only happen in exceptional cases	0.33
A bit reliable, but possible	1.0
A bit reliable, but it can happen	1.5
It can happen but is not commonplace	2.0
It is likely to happen	5.0
It may ,not be a surprise	8.0
Reliable, expected	10.0
Of course it will happen, unequivocally	15.0

Table 2. Frequently of the risk

Exposure number	The value of the factor the number of exposed ones
1-2	1.0
3-7	2.0
8-15	4.0
16-50	8.0
50 up	12.0

Table 3. Factors for the number of exposed workers

Table 4. Risk rate by calculatio

Risk category	Risk
Negligible risk (No risk control activities are required)	0-5
Small danger (Risk is low for workplace safety)	6-50
Medium risk Risk is present and should be tackled with measures to reduce it	51-250
High risk (Considerable risk to which protective measures must be laid down to reduce it)	251-500
An unbearable danger, extrem	Over 500

The risk at this level is negligible, so the work process must be stopped until its reduction)



2.1 Dedicated objects for work and following facilities

In the energy complex PP "Kosova" A there are facilities dedicated for work according to the specifics of the energy production, then the works for administrative work and the following facilities. Within the production facilities, there are numerous equipment and facilities of the character of the machinery, electrical, transport, Maintenance, construction etc, while in the frame of the accompanying objects there are hygienic facilities, food, wardrobes, storages, labs etc. All these technological and administrative process objects occurred in the space of this thermal energy complex.

2.2 Dangers and injuries are divided into groups, by type and nature.

- Mechanical hazards, which occur when using work equipment
- Risks that are related to job characteristics
- Risks that occur when using electricity

Damages that arise or appear in the work process are divided into;

- Chemical damages (dust, gases etc)
- Physical injuries (noise, vibrations, illumination, radiation, etc.)
- Biological damages (infections, during microorganism exposures),
- Damage from negative microclimate impacts (high or low temperature, humidity and velocity of airflow)
- Injuries arising from psychological aspects related to the workplace and
- Injuries related to the organization of work.

For each workplace, analytical processing of measurements was done and job estimates were made according to the degree of danger given in the equation (1).

The procedure for preparing the risk assessment document is based on international standards. The EU Directives on which are drafted laws and legal acts of Kosovo, in particular the EU Risk Assessment Directive no. 89/391 EEC.

3.0 Measurements of working conditions

During the measurement of PP "Kosova" A, the following measurements were performed at each measuring point:

Physical injurious: Noise, Illumination, Vibration and Ionized Radiation

Microclimatic Conditions: Air Temperature, Relative Humidity, Air Movement (Exit - Air Flow)

Chemical injurious: Dust - gravimetric method, Dust - Kniometric method, O₂, CO₂, CO, SO₂, NO₂ and NO_x gases

Biological injurious and hygienic - sanitary conditions: Bacteria, Mold and yeast, Viruses, parasites.

Working with technical gases always poses a risk to the personnel and as such requires devotion and responsibility for the protection at work. The most desirable case is when the gas is poisonous, flammable or explosive. Gases must be taken for actions that are foreseen by special regulations.

3.1 Risk assessment

On the basis of the assessed risk at the workplace, the employer must determine the necessary measures to avoid, reduce or avoid the risk as well as determine the persons responsible for their implementation.

Generally, workplaces are divided into countries with Risk and Damage.

The risk is due to mechanical, electrical, etc. factors Damage from chemical factors (dust, gases, etc.) Physical (noise, vibrations, illumination, radiation, etc.), Biological, and microclimatic influences

Hazardous sites by type, nature and risk factors are divided into these factors: Mechanical Factors, Electricity, Physical Factors, Chemical Factors, Biological Factors, Psychological Factors and Other Factors.

Mechanical factors: There is a lot of mechanical work on mematerial equipment and direct equipment in the working process and the technology used at work, which are considered places with workplace risk.

Electrical factors: From the factors that occur during the electric power generation operations in this company and affect the health of workers are:

- The risk of direct contact with electrical parts and equipment under voltage,
- The risk of indirect contact with electrical parts and equipment under voltage,
- Risk of high temperature effects that can cause electrical equipment and installations. (Short ties, explosion, electric arc, or electric sparks),
- Dangers of lightning and the consequences of atmospheri emptying,
- Risks from harmful impacts from electrostatic electricity etc.
- Other hazards that may arise regarding the use of electricity.

Physical Factors: Physical Damages Are; Exposure to noise, mechanical exposure by vibrations, atmospheric pressure (high or low temperature), microclimatic conditions, illumination, electromagnetic radiation exposure (heat, inefficient and insufficient light, X-rays, radioactive, ionized and non-radiated radiation Ionized) presence of pressurized fluids, harmful atmospheric impacts (working in a closed environment) etc.

Chemical factors: Materials containing chemical, dust, fog, organic and inorganic volatiles from production, liquid fuels, fuels and combustibles, industrial poisons (absorption, ingestion and absorbent of the harmful material, use of stretch and explosive materials, reactive substances And unstable, asbestos-related jobs, lack of oxygen, etc.

Description of risks and identified injurious in the workplace

Table.6. On the floor there are obstacles and objects, objects that hinder, work, free movement and can cause injury..

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
5	4	0.5	1	10
Risk Level				Small

Table 7. The Floor at the workplace is sometimes slippery, damp.

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
5	2.5	0.5	1	6.25

Risk level	Small
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Table 8. Shields in cars are not properly secured from where workers can be injured

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
2	2.5	2	1	10
Risk level				Small

4. In the work equipment there are unmarked electric devices with the appropriate symbol for EC 60417-5036 electrical devices with black lightning on the black triangle yellow background.

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
0.33	2.5	15	1	8.25
Risk level				Small

5. Work is done in close proximity to steam installations that are not isolated as they should be.

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
5	2.5	6	1	75
Risk level				medium

6. Work is done in close proximity to potentially hazardous electrical equipment

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
1	1	15	1	15
Risk level				Small

7. During the working process, an increased noise level is displayed

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
10	2.5	6	1	150
Risk level				medium

8. Possibility of contact with biological agents.

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
1	2.5	6	1	15
Risk level				Small

9. There is dust concentration in the workplace

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
2	2.5	6	1	30
Risk level				Small

10. Workplace means responsibility for people and equipment (exposure to stress)

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	
1.5	5	6	1	45
Risk level				Small
The risk level in this workplace:				Medium risk

COMPANY	Kosovo Energy Corporation		
DIVISION	Kosovo-A power plant		
Workplace	Condensation manipulator		
Short description of the job	Manipulant in condensing equipment		
Tools and working tools that are used	Gloves , helmets	contractor	
		7	

Description of risks and identified injurious in the workplace

1. On the floor there are obstacles and objects, obstructing objects, work, free movement and can cause injury

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
5	4	4	1	80
Risk level				Medium

2. The floor at the workplace is sometimes slippery, damp.

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
5	4	2	1	40
Risk level				Small

3. Shields in cars are not properly secured from where workers can be injured

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
2	2.5	2	1	10
Risk level				Small

4. On the work equipment there are unmarked electric buttons with the appropriate symbol for EC 60417-5036 electrical devices with black lightning on the black triangle yellow background.

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
0.33	4	15	1	10
Risk level				Small

5. Work is done in close proximity to steam installations that are not isolated as they should.

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk

5	2.5	6	1	75
Risk level				Medium

6. Work is done in close proximity to potentially hazardous electrical equipment

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
2	1	15	1	30
Risk level				Small

7. During the working process, an increased noise level is displayed

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
10	5	6	1	300
Risk level				High

8. Possibility of contact with biological agents.

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
5	2.5	6	1	75
Risk level				medium

9. There is dust concentration in the workplace

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
5	2.5	6	1	75
Risk level				Medium

10. In the section of the guild where intervention is required in case of emergency and damage to

Major lack of emergency light source alternatives - the battery.

Likely (L)	Frequently (F)	Damages (D)	Number of exposures (E)	Risk
10	1	10	1	100
Risk level				Medium

The risk level in this workplace: **High Risk**

Measurement results

For each risk identified and assessed with the degree of impact on security, medium risk, high risk and unacceptable risk, safeguards have been defined in the form of recommendations, which are provided at the end of certain groupings of Job positions by respective departments or departments.

Workplace Hazard Assessment for: Kosovo-A Power Plant Division, Production Department, Department of Electrical Maintenance, Department of Mechanical Maintenance, Department of Business Support Department: Project Management Engineering in TC A, Department of SI and MKZ, Department - Directorate TC A / PKU.

Measurements in the Production Department.

serial number	Evaluation of jobs in the production department	Number of workers
1	Workplaces included in the risk assessment.	35
2	Number of workers who finish	246
3	Workplaces with risks that require treatment for elimination, reduction or prevention.	35
4	Workplaces with increased degree of riskiness.	2

Department of Electrical Maintenance

Serial number	Evaluation of workplaces in the production department	Number of workers
1	Workplaces included in the risk assessment.	64
2	Number of workers who finish	90
3	Workplaces with risks that require treatment for elimination, reduction or prevention.	57
4	Workplaces with increased degree of riskiness.	7

Department of Mechanical Maintenance

Serial number	Evaluation of workplaces in the production department	Number of workers
1	Workplaces included in the risk assessment.	109
2	Number of workers who finish	269
3	Vende pune me rreziqe të cilat kërkojnë trajtim për eliminim, zvogëlim apo Parandalim të tij.	91
4	Workplaces with increased degree of riskiness.	16

Department of SI and MKZ

Serial number	Evaluation of workplaces in the production department	Number of workers
1	Workplaces included in the risk assessment	19
2	Number of workers who finish	81
3	Workplaces with risks that require treatment for elimination, reduction or prevention..	7

4	Workplaces with increased degree of riskiness.	5
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Department - Directorate TC A / PKU

Serial number	Evaluation of workplaces in the production department	Number of workers
1	Workplaces included in the risk assessment	25
2	Number of workers who finish	79
3	Workplaces with risks that require treatment for elimination, reduction or prevention..	13
4	Workplaces with increased degree of riskiness.	2

Department - Directorate PP-KA-PKU

Serial number	Evaluation of workplaces in the production department	Number of workers
1	Workplaces included in the risk assessment.	11
2	Number of workers who finish	25
3	Workplaces with risks that require treatment for elimination, reduction or prevention.	3
4	Workplaces with increased degree of riskiness	0

Department of Business Support

Serial number	Evaluation of workplaces in the production department	Number of workers
1	Workplaces included in the risk assessment..	15
2	Number of workers who finish	24
3	Workplaces with risks that require treatment for elimination, reduction or prevention.	4
4	Workplaces with increased degree of riskiness.	0

Methodology of assessment

Based on the methodology of the analytical evaluation of the works from the aspects of the general conditions of work and the damage caused by the factors listed above, the conditions of the job positions are assessed according to the categorization and classification. Each workplace is analyzed according to the results of the measured measurements and is evaluated based on the scores.

Determining the manner and measures for eliminating, reducing or preventing the risk

Risk prevention and compliance with laws are the basis upon which the protection of health and safety at work is based. Legislation in force, and in particular this document, based on implemented workplace measurements, on health and safety issues at workplaces, encourages and binds all relevant entities of the entities concerned according to their respective competencies and responsibilities in general, while representatives and employees For protection and security in particular for permanent engagement in eliminating, reducing or preventing the risk at workplaces where required.

TC "Kosovo" Is a company in which work processes are developed in some countries with specific specifications, working and with high risk, it is therefore necessary to assess the risks that may arise as a result of specific work interventions. The employer is obliged to prevent, eliminate or reduce the risks identified in the workplace and the working environment Minimum or at the lowest possible level.

For the elimination, reduction or prevention of risk, the employer undertakes the following measures:

- Realizes the application of general and special measures of safety at work, respectively other measures that the employer must undertake in order to avoid dangerous work resources;
- It sets the deadlines, the persons responsible for applying the measures, and the manner of controlling the application of these measures.
- Drafts a unique measures plan for all technological processes and serves as a basis for action of responsible persons in the work processes.
- Occupational safety measures are set, according to the dangers determined in the work process, the workplace and the number of employees.

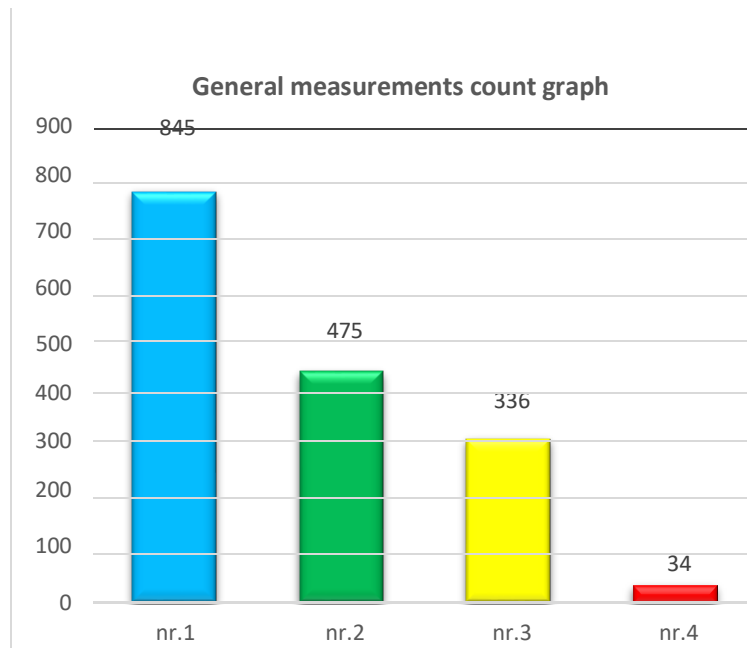
Measures for eliminating, reducing or avoiding risk are defined by these priorities;

- Eliminate or reduce risk when implementing the technological process
- Ensure the conditions for safe work and health at work
- Use of protective equipment and other means of protection
- Use of personal protective equipment
- Use of protective equipment and other means of protection
- Implementation of organizational protection measures (trainings, information, routine work development, warning signs, voice and light signals etc.

For all these workplaces , during analytical processing estimates and categorization of workplaces is shown in graph (1).

Table 23. Outcomes of workplace measurements.

nr.1	Number of workers for production of electricity	845
nr.2	Number of workers with low risk during electricity production.	475
nr.3	Number of workers who have medium risk during electricity production.	336
nr.4	Number of workers with high risk of electricity production.	34



Measures to control risks ranked at levels that can cause injury or illness to employees-Recommendations.

<i>Measures to control the risk of explosion</i>	<p>Keeping under regular supervision by technological process monitors, Maintaining explosive materials (H₂), working cleanliness and temperatures, Strict implementation of fire prevention measures in container spaces and flammable gas filling systems (hydrogen, etc.). Permanent and abundant supply with AKZ at such critical points</p>
Measures to control the risk of increased noise level	<p>Technical measures for reducing the noise level, Reducing to a minimum the exposure to noise, Useful of PPM for hearing protection by workers</p>
Measures to control the risk of increased dust concentration	<p>Technical choices that reduce the amount of dust generated Keeping the doors closed by the side of the cobble area, Neutral use of PPM to protect the respiratory organs from dust</p>
Measures to protect against dangers due to lack of sufficient lighting	<p>Regular lighting maintenance in closed working areas.</p>
On the floor there are obstacles and objects, uneven ground and different levels	<p>Work spaces should be kept clean and free of obstacles created by the jumps of different parts, Work spaces must be flat and without open holes, open channels, and inappropriate terrain that disable safe movement.</p>

<p>During the working process there is a possibility of accidental leakage of chemicals that are classified as toxic, harmful, corrosive and contact with them.</p>	<p>Regular maintenance and daily control of equipment containing chemicals, from the point of view of leakage or evaporation of chemicals, Continuous training and training of chemical workers Installation of ventilation equipment in technological areas where chemicals are used Adequate use of PPM for working with chemicals .</p>
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Assessment and measures to avoid workplace hazards and work related to the work are based on the provisions of the Law on Labor Safety, Health Protection of Employees and the Working Environment.

Duties and obligations of the employer;

In addition to the obligations of the employer, there are also obligations of the employees who have obligations to the employer for the maximum correct use of the equipment and facilities and working tools, equipment, dangerous substances, transport equipment, plants, buildings, buildings and other means And the use of Personal Protective Equipment.

Recommendation

Based on the results obtained during the measurements and the categorization of sites classified as high risk, the employer is obliged to take measures to improve hygienic and sanitary conditions in order to enable the elimination of biological agents and to protect the health of the workers.

We prefer disinfection as a process that hinders the development of such agents as maintenance and disinfection of refrigerators, disinfection of water gallons, cleaning and disinfection of sanitary wires. While in some other places like in the fitness room, space cleaning and disinfection with industry-friendly disinfectants are recommended, 96% alcohol can be used and alcohol at 70%.

Take action on; Eliminating dust from the workers in the places where they work, placing containers in adequate places, lack of adequate waste bin, drinking water, storage of food in the fridge and sanitary napkins Etc.

Psychological factors; Are factors that are conditioned by the work process, labor intensity.

Other factors; The danger caused by other people (eg, personnel violence), under pressure, severe weather conditions, hygiene-sanitary conditions in the workplace, etc.

Measures to control risks ranked at levels that can cause injuries or illnesses to employees

Measures to control the risk of explosion

Keeping under regular supervision by technological process monitors,

Maintaining explosive materials (H2), working cleanliness and temperatures,

Strict implementation of fire prevention measures in container spaces and flammable gas filling systems (hydrogen, etc.).

Measures to control the risk of increased noise level

Technical measures for reducing the noise level,

Reducing to a minimum the exposure to noise,

Useful of PPM for hearing protection by workers

Measures to control the risk of increased dust concentration
Technical choices that reduce the amount of dust generated
Keeping the doors closed by the side of the cobble area,
Neutral use of PPM to protect the respiratory organs from dust

Measures to protect against dangers due to lack of sufficient lighting
Regular lighting maintenance in closed working areas.

On the floor there are obstacles and objects, uneven ground and different levels
Work spaces should be kept clean and free of obstacles created by the jumps of different parts,
Work spaces must be flat and without open holes, open channels, and inappropriate terrain that
disable safe movement.

During the working process there is a possibility of accidental leakage of chemical substances
classified as toxic, harmful, corrosive and contact with them. Regular maintenance and daily
control of equipment containing chemicals, from the point of view of leakage or evaporation
Chemicals,
Continuous training and qualification of workers who work with chemicals.
Installation of ventilation equipment in technological areas where chemicals are used
Adequate use of PPM for working with chemicals.

Conclusion

Safety, Health and Preventing the Adverse Effects of Hazardous Workers at Work are the ultimate goal of risk assessment procedures at work. With the aim of improving the working conditions, the 7th of October International Day for Safety and Health Protection at Work . The largest international organizations dealing with labor issues continuously research and define forms and methodologies for protecting workers in workplaces through advancing technological processes and creating better working conditions through normative laws, Directives, regulations, administrative instructions etc. to the convention. The company TC "Kosova" A, within the internal organization, has established a department for protection and security, which acts and undertakes technical measures for safety and healthprotection from the operations generated by the work processes. For the purpose of fulfilling the legal obligation for protection and safety at work, at TC A are carried out measurements ofworking conditions at the measuring points-places designated by the employer. The measurements were performed according to the standards and the legislation for risk identification in the workplace. The risk assessment at work was carried out in accordance withthe legal acts in force. Classification of hazardous sites is made according to the legal criteria, while the company is obliged to plan according to the priorities Eliminating or reducing them at the lowest possible level. Risk assessment is not done once and ends, it needs to be reviewedand refreshed as needed to adapt to the level of change in work activity and changes in the organization of the work process and new job situations. The employer in the declaration formis obliged to apply all the prescribed measures for safe and healthy work in the workplace andthe working environment in accordance with the risk assessment act, as well as keep records ofwork injuries, occupational diseases , Accidents in his working subject, etc.

Based on the measured results of workplace hazards, it can be seen that we are dealing with some countries that do not respond to the allowed standards of international standards.

Employer TC A should continue to maintain full statistics on work injuries, Occupational diseases and other diseases presented by the work.

Highly hazardous workplace status remains until when the results of the specific measurements carried out during the monitoring are found to have improved the working conditions and that with the further work there is no risk to the life and health of workers who work in the country Working with high-risk workplace status. The workshop is responsible for taking care of his / her safety and health and other persons at work, in accordance with the instructions given to him by the employer.

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Small and Medium Enterprises: Importance for the Kosovo Economy

Afrim Loku¹, Nadire Loku², Naim Baftiu³

¹University of Applied Sciences in Ferizaj, St. Universiteti 70000, Ferizaj, Kosovo,

²University for Business and Technology (UBT College), St. Rexhep Krasniqi 10000, Prishtina, Kosovo, ³University "Ukshin Hoti" Prizren/Kosovo

afrim.loku@ushaf.net¹, nadire_loku@hotmail.com², naim.baftiu@uni-prizren.com³

Abstract. This study aims to broaden the understanding of the importance of SMEs (Small and Medium Enterprises) for the economy of Kosovo, especially in terms of economic development, labor market and innovation. The study was conducted through analysis of documentary sources such as relevant reports of national and international institutions as well as relevant scientific papers. The scientific contribution of this study is theoretical and aims to help researchers of the field for an expanded understanding of SMEs over national economies. This study provides a concise framework of important elements on the economic impact of SMEs on Kosovo's economy. Examination of available data has shown that the SME sector plays a crucial role for the Kosovo economy. The key role of SMEs was emphasized in terms of overall value generated and the labor market while innovation remains a challenge to be addressed by stakeholders.

Keywords. SMEs, economic development, labor market, innovation

I. Introduction

Small and medium-sized entities are the most vital and important sector in a country's economy. They are the backbone of an economy and a key factor for growth and employment. SMEs provide and generate employment opportunities, especially in times of recession; they are a major source of modernization, innovation and entrepreneurial spirit; they create links between individuals' innovative efforts and competitiveness and play a very important role in the future development of businesses. In short, dynamic and healthy market economies are totally reliant on small and medium-sized entities. The economy which is depended on remittances, public investment and consumption activities also remains a general challenge for the economy of Kosovo. This structure of economy it's followed by challenges of the private sector which also have hindered the contribution of the business community to economic growth.

The main problem that prompted this study is the scarcity and clarity of the impact of SMEs on the economy of Kosovo, which makes it difficult to further expand studies on the importance of SMEs. This study addresses three key issues. Firstly, it is the aim of this paper to give a brief exposition of some arguments in favour of and against the economic importance of the small business sector to the Kosovo economy. Secondly, it examines the overall role of SMEs in Kosovo's economy. Thirdly, it deals with the role of the SME sector in employment,

and it analyses their role toward innovations. The subject of this paper is Kosovo SME sector, economic development, employment and innovation.

1.1. Literature review

Classification of SMEs: To better analyse the role of SMEs we must first define them. An enterprise is defined as any legal, independent, organized entity of persons, property, obligation and economic activities oriented on realization of profit. (Khatuna & Jinoria, 2014). Classification of enterprises are categorized based on different factors and criteria's, such as location, size, age, structure, organization, number of employees, sales volume, worth of assets, ownership through innovation and technology (Rahman, 2001). The most used criterion to distinguish between large and small businesses is the number of employees (Hatten, 2011). According to Bolton Report 1971, two main approaches are suggested to the business entity definition: quantitative and qualitative approach, (Carter & Jones-Evans, 2006). Nations, policymakers, academics, statistical agencies, and international institutions mainly apply quantitative criteria when defining SMEs. European Commission in 2003 determine the number of employees as main criterion to define an enterprise, however in 2005 this institution added two more financial criteria: annual turnover and annual balance sheet (European Commission: 2003, 2005).

Importance of SMEs for economy, employment and innovation: SMEs are widely considered as the backbone for most economies. In general, the role of SME-s in industrialized and developed nations has increased continuously over the past decades. Small and medium enterprises have been the subject of discussion for many relevant international actors and under the attention of academics (Dragnić D., 2009, 39). The main characteristics of SMEs tend to derive from their limitation such as personalized management (owner/manager), constraints on resources (management/organization, human resources, finance) and technological capabilities, limited market impact, and greater flexibility to the external environment (Dragnic, 2014, p. 122).

Small and medium sized are widespread around the world and represent more than 90% of the total enterprises in almost every nation (World Bank, 2020). According to Annual Report on European SMEs, there are more than 25 million SMEs which accounts 99.8% of total enterprises in EU-28, and employ around 100 million people or (66%) of total employment in EU-28, and generate slightly less than three-fifth of the value added in the non-financial business sector. Despite the fact that they provide economic growth, generate employment, impacts innovation process, bring new ideas and other benefits, there is a fact that SMEs play a major role particularly in developing countries (World Bank, 2020).

However, Kosovo lags behind the region relatively economically and socially and is considered a potential source of social and political instability (KEC, 2019, p.9). Slow GDP growth due to weak economic policies and political instability has resulted in high levels of unemployment and informal economy (1/3 of GDP according to EC, 2019, p.47), poor education, weak business climate and infrastructure.

Nowadays the word innovation is synonymous with the word digitalization. The way business is developed today is very different from the way it has been. Innovations are changing economies, markets, and rediscovering relationships between organizations and suppliers, consumers, thus becoming a critical mechanism for increasing innovation and job creation (EC, 2019, 4). Digital transformation is a major challenge for SMEs, especially those traditional and non-innovative such as those in Kosovo who are facing the risk of digital transformation losing the ability to compete (KCHC, 2019, 7).

2. Methodology

The methodological approach of this paper is based on secondary data analysis and examination of documentary sources, such as reports from national and international relevant institutions, and other research publications in the area of SMEs. This study has analytical character and aims to review the previous studies by various actors which serves to the theoretical part of the study. The research purpose is also to give an overview of the SME role related to economic growth, challenges and constraints toward employment opportunities and innovation. The researcher first examines and interpret concepts and data for the relevance of SMEs toward economy in general. Secondly the author discuss and analyze the relevance of SMEs toward labor market and innovation generally, to try to realize the ultimate goal of our study, and draw conclusions based on result analysis.

3. Results and discussions

3.1. The SMEs' significance for the Kosovo economy

In Kosovo the classification of SMEs is defined by law on foreign investment which entered into force in 2014. This law defines SMEs by the sole criterion of the size of the number of employees, which is in line with the definition of the EU. (OECD, 2019)

Table 1. Classification of SMEs in Republic of Kosovo

	EU definition	Kosovo definition
Micro	< 10 employees, turnover or balance sheet total ≤ EUR 2 m	< 10 employees
Small	< 50 employees, turnover or balance sheet total ≤ EUR 10 m	< 50 employees
Medium	< 250 employees, turnover or balance sheet total ≤ EUR 43 m	< 250 employees

Source: Republic of Kosovo (2014 [23]), Law No. 04/L-220 on Foreign Investment, <https://mti.rksgov.net/desk/inc/media/1916AE1F-48E8-451D-A328-CA350EC4D7D2.pdf>;

Despite facing a high number of challenges and barriers, SMEs are expected to be the largest contributors to the economy and employment opportunities. A private sector which is dominated by SMEs and is considered as a pillar of economic growth, is also considered to be suffering from a state of stagnation and has failed to grow relative to its potential (Dobranja, 2019).

Table 2. Business demography indicators

Enterprise size	Enterprise		Value added	
	Number	Share	Million E	Share
Micro	34,611	93,1%	39,9	13,2%
Small	2,182	5,9%	61,5	20,3%
Medium	322	1,0%	144,4	47,5%
Total SMEs	37115	100,0%	303,3	100,0%

Source: EC (2019) SBA Factsheet. Country Report. p.3

Although, SMEs play a special role in the private sector, composing about 99.9% of all firms (KAS, 2020). With more than 93% of total business stock, is composed by micro-enterprises which is the largest sector in non-financial business economy (European Commission, 2019, p.3), while small and medium sized enterprise comprise 5.9% and 0.1% respectively. Kosovo SMEs generate 81,0% of total value added for the private sector with medium enterprises as largest contributor with 47% of total value added (European Commission, 2019, p.3). However, the value added created by micro-enterprise remains low compared to its peers in region, meanwhile, it has the largest share of SME exports of all the WBT economies, at 97.3%, with the dominant share coming from micro enterprises 54.9% (OECD, 2019, p.655). Given the fact that micro-enterprises dominate, and while their contribution remains small, stakeholders should be committed to creating favorable conditions for micro-enterprises to grow by creating a stability that enables future growth of value added, employment and exports.

Services are largest and most important sector of Kosovo's economy contributing significantly to GDP (72.63% of the GDP) and job creation (85.3% of the employment in Kosovo), driven by construction, real estate, and retail (MTI, 2019). Almost half of businesses in Kosovo (45.4%) belong to the trade and retail sector, the other services sector represents about 15% of enterprises, while manufacturing represents about 14% of enterprises in the business sector according to Kosovo Agency of Statistics.

Most enterprises in the trade and retail sector are mainly focused on food, beverages and retail. While most of the companies in service industry mainly operate in catering services, which is considered that the majority of businesses in the construction are involved in the construction of residential buildings. Despite the potential of the sectors that these enterprises are operating, they are mainly focused on consumption activities, which impacts directly to the growth of trade deficit, giving the fact that sectors with potential for employment growth and exports like manufacturing and information and communication technology continue to be underdeveloped and below potential levels.

This poor performance is attributed to the impact of internal and external barriers. From an internal perspective, the SME sector is characterized by traditional and non-innovative enterprises which often suffer from a lack of skills related to business activities, access to finance, innovation and technological skills, etc. Whereas, the external barriers that limit the development of SMEs and the utilization of its potential have to do with corruption, informality and unfair competition as well as the limited market and weak business climate, pandemic situation, political instability. Consequently, the continuous improvement of the business environment has not been sufficient for SMEs to increase the importance of this sector further.

3.2. The SMEs' contribution to job market and innovation

The SME sector also plays an important role in the labor market in Kosovo. Although not enough to reduce the unemployment rate which is currently at the level of 24.5%. This situation is also reflected by the education system which is not responding sufficiently to labor market needs.

Table 3. Employment in SME sector

Enterprise size	Numer of employee	Share
Micro	62,450	34,9%
Small	40,727	22,8%
Medium	33,075	18,5%
Total	136,252	76,2%

Source: EC (2019) SBA Factsheet. Country Report. p.3

Kosovo is considered a state of micro-enterprises based on the fact that about 99% of enterprises employ 1-9 workers, or about 76% of total business sector employment in Kosovo (EC, 2019, p.3), and around 55% of total employment in Kosovo (KAS, 2020). While the small and medium sized proportion (employing 10-249 workers) is only 1% (EC, 2019, p. 51). This is reflected in the fact that about 80% of enterprises have individual ownership according to the Open Data Platform, which in most cases have a characteristic of self-employment rather than their growth as an integral part.

The SME sector is considered a promising sector in terms of employment growth, however, based on the fact that the existing enterprises are small and do not continue to grow their staff and business, it remains unclear whether the SME sector will generate in the future the necessary employment to ensure higher growth and impact in terms of the labor market and overall economic development. SME sector have been subject of a major criticism, concerning the working conditions and the treatment of labor force (Dita Dobranja, 2019).

Based on our above states that most of the firms in Kosovo are traditional and non-innovative, Kosovo is still in its initial phases of innovation development. However, some steps are taken by the institutions with the establishment of the Ministry for Innovation and Entrepreneurship (MIE) in 2017 which has underpinned the institutional framework for streamlining innovation in policy making. Kosovo has made some progress with launching the programme for direct financial support for innovative businesses, start-ups and NGOs, however a limited number of entities supported it will not help overall improvement in the innovation and entrepreneurship without ant long term strategy (EC, 2019, p.28) Widespread informality leads to unfair competition, hindering access to finance and the ability of registered businesses to grow and innovate (EC, 2019, p.7)

The ICT sector is expected to take the lead in the innovation road of Kosovo. It is the one of few sectors with positive trade balance, 78% of already existing companies export their services (ECIKS, 2019) and has started to play an increasing role in Kosovo's economic growth.

Despite this situation, international community especially European Commission is helping out with financial measures to support expansion of ICT infrastructure network, innovation and entrepreneurship centers, improve quality of vocational and education training and other educational reforms to help overall environment for social development (EC, 2019, p.29). According to Kosovo Chamber of Commerce, management/owners of SMEs are aware and are working in the direction of digital transformation to improve their business performance. However, skilled workforce, knowledge and financial resources are main obstacle to digitally transform their businesses.

4. Conclusions

SME sector plays an important role in the economic development and employment of Kosovo, while innovation activity of SMEs remains challenging and its necessary to take strategic and concrete actions by relevant actors to unlock the potential in the most important and promising sector of the economy.

Kosovo's SMEs lack long-term objectives, innovation development, low product range, trade and services orientation, inadequate resource management, low level of product diversification, access to finance, informal economy.

Utilizing and expanding the potential of sectors such as manufacturing and information and technology would help the overall contribution of SME toward stable economic development, generate employment, and increase innovation activity toward healthy export rates.

The SME development agenda must be considered with urgent and concrete priority by local and international institutions to overcome the challenges of unemployment, high trade deficit, consumption in terms of development and increasing their role in the overall economy of the country. Kosovo's medium-term outlook is estimated to be positive and growing. However, Kosovo needs to further engage in creating a sustainable political and fiscal environment and create a better business climate that enables productive investment and SMEs performance enhancement.


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Article

VEGF Triggers Transient Induction of Autophagy in Endothelial Cells via AMPK 1

Katrin Spengler ¹, Nderim Kryeziu ¹, Silke Große ¹, Alexander S. Mosig ²  and Regine Heller ^{1,*}

¹ Institute of Molecular Cell Biology, Center for Molecular Biomedicine, Jena University Hospital, 07743 Jena, Germany; Katrin.Spengler@med.uni-jena.de (K.S.); nderim_kryeziu@hotmail.com (N.K.); Silke.Lindenmueller@med.uni-jena.de (S.G.)

² Institute of Biochemistry II and Center for Sepsis Control and Care, Jena University Hospital, 07743 Jena, Germany; Alexander.Mosig@med.uni-jena.de

* Correspondence: Regine.Heller@med.uni-jena.de; Tel.: +49-3641-939-5633

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Abstract: AMP-activated protein kinase (AMPK) is activated by vascular endothelial growth factor (VEGF) in endothelial cells and it is significantly involved in VEGF-induced angiogenesis. This study investigates whether the VEGF/AMPK pathway regulates autophagy in endothelial cells and whether this is linked to its pro-angiogenic role. We show that VEGF leads to AMPK 1-dependent phosphorylation of Unc-51-like kinase 1 (ULK1) at its serine residue 556 and to the subsequent phosphorylation of the ULK1 substrate ATG14. This triggers initiation of autophagy as shown by phosphorylation of ATG16L1 and conjugation of the microtubule-associated protein light chain 3B, which indicates autophagosome formation; this is followed by increased autophagic flux measured in the presence of bafilomycin A1 and by reduced expression of the autophagy substrate p62. VEGF-induced autophagy is transient and probably terminated by mechanistic target of rapamycin (mTOR), which is activated by VEGF in a delayed manner. We show that functional autophagy is required for VEGF-induced angiogenesis and may have specific functions in addition to maintaining homeostasis. In line with this, inhibition of autophagy impaired VEGF-mediated formation of the Notch intracellular domain, a critical regulator of angiogenesis. Our study characterizes autophagy induction as a pro-angiogenic function of the VEGF/AMPK pathway and suggests that timely activation of autophagy-initiating pathways may help to initiate angiogenesis.

Keywords: AMPK; autophagy; angiogenesis; VEGF; mTOR; ULK1

1. Introduction

Macroautophagy (herein, autophagy) is a cellular self-digestion process by which intracellular components such as long-lived proteins or dysfunctional organelles are sequestered in double-membrane autophagosomes and targeted to lysosomes for degradation [1]. While basal autophagy maintains cellular homeostasis under normal growth conditions, autophagy activated by nutrient or energy depletion serves to recycle essential biomolecules for cell survival and growth [2–5]. Autophagy stimulation is mediated via nutrient-sensing pathways involving the inhibition of mechanistic target of rapamycin (mTOR) and/or the activation of AMP-activated protein kinase (AMPK). mTOR and AMPK are serine (S)/threonine (T) kinases, which both target the Unc-51-like kinase 1 (ULK1), a key initiator of autophagy, and which also negatively regulate each other [6]. While mTOR inhibits ULK1 activity by phosphorylating its S758 residue (human sequence, corresponding to murine S757), AMPK triggers activation of ULK1 via phosphorylation of several serine sites including S556 (human sequence, corresponding to murine S555) [7–11], although inhibitory effects of AMPK have also been observed [12]. Once ULK1 is activated, it phosphorylates several components of the

vacuolar protein sorting 34/phosphoinositide 3-kinase (VPS34/PI3K) complex leading to generation of phosphatidylinositol 3-phosphate (PI3P) and nucleation of the autophagosome [13,14]. The formation of the autophagosome is then completed by protein conjugation and lipidation complexes that involve the conversion of the microtubule-associated protein light chain 3B-I (LC3B-I) to LC3B-II [15]. The autophagosome finally fuses with a lysosome to allow degradation of the cargo by lysosomal proteases [16].

In endothelial cells, autophagy is regulated by laminar shear stress [17,18] and protects endothelial cells from oxidative stress, high glucose or excess lipid accumulation [19–24]. Several vasculo-protective compounds such as epigallocatechin gallate, resveratrol and curcumin [23,25,26] or endothelial stressors, for example, oxidized low density lipoproteins [27,28] or advanced glycation end products [29] have been shown to stimulate autophagy thereby strengthening endothelial resistance or compensating for the detrimental effects of cellular stress, respectively. Loss of autophagy has been reported to cause endothelial dysfunction in aged human and mouse endothelial cells [30,31] and to accelerate atherosclerotic plaque formation in mice [32]. Autophagy seems to be required for maintaining endothelial functions such as nitric oxide (NO) biosynthesis [33–35], endothelial barrier function [36,37], secretion of von Willebrand factor [38], and angiogenesis [39–44]. In contrast, an anti-angiogenic effect of autophagy induction has also been reported [45–47].

The induction of autophagy in endothelial cells by various agonists often involves the activation of AMPK, which suggests that autophagy is one of the mechanisms by which AMPK accomplishes its known anti-inflammatory and anti-atherogenic effects [21,25,40,47–50]. AMPK is ubiquitously expressed and mainly known as a sensor and regulator of the cellular energy status [51]. It maintains cellular ATP homeostasis by activating catabolic pathways and inhibiting anabolic pathways via phosphorylation of proteins such as acetyl-CoA carboxylase (ACC), an important regulator of fatty acid metabolism [52]. Our previous work and other studies have shown that vascular endothelial growth factor (VEGF), an important angiogenic stimulus, is a potent agonist of AMPK activation and that the catalytic isoform AMPK 1 is essentially involved in VEGF-induced angiogenesis [53–55]. The mechanisms underlying the pro-angiogenic role of AMPK are still poorly understood. One explanation is that AMPK inhibits glutamine:fructose-6-phosphate amidotransferase 1 (GFAT1), leading to reduced formation of O-linked -N-acetylglucosamine-modified proteins, which normally negatively affect angiogenesis [56]. Another reason may be that AMPK activates endothelial NO synthase (eNOS) and promotes angiogenesis by enhanced NO production, although data on the interaction of AMPK with eNOS are conflicting [53,57–61]. In the present study, we have addressed the hypothesis that the VEGF/AMPK pathway regulates autophagy in endothelial cells and that stimulation of autophagy contributes to the pro-angiogenic function of this pathway. Our data show that the growth factor VEGF leads to transient induction of autophagy in endothelial cells via sequential activation of AMPK and mTOR pathways leading to consecutive phosphorylation of ULK1 at activating (S556) and inhibiting (S758) sites.

2. Materials and Methods

2.1. Chemicals

M199 was purchased from Lonza (Verviers, Belgium), fetal calf serum (FCS) and human serum were from Sigma (Taufkirchen, Germany) and endothelial mitogen was purchased from Biomedical Technologies Inc. (Stoughton, MA, USA). The siRNAs against AMPK 1, AMPK 2, ULK1, BECN1 and non-targeting control siRNA were SMARTpool-siRNAs obtained from GE Healthcare, Dharmacon RNAi and Gene Expression (Lafayette, CO, USA). Recombinant human VEGF-165 was obtained from R&D Systems GmbH (Wiesbaden, Germany). Protease inhibitor mixture complete, EDTA-free, was obtained from Roche Diagnostics (Mannheim, Germany) and used in protein lysates, while a protease inhibitor cocktail obtained from Sigma (Taufkirchen, Germany) was administered to protect cytokine degradation in supernatants. Propidium iodide, RNase A, DL-buthionine-(S,R)-sulfoximine (BSO),

5,5⁰-Dithiobis(2-nitrobenzoic acid) (DTNB), NADPH, 2-deoxyglucose (2-DG), thrombin and aprotinin were purchased from Sigma (Taufkirchen, Germany). Bafilomycin A1 was from Enzo Life Sciences (Lörrach, Germany) and fibrinogen was from Merck/Millipore (Darmstadt, Germany). Bovine serum albumin-C (BSA-C) and goat serum were obtained from Aurion (Wageningen, The Netherlands) and Cell Signaling Technology (Frankfurt, Germany), respectively.

2.2. Antibodies

Rabbit monoclonal antibodies against -actin, p62, BECN1, p-ULK1 (S555* (mouse), S556 (human)), p-ATG14 (S29), ATG14, ATG16L1, p-AMPK (T172), p-VEGFR2 (Y1175), VEGFR2, p-eNOS (S1177), ACC, cleaved Notch1 (V1744) (NICD), rabbit polyclonal antibodies against p-ULK1, (S757* (mouse), S758 (human), ULK1, AMPK, AMPK 1, AMPK 2, p-PLC (Y783), PLC, p-p70S6K (T389), p70S6K, p-ACC (S79) and mouse monoclonal antibodies against LC3B, p-ERK1/2 (T202/Y204) and ERK1/2 were obtained from Cell Signaling Technology (Frankfurt, Germany). The antibodies labelled with * are named after the murine sequences by the manufacturer but also recognize the respective human sequences. The LC3B antibody detected mainly the type II form of LC3B under the applied conditions. Rabbit monoclonal antibody against p-ATG16L1 (S278) and mouse polyclonal antibody against eNOS were from Abcam (Cambridge, UK) and BD Transduction Laboratories (Heidelberg, Germany), respectively. Peroxidase-labeled anti-mouse and anti-rabbit IgG were from Kirkegaard and Perry Laboratories, Inc. (Gaithersburg, MD, USA). Secondary AlexaFluor^{fi}488-conjugated goat anti-rabbit IgG was from Thermo Scientific (Waltham, MA, USA).

2.3. Cell Culture

Human umbilical vein endothelial cells (HUVEC) were isolated from anonymously acquired umbilical cords according to the Declaration of Helsinki, "Ethical principles for Medical Research Involving Human Subjects" (1964). The study was approved by the Jena University Hospital Ethics Committee (no. 3130-05/11) and donors were informed and gave written consent. For cell preparation, umbilical cord veins were cleaned with 0.9% NaCl solution and cells were detached with 0.01% collagenase dissolved in M199 for 3 min at 37 °C. Veins were then rinsed with M199/10% FCS and the cell suspension was centrifuged (500 g, 6 min). The pellet was resuspended in M199/10% FCS and seeded on a cell culture flask coated with 0.2% gelatin. After 24 h, cells were washed and cultured in full growth medium (M199, 17.5% FCS, 2.5% human serum, 7.5 g/mL endothelial mitogen, 7.5 U/mL heparin, 680 M glutamine, 100 M vitamin C, 100 U/mL penicillin, 100 g/mL streptomycin). In general, HUVEC from the second passage were seeded at a density of 27,500/cm² and used for experiments three days after seeding if not otherwise indicated. For transfection with siRNA, the seeding density was 23,000/cm², for cell cycle analysis 15,000/cm² if cell proliferation was investigated and 23,000/cm² if survival was analyzed. For the immunofluorescence studies, 50,000 cells/cm² were seeded on coverslips. Most experiments were performed with cells on 30 mm culture dishes. For spheroid generation, ATP measurements or glutathione (GSH) determination, 96-well plates, 24-well plates or 60 mm dishes were employed, respectively.

2.4. Cell Treatment

HUVEC were incubated in full growth medium for monitoring basal autophagy and the effect of autophagy inhibition on basal cell functions (growth, survival, cytokines, GSH, ATP, validation of downregulation, mRNA expression). Experiments, in which VEGF was used as a stimulus, were either performed in the absence of serum (short-term studies, <30 min) or in the presence of 2% FCS (long-term studies, >30 min). Stimulation in serum-free medium was done in M199 containing 0.25% human serum albumin (HSA) (signaling studies shown in Figures 2 and 3) or in Hepes buffer (10 mM Hepes (pH 7.4), 145 mM NaCl, 5 mM KCl, 1 mM MgSO₄, 1.5 mM CaCl₂, 10 mM glucose) supplemented with 0.25% HSA (signaling studies shown in Figure 5).

2.5. siRNA Transfection

HUVEC were transfected 24 h after seeding with 0.5 g/mL of non-targeting or specific siRNA using SAINT-sRNA transfection reagent (Synvolux Therapeutics B.V., Groningen, The Netherlands). If two proteins, i.e., ULK1 and BECN1, were downregulated at the same time by using 0.5 g/mL specific siRNA for each target, 1 g/mL control siRNA was applied. The transfection solution was prepared by first combining siRNA diluted in 100 L Hepes-buffered saline (HBS) with 20 L SAINT-sRNA diluted in 80 L HBS and then adding 800 L of M199 supplemented with 0.25% HSA to this mixture. HUVEC were washed twice with Hanks' Balanced Salt Solution (HBSS) and the transfection solution was added. After 4 h, 2 mL of full growth medium was added and cells were cultured for 72 h before the experiment was performed.

2.6. Cell Lysis and Western Blot

HUVEC were lysed in ice-cold Tris buffer (50 mM Tris (pH 7.4), 2 mM EDTA, 1 mM EGTA, 50 mM NaF, 10 mM Na₄P₂O₇, 1 mM Na₃VO₄, 1 mM DTT, 1% Triton X-100, 0.1% SDS, 1 mM PMSF, 10 U/mL protease inhibitor cocktail (Roche) for 15 min on ice and scraped. After centrifugation of the lysates (700 g, 6 min), the protein content of the supernatants was determined using Lowry reagents (DC™ Protein Assay kit) and bovine serum albumin (BSA) as standard. Supernatants were supplemented with Laemmli buffer, subjected to SDS-PAGE (25–50 g lysate protein/lane) and transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked for 1 h in Tris-buffered saline/Tween (TBST) buffer (20 mM Tris (pH 7.6), 137 mM NaCl, 0.1% (v/v) Tween[®] 20) containing 5% non-fat dried skimmed milk and then incubated overnight at 4 °C with primary antibodies. Antibody dilutions were prepared in TBST containing 5% BSA. Following incubation with the respective horseradish peroxidase-conjugated secondary antibodies for 1 h, signal detection was performed using the enhanced chemiluminescence (ECL) reagent (GE Healthcare, Chicago, IL, USA) or Western Lightning Plus-ECL reagent (Perkin Elmer, Waltham, MA, US). For each staining, dilution series of lysates, antibody dilution and exposure times were tested prior to experiments and conditions were chosen that allowed protein band detection in the linear range. Quantification was carried out by densitometry using ImageJ software. If applicable, ratios between phosphorylated protein and total protein were calculated. LC3B conjugation was evaluated by analyzing LC3B type II, i.e., the lower LC3B-positive band if double bands were seen.

2.7. Cytokine Measurement

Experiments were started 48 h after transfection of cells with siRNA against ULK1 and BECN1 by adding 600 L fresh growth medium and an additional incubation for 24 h. Supernatants were harvested and supplemented with protease inhibitor cocktail (Sigma, 1:500). Cytokines were measured using the BD Cytometric Bead Array (CBA) Human Soluble Protein Master Buffer Kit for IL-8 and the BD CBA Human Enhanced Sensitivity Master Buffer Kit for IL-1 and TNF- α , respectively (BD Biosciences, Heidelberg, Germany). For normalization, parallel dishes were lysed with solubilization buffer (100 mM NaOH, 1.9 M Na₂CO₃, 1% SDS) and protein content was determined according to Lowry.

2.8. GSH Measurement

After transfection of cells with siRNA to downregulate autophagy, cells were washed twice with phosphate-buffered saline (PBS) and lysed with 90 L of 0.01 N HCl. Cells were harvested and centrifuged (15,000 g, 10 min). Sulfosalicylic acid (27.2 L) was added to 80 L of the supernatants and incubated on ice for 5 min. Thereafter, proteins were precipitated by centrifugation (15,000 g, 5 min). Supernatant (40 L) was mixed with 340 L reaction buffer (200 mM Tris, 1 mM EDTA, 1.5 mM NADPH) and 20 L of 10 mM DTNB. The assay mixture was transferred to 96-well plates (100 L/well, triplicates) and 20 L glutathione reductase (10 U/mL) was added per well. The absorption at 412 nm

was measured over a 12 min time period and GSH concentrations were determined on the basis of a calibration curve. Cells were lysed with solubilization buffer and protein content was determined according to Lowry for normalization.

2.9. Cell Cycle Analysis

Cells were trypsinized and pooled with cells from culture supernatants and washing solutions if subG₁ fractions were analyzed. The cell suspensions were centrifuged (500 g, 6 min) and the obtained cell pellets washed twice with PBS and resuspended in 200 L cold PBS. Then, 2 mL 70% ethanol was added dropwise and mixed carefully and samples were incubated for 1 h at 4 C under gentle shaking. Pellets were washed twice in cold PBS with 0.5% Tween^{fi} 20, resuspended in 100 L RNase A solution (200 g/mL) and incubated at 37 C for 15 min. Then, 100 L propidium iodide (150 g/mL) was added and incubated for 10 min in the dark. Samples were then analyzed by flow cytometry.

2.10. Mitochondrial ROS Measurement

Mitochondrial ROS levels were detected using the MitoSOX Red Mitochondrial Superoxide Indicator from Thermo Scientific (Waltham, MA, USA). Experiments were performed according to the manufacturer's protocol. In brief, cells were washed once with PBS and incubated with staining solution for 30 min. After trypsinization cell pellets were resuspended in PBS and subjected to flow cytometric analysis.

2.11. ATP Measurement

After transfection with siRNA against ULK1 and BECN1 and additional experimental incubations, cell proteins were denaturated by adding 200 L ethanol per well. After evaporation of the ethanol, 100 L Tris buffer contained in the assay kit was added and cells were subjected to one freezing/thawing cycle in liquid nitrogen, scraping and centrifugation. The ATP content of the soluble fraction was determined using the ATP Kit SL from Biotherma (Handen, Sweden) according to the manufacturer's protocol. For normalization, cells in identically treated wells were lysed with solubilization buffer and the protein content was determined according to Lowry.

2.12. Spheroid Assay

Spheroids were generated as described previously [62]. In brief, control or siRNA-transfected cells suspended in growth medium were mixed with methyl cellulose (stock solution 12 mg/mL) at a 4:1 ratio and 3000 cells/well were seeded in 96-well round-bottom plates. After 24 h, spheroids were collected, centrifuged (200 g, 4 min) and washed with Hepes buffer. Then, fibrinogen solution (1.8 mg/mL in Hepes buffer) containing 20 U/mL aprotinin was added to the spheroids to obtain a suspension with approximately 100 spheroids per ml. Then, 300 L of this suspension together with 0.2 U thrombin were added per well of a 24-well plate. The plate was incubated for 20 min at 37 C to allow the formation of a fibrin gel. To equilibrate the gel with medium and wash out thrombin, M199 containing 2% FCS, 680 M glutamine, 100 U/mL penicillin and 100 g/mL streptomycin was added twice for 15 min. Thereafter, spheroids were cultured in the same medium and stimulated with 50 ng/mL VEGF for 24–48 h. Finally, spheroids were fixed on ice by adding 1 mL 4% paraformaldehyde per well for 10 min. After two washing steps with PBS, spheroid sprouting was viewed by light microscopy and pictures were taken (AxioVert 200, Carl Zeiss, Oberkochen, Germany). The number and length of sprouts were analyzed using cellSens image analysis software (Olympus, Tokyo, Japan).

2.13. qRT-PCR

RNA was extracted using the NucleoSpin^{fi} Kit from Machery-Nagel (Düren, Germany). cDNA from 1500 ng RNA was synthesized with the First Strand cDNA Synthesis Kit from Thermo Scientific (Waltham, MA, USA). Quantitative RT-PCR was performed with the Maxima SYBR

Green/ROX qPCR Master Mix (2X) from Thermo Scientific using primers for LC3B (forward 5⁰GATGTCCGACTTATTCGAGAGC3⁰⁰, reverse 5⁰TTGAGCTGTAAGCGCCTTCTA3⁰) and -actin (forward 5⁰GGGACGACATGGAGAAAATCTG3⁰, reverse 5⁰GAAGGTCTCAAACATGATCTGGG3⁰). LC3B expression was normalized to the expression of -actin.

2.14. LC3B Immunofluorescence

Seventy-two hours after transfection with siRNA, cells were washed with HEPES buffer and fixed with 500 L ice-cold methanol for 10 min at 4 °C. After two washes with ice-cold PBS 300 L blocking solution (10% BSA-C, 5% goat serum in PBS) was added and coverslips were incubated for 30 min at room temperature. Cells were washed twice with PBS followed by incubation with 50 L of LC3B antibody solution (1:200 in blocking solution) for 2 h. Subsequently, cells were washed twice with PBS, incubated with 50 L AF488-conjugated secondary antibody (1:500 in blocking solution) for 1 h and mounted on slides. Pictures were taken with the Olympus BX61 microscope (Olympus, Tokyo, Japan).

2.15. Statistics

Data are given as mean values SEM from at least three independent experiments. For testing statistical significance one-way or two-way repeated measurement ANOVA with Holm–Šidák post hoc testing were used to compare two or more conditions, respectively. A p-value of <0.05 was accepted as statistically significant.

3. Results

3.1. Functional Autophagy is a Prerequisite for Endothelial Homeostasis

In the first series of experiments, we characterized autophagy in human umbilical vein endothelial cells cultured in full growth medium. These cells exhibited substantial basal autophagic flux, which was shown as time-dependent accumulation of conjugated LC3B in the presence of the lysosomal inhibitor bafilomycin A1 (Figure 1A). To study the role of autophagy, ULK1 and beclin 1 (BECN1), two molecules involved in autophagy initiation, were downregulated by specific siRNA (Figure 1B). This led to a decrease in LC3B conjugation, a decline in LC3B-positive autophagosomes and an accumulation of p62, a well-known autophagy substrate, indicating a significant reduction in autophagy in comparison to control cells (Figure 1C,D). Autophagy inhibition was paralleled by enhanced secretion of the pro-inflammatory cytokines, tumor necrosis factor- (TNF-), interleukin-1 (IL-1) and interleukin-8 (IL-8), decreased levels of the endogenous antioxidant glutathione (GSH) suggesting impaired antioxidant defense, and impaired cell survival (Figure 1E–G). Mitochondrial formation of reactive oxygen species (ROS) was not enhanced under these conditions (Figure 1H). Downregulation of ULK1 and BECN1 had little effect on ATP availability. When endothelial cells were additionally challenged with the glycolysis inhibitor 2-deoxyglucose (2-DG), which led to ATP reduction, no difference in ATP levels between control cells and autophagy-deficient cells was seen (Figure 1I). Together, these data underline the important homeostatic role of basal autophagy in endothelial cells but do not suggest that this process has an influence on energy metabolism.

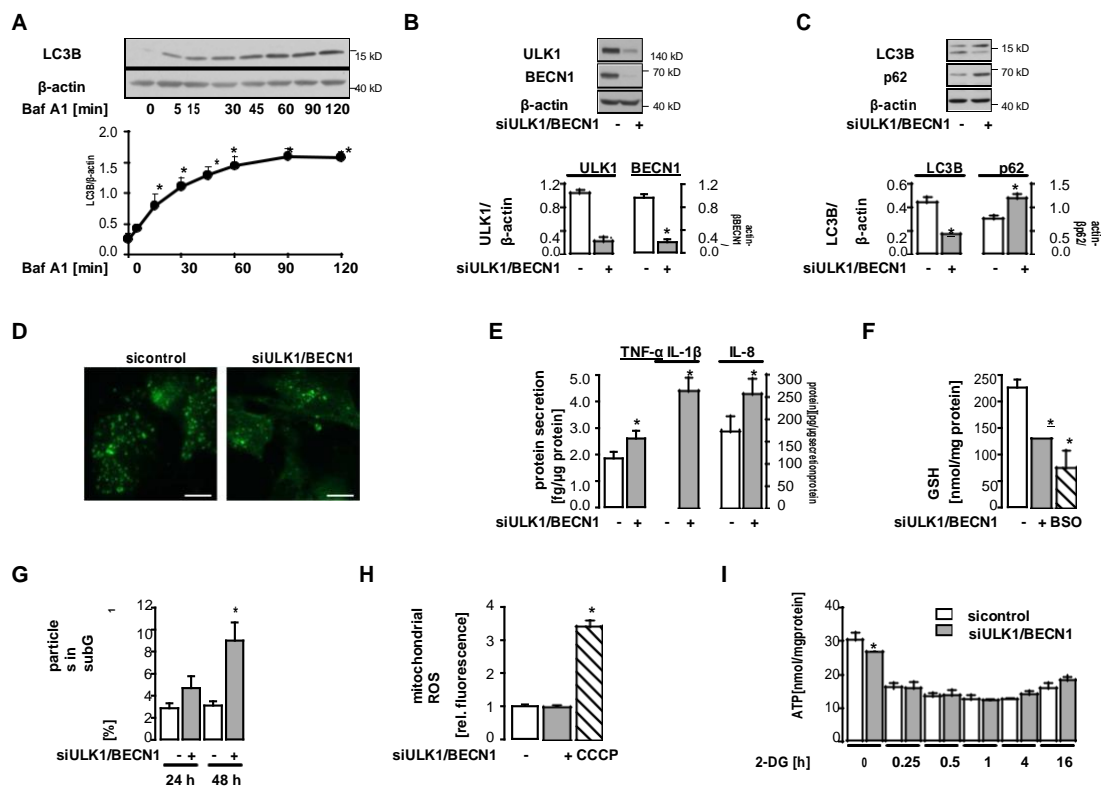


Figure 1. Functional autophagy is a prerequisite for endothelial homeostasis. **(A)** Human umbilical vein endothelial cells (HUVEC) were treated with 50 nM bafilomycin A1 (Baf A1) for the indicated times. Lysates were subjected to Western blot analyses of LC3B and β -actin. Representative blots and densitometric evaluation are shown (mean values + SEM, $n = 5$), * $p < 0.05$ vs. untreated control. **(B–I)** HUVEC were transfected with control-siRNA or Unc-51-like kinase 1 (ULK1)- plus beclin 1 (BECN1)-siRNA for 72 h and analyzed thereafter **(B–F, H–I)** or after cultivation for 24 or 48 h **(G)**. **(B–C)** Cell lysates were analyzed for the indicated proteins in Western blots. Representative blots and densitometric evaluation are shown (mean values + SEM, $n = 5$). **(D)** Cells were stained for LC3B, whose accumulation in punctae reflects the formation of autophagosomes. Representative immunofluorescent images are shown ($n = 2$), scale bar = 10 μ m. **(E)** Cytokines were quantified in cell supernatants by multiplex bead-based flow cytometric analyses (mean values + SEM, $n = 5$). **(F)** Glutathione (GSH) levels of cell lysates were determined in a colorimetric assay (mean values + SEM, $n = 3$). The positive control was treated with 100 μ M DL-buthionine-(S,R)-sulfoximine (BSO, inhibitor of GSH synthesis) for 12 h. **(G)** Cells were stained with propidium iodide and analyzed by flow cytometry. The percentage of particles in the subG1 fraction is shown (mean values + SEM, $n = 5$). **(H)** Mitochondrial production of reactive oxygen species was detected by MitoSOX-based flow cytometry. Treatment of cells with 100 μ M carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) for 1 h served as positive control. **(I)** Cells were treated with 20 mM 2-deoxyglucose (2-DG) for the indicated times. ATP levels in cell extracts were measured using a luciferase-based assay (mean values + SEM, $n = 3$). Compared to untreated controls, 2-DG led to a significant reduction of ATP levels under all conditions ($p < 0.001$, not indicated in the graph). **(B–I)** * $p < 0.05$ vs. control-siRNA-treated cells.

3.2. VEGF Initiates Functional Autophagy in Endothelial Cells via Phosphorylation of ULK1 at S556

Since autophagy is known to be controlled by AMPK and mTOR, we asked how the growth factor VEGF, known to activate both pathways [53,55,63] affects autophagy. Figure 2A,B show that VEGF triggered transient phosphorylation of ULK1 and its substrate ATG14, a member of the VPS34 complex [64], at S556 and S29, respectively, denoting the initiation of autophagy. Accordingly, a transitory phosphorylation of ATG16L1, a part of the LC3B lipidation complex [65,66], at S278 and conjugation of LC3B, which both point to the formation of autophagosomes in response to VEGF, were

observed (Figure 2C,D). Further, an early increase in autophagic flux in cells stimulated with VEGF in the presence of bafilomycin A1, and in parallel, a lower expression of p62 upon VEGF treatment were detected, indicating functional autophagy (Figure 2E,F). The transient nature of autophagy induction may be related to the inhibitory phosphorylation of ULK1 at S758, the mTOR phosphorylation site, which occurred with a time lag in response to VEGF and may terminate activation of ULK1 (Figure 2G).

Figure 2. VEGF, a physiological AMPK agonist, initiates autophagy in endothelial cells Together, these data show that VEGF induced autophagy in endothelial cells via ULK1 phosphorylation via phosphorylation of ULK1 at S556. at S556.

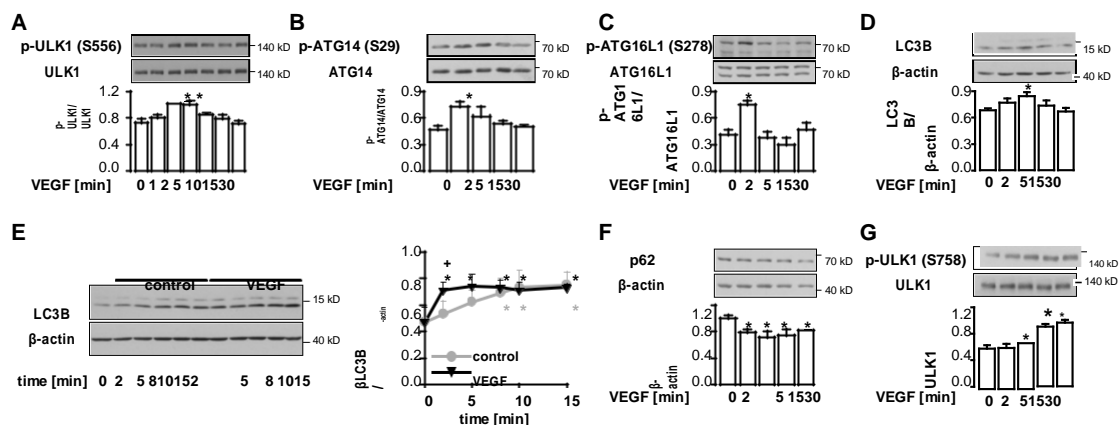


Figure 2. Vascular endothelial growth factor (VEGF), a physiological AMP-activated protein kinase (AMPK) agonist, initiates autophagy in endothelial cells via phosphorylation of ULK1 at S556. (**A–D, F–G**) HUVEC were stimulated with 50 ng/mL VEGF for the indicated times, lysed and subjected to Western blot analyses of the indicated proteins. (**E**) HUVEC were pretreated with 50 nM bafilomycin A1 for 15 min (time = 0), subsequently stimulated with 50 ng/mL VEGF or vehicle (control) for the indicated times, lysed and subjected to Western blot analyses of LC3B. (**A–G**) Representative blots and densitometric evaluation are shown (mean values + SEM, $n = 3$ (**B,G**), $n = 4$ (**A, C, D, F**), $n = 5$ (**E**)), * $p < 0.05$ vs. unstimulated control, + $p < 0.05$ vs. unstimulated control pretreated with bafilomycin A1.

3.3. VEGF-Induced Initiation of Autophagy depends on AMPK 1

To understand the role of AMPK in autophagy initiation by VEGF, we applied specific siRNAs to downregulate the AMPK isoforms 1 or 2 (94% or 81% reduction, respectively) (Figure 3A,B) and compared VEGF-triggered ULK1 phosphorylation between AMPK 1- or 2-depleted and control cells. Phosphorylation of ULK1 at S556 was completely prevented when the AMPK isoform 1 was downregulated, demonstrating that it was mediated by AMPK 1 (Figure 3C). Thus, VEGF led to a transient stimulation of autophagy via activation of AMPK 1. In contrast, depletion of AMPK 2 had no effect (Figure 3D). VEGF did not induce LC3B conjugation in cells, in which AMPK 1 or AMPK 2 were downregulated (Figure 3E,F). However, under these conditions, we observed an increased basal expression and conjugation of LC3B compared to control cells as reported previously [67,68] (Figure 3E–G). This was not accompanied by an alteration of the basal autophagic flux in AMPK 1- or AMPK 2-depleted cells (Figure 3H,I).

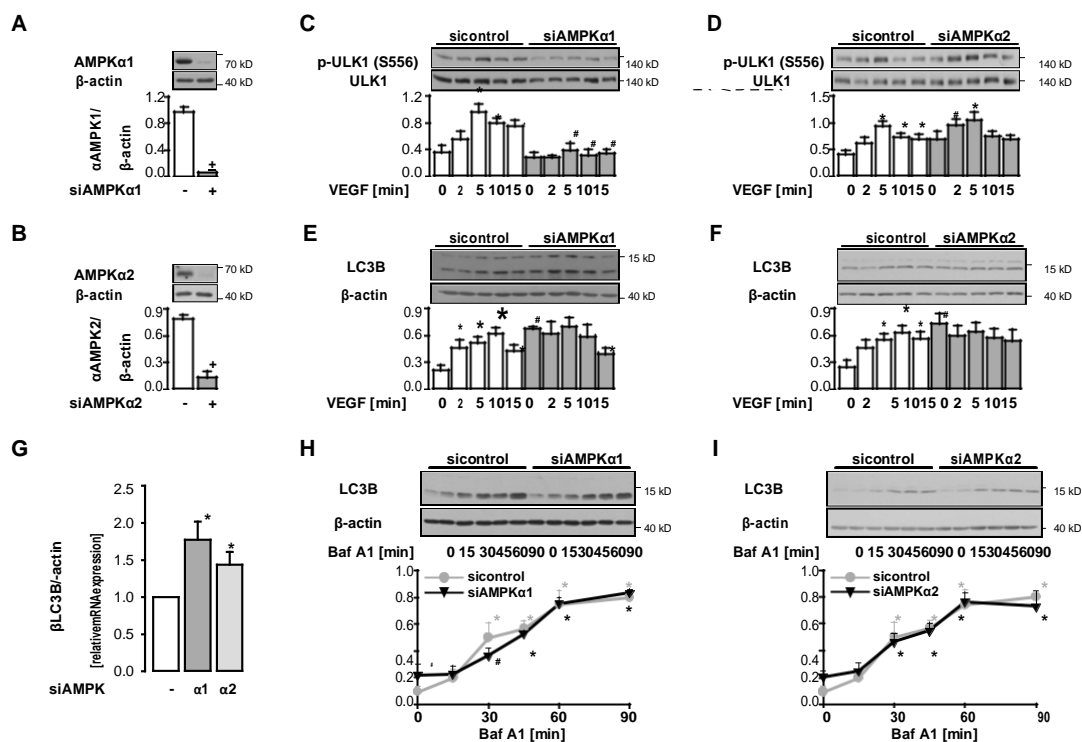
Figure 3. VEGF-induced ULK1 phosphorylation is mediated by AMPK α 1.

Figure 3. VEGF-induced ULK1 phosphorylation is mediated by AMPK 1. (A–I) HUVEC were transfected with control-siRNA, AMPK 1-siRNA or AMPK 2-siRNA, respectively. (A–F) Cells were directly lysed (A–B) or stimulated with 50 ng/mL VEGF for the indicated times (C–F) and subjected to Western blot analyses of the indicated proteins. Representative blots and densitometric evaluation are shown (mean values + SEM, $n = 3$ (C), $n = 4$ (A,B,D), $n = 5$ (E–F)), * $p < 0.05$ vs. control-siRNA, * $p < 0.05$ vs. unstimulated control, # $p < 0.05$ vs. the respective VEGF-stimulated sample transfected with control-siRNA. (G) RNA was extracted, transcribed into cDNA and qRT-PCR was performed. Mean values + SEM of LC3B mRNA levels normalized to β -actin are shown, $n = 4$, * $p < 0.05$ vs. control-siRNA. (H–I) Cells were treated with 50 nM bafilomycin A1 (Baf A1) for the indicated times, lysed and subjected to Western blot analyses of LC3B. Representative blots and densitometric evaluation are shown (mean values + SEM, $n = 3$), * $p < 0.05$ vs. respective controls without Baf A1 treatment, # $p < 0.05$ vs. respective control-siRNA-treated sample.

3.4. VEGF-Induced Angiogenesis Requires Functional Autophagy

We next addressed the question of whether autophagy is required for VEGF-induced angiogenesis by employing a spheroid assay. VEGF induced considerable sprouting in control spheroids but had little effect in spheroids generated from ULK1/BECN1-depleted cells (Figure 4A). In contrast, VEGF was still able to stimulate proliferation and survival in autophagy-deficient cells, although basal proliferation and survival were substantially impaired, probably due to disturbed homeostasis (Figure 4B,C). Angiogenesis was also repressed by the lysosomal inhibitor bafilomycin A1 at a low concentration, which impaired autophagy but did not affect basal survival (Figure 4D–F).

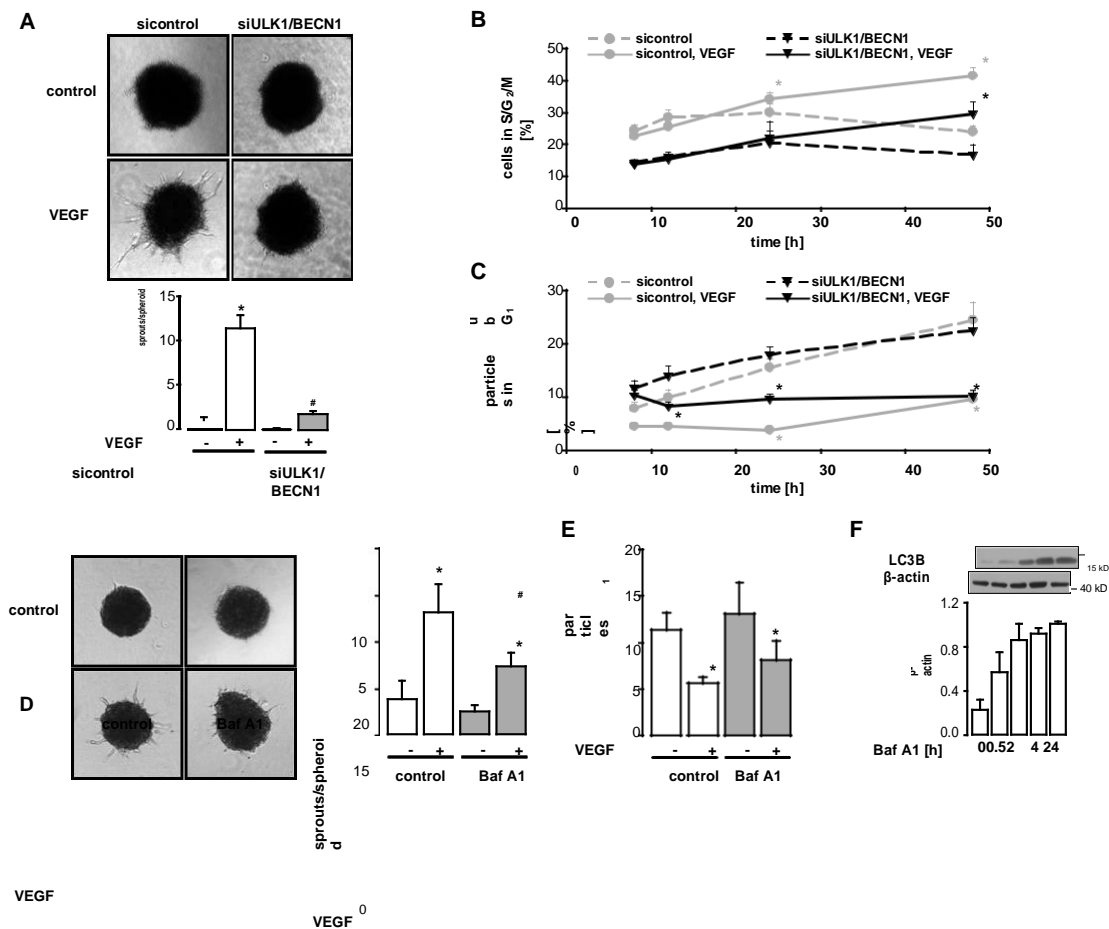


Figure 4. VEGF-induced angiogenesis requires functional autophagy. (A–C) HUVEC were transfected with control-siRNA or ULK1- plus BECN1-siRNA for 72 h. (A) Spheroids generated from transfected cells were stimulated with VEGF (50 ng/mL, 48 h) and sprouting was analyzed. Representative pictures and quantification of sprout numbers per spheroid are shown (mean values + SEM, n = 4), * p < 0.05 vs. respective unstimulated control, # p < 0.05 vs. VEGF-stimulated sample transfected with control-siRNA. (B–C) Cells were stimulated with 50 ng/mL VEGF for the indicated times and subjected to cell cycle analysis. The proportion of cells in the proliferative phases S/G₂/M (B) or particles in the subG₁ fraction (C) is shown (mean values + SEM, n = 4), * p < 0.05 vs. respective values at 8 h after VEGF addition. (D–E) HUVEC spheroids (D) or cultured HUVEC (E) were pretreated with 5 nM bafilomycin A1 (Baf A1) for 30 min and stimulated with 50 ng/mL VEGF for 24 h. (D) Representative pictures and the quantification of sprouts per spheroid are shown (mean values + SEM, n = 5). (E) Cells were stained with propidium iodide and subjected to flow cytometry analysis. The proportion of particles in the subG₁ fraction is shown (mean values + SEM, n = 5). (D–E) * p < 0.05 vs. respective unstimulated control, # p < 0.05 vs. respective non-Baf A1-treated sample. (F) Cells were treated with 5 nM Baf A1 for the indicated times, lysed and subjected to Western blot analysis. Representative blots and densitometric evaluation are shown (mean values + SEM, n = 2).

3.5. Autophagy Interferes with VEGF-Induced Notch Signaling

To test the effect of autophagy on major angiogenic signaling pathways such as activation of VEGF receptor 2 (VEGFR2), phospholipase C (PLC), extracellular signal-regulated kinase 1/2 (ERK1/2), eNOS, p70 ribosomal protein S6 kinase (p70S6K), AMPK and ACC, control cells and ULK1/BECN1-depleted cells were stimulated with VEGF. VEGF led to a transient phosphorylation with a maximum at 2–5 min for most of the investigated proteins (Figure 5A–F). In contrast, the phosphorylation of the mTOR target p70S6K only peaked at 15 min indicating delayed activation of the mTOR pathway (Figure 5G). Almost no differences in the expression and VEGF-induced phosphorylation of the investigated

proteins were seen between control cells and autophagy-deficient cells, which indicated that the respective signaling pathways were not controlled by autophagy.

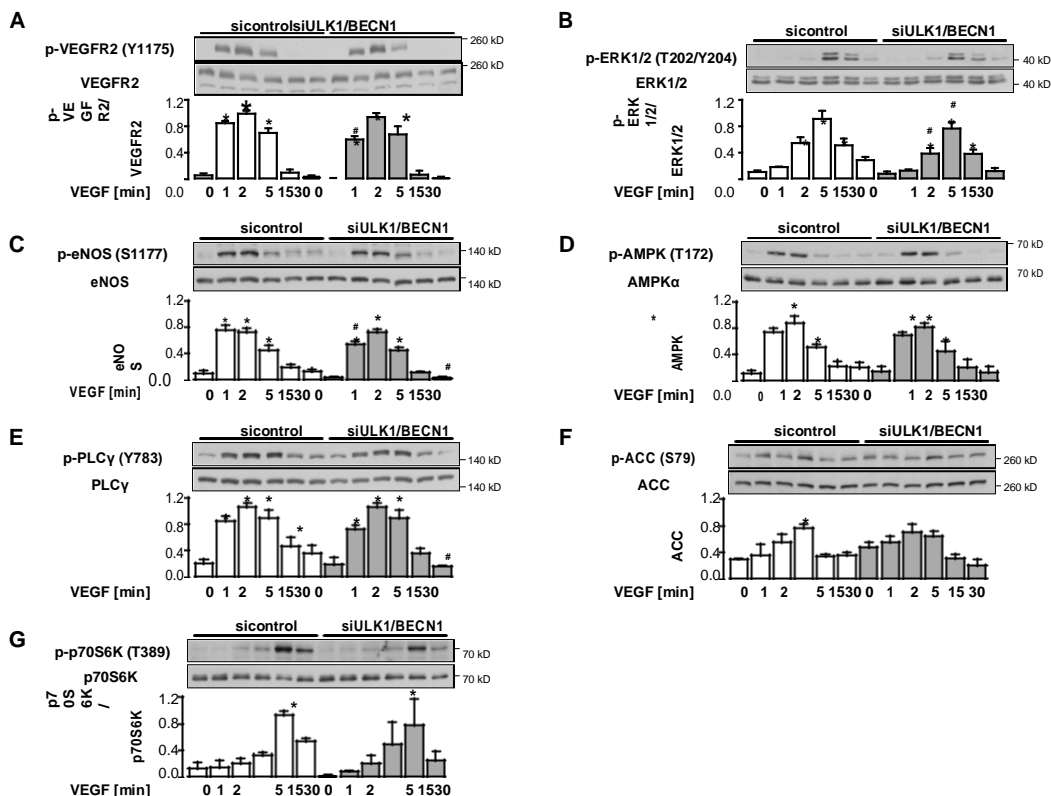


Figure 5. Autophagy has a minor impact on VEGF-induced signaling pathways. (A–G) HUVEC were transfected with control-siRNA or ULK1- plus BECN1-siRNA for 72 h and stimulated with 50 ng/mL VEGF for the indicated times. Cell lysates were subjected to Western blot analyses of the indicated proteins. Representative blots and densitometric evaluations are shown (mean values + SEM, n = 4), * p < 0.05 vs. respective unstimulated control, # p < 0.05 vs. respective samples treated with control-siRNA.

We also evaluated VEGF-induced Notch signaling, a pathway that controls endothelial differentiation and stabilization during angiogenesis, by monitoring the formation of the Notch intracellular domain (NICD). NICD accumulated time-dependently in response to VEGF in control cells and reached a maximum at 24 h (Figure 6A). At this time point, NICD abundance was significantly lower in cells in which ULK1 and BECN1 were downregulated (Figure 6B). Together, these data suggest that the failure in VEGF-induced sprout formation in conditions where autophagy is inhibited may not be related to reduced VEGF responsiveness of endothelial cells in general, but rather it may be related to disturbed basal homeostasis and to interference with specific angiogenic processes such as Notch processing.

Figure 6. Autophagy interferes with the Notch signaling pathway.

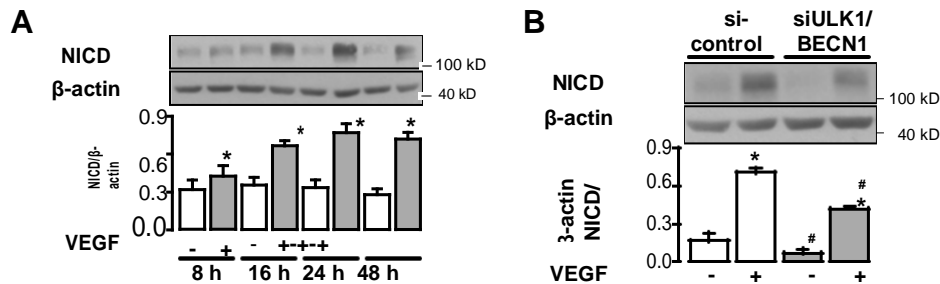


Figure 6. Autophagy interferes with the Notch signaling pathway. (A) HUVEC were stimulated with 50 ng/mL VEGF for the indicated times. (A) Cells were transfected with control-siRNA or ULK1- plus BECN1-siRNA and stimulated with 50 ng/mL VEGF for 24 h. (A–B) Cells were lysed and subjected to Western blot analyses of the Notch intracellular domain (NICD). Representative blots and densitometric evaluation are shown (mean values + SEM, n = 5 (A), n = 4 (B)), * p < 0.05 vs. respective unstimulated control, # p < 0.05 vs. respective samples treated with control-siRNA.

4. Discussion

Autophagy is induced by inhibition of mTOR upon amino acid depletion or activation of AMPK upon glucose starvation. These two nutrient-sensing pathways converge on ULK1, a key initiator of autophagy, and regulate ULK1 activity by phosphorylating serine residues that either inhibit or activate the enzyme [6]. Growth factors such as VEGF are known to trigger the Akt/mTOR pathway [69], which mediates the inhibitory phosphorylation of ULK1, suggesting that they slow down autophagy. In line with this, growth factor removal has been reported to activate autophagy [70]. However, as shown previously by our group and others, VEGF is also a potent stimulus for AMPK activation [53,55], which in turn may lead to ULK1 activation. Thus, the question arose of how the angiogenic factor VEGF, which is activating both AMPK and mTOR pathways, affects autophagy.

Here, we report for the first time that VEGF stimulates autophagy via an AMPK 1-dependent mechanism. Autophagy activation was verified by VEGF-induced phosphorylation of ULK1 and its substrate ATG14 at sites known to be involved in the initiation of autophagy (S556 and S29, respectively) [7–11,64], by phosphorylation of ATG16L1, a component of the LC3B lipidation complex [65,66] and by conjugation of LC3B, a marker of autophagosome formation. Furthermore, functional autophagy was confirmed by an increase in autophagic flux soon after VEGF stimulation and a decrease in p62 expression, which indicates its enhanced autophagic degradation in response to VEGF. ULK1 phosphorylation triggered by VEGF was prevented when AMPK 1 was downregulated by specific siRNA, while downregulation of AMPK 2 had no effect. These data underline the role of AMPK 1 in VEGF-induced initiation of autophagy. In line with this, VEGF did not stimulate LC3B conjugation in AMPK 1-depleted cells, although the upregulation of LC3B in cells in which AMPK isoforms were downregulated, hampers the interpretation of these data.

The autophagy induction in response to VEGF was transient. This may be related to mTOR having a counteracting role, which was stimulated by VEGF in a delayed manner. AMPK activity upon VEGF stimulation returned to basal levels at the same time as mTOR activity increased, which was monitored as phosphorylation of its target p70S6K [71]. In line with this, an inhibitory phosphorylation of ULK1 at S758 known to be mediated by mTOR occurred with a time delay upon VEGF stimulation and may be involved in terminating VEGF-initiated autophagy induction. In fact, mTOR-mediated phosphorylation of ULK1 at S757 (murine sequence) has previously been found to block autophagy by disrupting the interaction of AMPK with ULK1, thereby preventing ULK1 phosphorylation at S555 (murine sequence) by AMPK [7]. Thus, the sequential activation of AMPK and mTOR by VEGF may allow transient activation of autophagy, which is necessary for the induction of angiogenesis before growth-promoting pathways are initiated.

The present study reveals that induction of autophagy may be part of the pro-angiogenic processes induced by the VEGF/AMPK pathway. One function of enhanced autophagy in response to VEGF may

be to create permissive conditions for the initiation of angiogenic sprouting by proteolytic clearance of damaged proteins. It is known that only fully functional endothelial cells are able to start sprouting and forming new blood vessels, while endothelial dysfunction impairs the angiogenic response [72,73]. In line with previous studies [19,31,33–37] our data confirm that autophagy is important to maintain endothelial functions, although it did not affect cellular energy state and mitochondrial ROS production. Autophagy blockade results in inflammatory stress, reduced antioxidative defense, reduced growth, lower survival, and finally, reduced angiogenic capacity. Conversely, stimulation of angiogenesis by chemerin, the angiogenic factor AGGF1 or by hypoxia has previously been reported to promote angiogenesis [40,43,44]. Thus, induction of autophagy may stabilize cellular homeostasis, which is required for angiogenic responses.

In addition, VEGF-induced stimulation of autophagy is likely to affect angiogenesis beyond maintaining homeostasis. It may readily provide building blocks for macromolecule synthesis necessary for sprouting. Activation of autophagy may also be required to process angiogenic molecules and support angiogenic pathways. It has already been reported that autophagy leads to degradation of angiogenic molecules like VEGFR2 and gastrin-releasing peptide (GRP) and interferes with the Wnt pathway [74–76], although these processes affected angiogenesis in a negative manner. In our study, VEGF-induced signaling pathways such as activation of VEGFR2, PLC, ERK1/2, eNOS, p70S6K and AMPK as well as the expression of these proteins were comparable in autophagy-deficient and control cells, and thus probably not regulated by autophagy. Cells with inhibited autophagy were still able to proliferate and to respond to the survival signals of VEGF to the same extent as control cells, although they started from lower basal levels. However, VEGF-induced sprouting was almost completely blocked in autophagy-deficient cells, which points to the regulatory role of autophagy in the differentiation of endothelial cells to capillary-like sprouts.

The Notch pathway is essential for regulating endothelial differentiation during angiogenesis. It is activated in cells adjacent to VEGF-activated cells leading to the release of NICD, which in turn mediates reduced responsiveness of cells to VEGF [77]. Via this pathway, tip/stalk cell specification and junctional rearrangement during angiogenesis as well as the switch from cell proliferation to vessel maturation and stabilization are controlled. Notch signaling requires tight regulation since its inhibition results in uncontrolled tip cell formation and impaired maturation, while its overstimulation leads to the breakdown of VEGFR2-mediated signaling. In stem cells, autophagy has been reported to prevent hyperactivation of the Notch pathway by recruiting the Notch1 receptor to autophagosome-precursor vesicles and mediating its subsequent degradation [78]. Enhanced degradation of Notch1 or NICD via autophagy has also been observed in other cell types [72,73,79,80]. In contrast, our data suggest that autophagy supports Notch signaling. In our study, VEGF activated the Notch pathway in endothelial monolayers in a time-dependent manner, which may facilitate cell quiescence and junctional stabilization. Importantly, VEGF-induced NICD generation was significantly reduced in autophagy-depleted cells. Thus, the observed induction of autophagy by VEGF may be linked to NICD processing and ensure an appropriate adjustment of Notch signaling to the requirements of the complex angiogenic process. This will have to be substantiated in further experiments using three-dimensional models, which allow discrimination between tip and stalk cells, and monitoring the angiogenic process over time.

In summary, our data show that the growth factor VEGF is able to transiently stimulate autophagy via AMPK before it activates the mTOR pathway to induce cellular growth. Autophagy may have different functions in mediating angiogenesis including cellular homeostasis, the fast supply of macromolecules and processing of angiogenic molecules. One of these may be NICD, which regulates tip/stalk cell specification and vessel maturation. Our data highlight the importance of a well-balanced stimulation of AMPK and mTOR pathways in response to VEGF, which allows timely activation/inactivation of ULK1, a key player in autophagy initiation. As a consequence, temporary autophagy induction occurs and may promote angiogenic processes.

Author Contributions: K.S. was involved in conceptualization of the project, performed experiments, analyzed and validated the data, designed the figures and contributed to writing and editing the manuscript; N.K., S.G. participated in the experimental work and data analyses; A.S.M. contributed to the methodological aspects; R.H. initiated the project, conducted conceptual planning, acquired funding, supervised experimental work, discussed the data, and wrote and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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ICS XXIII, 23-rd International Conference on Interdisciplinary Studies Post Pandemic World: Towards a Digital Society” 2-3 April 2021, Brussels. Presentation Certificate Nderim Rizanaj for presenting the scientific contribution titled: Impact Analysis of the Subscale of Aggression in Depression and Anxiety, Among Adolescents Aged 16-18 Years

<https://euser.org/icis23>

Analizimi i ndikimit të nënshkallëve të agresionit në depresionin dhe ankthin, te adoleshentët e moshës 16-18 vjeçare.

Rast studimi: Shkolla e mesme e lartë “Luciano Motroni” Prizren

Dr.sc.Nderim Rizanaj

Doktor në Psikologji Pedagogji

Abstrakt

Agresioni është një fenomen i cili ngërthen në vete disa nënshkallë shumë domethënëse dhe që mund të manifestohen përmes sjelljeve të ashpra, armiqësisë me shokët/shoqet, zemërimit, reagimeve të pakontrolluara/jokonsistente si dhe agresionit verbal e indirekt. Këta faktor duhet shiquar me vëmendje dhe duhet përcjellur me hulumtime, me qëllim që t’u ipet hapësirë të rinjëve të përmirësojnë sjelljet dhe të arrijnë suksese të larta, në të kundërtën kjo ndikon në ankthin dhe depresionin e adoleshentëve.

Qëllimi i këtij studimi është të analizojmë efektet të cilat i shkaktojnë nënshkallët e agresionit si, zemërimi, armiqësia, agresioni verbal, agresioni indirekt, reagimi jokosistent në ankthin dhe depresionin e adoleshentëve të moshës 16-18 vjeçare. Në këtë hulumtim janë përfshi tri grupe të nxënësve, 16 vjeç, 17 vjeç dhe 18 vjeç, ku për secilin grup janë përfshi nga 50 adoleshentë, pra gjithsej mostra është 150 adoleshentë të moshës 16-18 vjeçare. Hulumtimi i takon tipit sasior dhe si studim rasti është zgjedhur shkolla e mesme e lartë “Luciano Motroni” në komunën e Prizrenit gjatë muajit Janar të vitit 2020.

Rezultatet e hulumtimit tregojnë se nënshkallët e depresionit si agresioni verbal ka ndikim në ankthin dhe depresionin ($\rho=.163^*$, $p \text{ value}=.047$), zemërimi ($\rho=.385^{**}$, $p \text{ value}=.000$), armiqësia ($\rho=.174^*$, $p \text{ value}=.033$), agresioni indirekt ($\rho=.261^{**}$, $p \text{ value}=.001$) dhe reagimi jokosistent ($\rho=.170^*$, $p \text{ value}=.037$) të cilat janë statistikisht signifikante në 1% dhe 5% të nivelit të besueshmërisë. *Krahasuar me një hulumtim të realizuar në vitin 2019, të titulluar “Association between anxiety and aggression in*

*adolescents: a cross-sectional study*¹, shohim se agresioni ka një ndikim të rëndësishëm në ankthin e të rinjëve. Pra, nënshkallët e agresionit paraqesin një lidhje të rëndësishme signifikante në ankthin dhe depresionin e adoleshentëve.

Në fund konkludojmë se nënshkallët e agresionit kanë ndikim statistikisht të rëndësishëm në ankth dhe depresionin e adoleshentëve tek të rinjtë e moshës 16-18 vjeçare, të komunës së Prizrenit. Ajo që rekomandojmë janë trajnimet dhe përkrahja e të rinjëve gjatë rrugëtimit të tyre të shkollimit të mesëm.

Fjalët kyçe: *Adoleshentët, agresioni, nënshkallët e agresionit, depresioni dhe ankthi*

¹ <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6471775/>

Hyrje

Termi agresivitet është përdorur shpesh për të përshkruar aktivitetet e sjelljes së të tjerëve, si dhe të vetvetes. Në psikologji, koncepti i agresionit i referohet një shumëllojshmërie të sjelljes që rezulton në dëmtim emocional, si dhe fizik ndaj një individi. Psikologët socialë e shpjegojnë agresionin si një veprim që është planifikuar të dëmtojë ata njerëz që nuk duan të dëmtohen. (Baron, R. A., & Richardson, D. R., 1994).

Agresioni paraqitet në çdo kohë dhe është shumë me rëndësi që të kuptohet se ajo manifestohet dhe zgjerohet përmes mendimeve, fantazive të njerëzve, dëshirave të tyre, mendimeve dhe sjelles së tyre të përditshme. (Semprini F, Giovanni AF, Sonino N., 2010). Gjatë adoleshencës, agresivitetit paraqitet shpesh si një përpjekje e vetëdijshme për të arritur qëllimet dhe e pavetëdijshme për të arritur një autonomi të tyre dhe këtë e shprehin shumë mirë autorë të shumtë rreth botës. (Semprini F, Giovanni AF, Sonino N., 2010) .

Agresiviteti në përgjithësi tek të rinjtë, shfaqet përmes rebelimeve të tyre ndaj shoqërisë dhe mjedisit në përgjithësi, duke filluar me probleme në shtëpi me mosbindje ndaj prindërve, agresione të ndryshme, duke filluar nga agresioni verbal, indirekt si dhe aspekte të tjera të rrezikshme. (Csorba J, Dinya E, Plener P., 2009). Aggressive behavior is often associated with lower verbal and cognitive abilities Sipas (LJ., 2008) agresioni paraqitet si një aftësi e zhvilluar me aftësi të ulët verbale dhe kognitive.

Sipas (Carrion, 2012) e përshkruan zemërimin si një gjendje emocionale që në intensitet mund të lëviz nga acarimi i butë në zemërim të tërbuar. Zemërimi përcillet me reaksione fizike duke përfshirë të rrahurat e shpejtuara të zemrës, tensionin e rritur të gjakut dhe nivelet e rritura të adrenalinës dhe noradrenalinës. Zemërimi është një reaksion (fiziologjik dhe psikik) i një kërcënimi të perceptuar ndaj vetes ose të njerëzve të tjerë me rëndësi, të tashmes, të kaluarës apo të së ardhmes. Kërcënimi mund të duket i vërtetë, i diskutuar ose i imagjinuar. Zemërimi shpesh është një përgjigje ndaj perceptimit të kërcënimit për shkak të konfliktit fizik, padrejtësisë, neglizhencës, poshtërimit apo tradhtisë midis grindjeve të tjera. Shprehja e zemërimit mund të bëhet përmes sjelljeve aktive ose pasive. Në rastin e emocionit "aktiv", personi i zemëruar "shprehet" me gojë ose fizikisht ndaj subjektit të synuar. Kur zemërimi është një emocion "pasiv" kjo karakterizohet nga heshtja, sjelljet pasive-agresive (armiçësi) dhe tension."

Sipas një raporti hulamtues të kryer nga (Kosovës, 2016) , mungesat dhe dhuna në përgjithësi, duke theksuar dhunën verbale dhe psikologjike të nxënësve ndaj nxënësve (dhuna indirekte/instrumentale), është më e theksuar se sa dhuna fizike (dhuna direkte) dhe karakterizohet me fyerje, tallje, përdorim të emrave fytes, si dhe përhapje thashethemesh dhe informacionesh personale. Gjithashtu prania e armëve të ftohta është shumë më e madhe. Nga të gjithë nxënësit e anketuar si pjesë e këtij hulumtimi, 16.26% janë shprehur se kanë qenë viktima të dhunës, kërcënimit dhe ngacmimit. Megjithëse ekzistojnë mjaft hulumtime që ofrojnë të dhëna se përdorimi i dhunës të formave të ndryshme në mes të nxënësve vazhdon të jetë e pranishme në mjediset edukative – arsimore në të gjitha shkolla e Kosovës, nuk ka pasur ndonjë hulumtim që flet për rolin e ruminimit në përgjithësi dhe në veçanti të ruminimit në zemërim, në paraqitje dhe zhvillim të dhunës të formave të ndryshme. Adoleshenca është një periudhë shumë kritike në të cilën të rinjtë apo adolehentët, pësojnë ndryshime sociale, emocionale dhe fizike, e të cilët në shumicën e rasteve mund të krijojnë përciptime negative dhe sjellje agresive, të cilat përcillen më pastaj me problem. (Peter RE, Crocker CMS, Kowalski KC, McDonough MH, Nanette K., 2006).

Kohët e fundit, hulamtuesit kanë filluar të hulumtojnë rolin e mundshëm të ruminimit, apo tendencën për të menduar për përvojat dhe ndjenjat që përcillen me ato përvoja në sjellje agresive. Është paksa e natyrshme që njerëzit të reflektojnë mbi situatat që përcillen me përvoja të dhimbshme, negative apo shqetësuese. Duke e bërë këtë individët përpiqen që të shohin situatat për të cilat ruminojmë nga një perspektivë e re dhe kështu ta zvogëlojmë shqetësimin që ndjenjë (Nolen-Hoeksema, S., Wisco, B.E., Lyubomirsky, S. , 2008) . Por ajo çfarë zakonisht ndodh është që në vend se të ndihemi të çliruar nga ky proces, ne i mendojmë situatat shqetësuese duke i përsëritur ato në kokën tonë dhe kështu përfundojmë edhe më të mërëzitur, zemëruar apo më të shqetësuar. Për shembull ne e përsërisim skenën e një bisede konfliktuoze me dikë duke e analizuar në detaje, duke shqyrtuar nëpër kokë versione të ndryshme të një konfrontimi me atë person, edhe pse në realitet ndoshta kurrë nuk do të ndodh ai konfrontim. (Csorba J, Dinya E, Plener P., 2009)

Megjithatë, konstrukti i ruminimit si i tillë është paksa i gjerë, ashtu që përfshin tendencën të fokusohet në shumë gjendje negative të disponimit e jo në një emocion në veçanti. Për këtë arsye disa hulamtues janë fokusuar në ruminimin në zemërim, tendencën për tu fokusuar në mendimet që lidhen me gjendjen emocionale gjatë një episodi zemërimi. Në përgjithësi, nëse zemërimi shihet si një emocion, ruminimi në zemërim mund të definohet si “të mendosh për këtë emocion” (Sukhodolsky, Golub and Cromwell, 2000)

Qëllimi i hulumtimit

Qëllimi i këtij studimi është të analizojmë efektet të cilat i shkaktojnë nënshkallët e agresionit si, zemërimi, armiqësia, agresioni verbal, agresioni indirekt, reagimi jo konsistent në ankthin dhe depresionin e adoleshentëve të moshës 16-18 vjeçare. Pra të zbulohen nëse variablat apo nënshkallët e agresionit kontribuojnë në zhvillimin e fenomenit të dhunës tek adoleshentët.

Pyetja hulumtuese

- 1) Nxënësit me agresion të lartë, gjegjësisht me agresion verbal, zemërimi, armiqësi, agresioni indirekt dhe jokonsistent, a do të shfaqin nivel të lartë të ankthit dhe depresionit ?

Hipoteza e hulumtimit:

H01. Nënshkallët e agresionit si agresioni verbal, armiqësia , zemërimi, agresioni indirekt dhe jokonsistent kanë ndikim negativ në rritjen e ankthit/depresionit tek të rinjtë e moshës 16-18 vjeç në shkollat e mesme të komunës së Prizrenit.

Metodologjia e hulumtimit

Metodologjia e hulumtimit i takon tipit sasior. Ky studim është kryer me adoleshentë apo nxënës të moshës 16-18 vjeçare. Si studim rasti është marrë shkolla e mesme e lartë "Luciano Motroni" në komunën e Prizrenit. Numri i pjesëmarrësve është 150 nxënës, 50 nxënës për çdo paralele, 50 nga klasa 10 apo mosha 16 vjeçare, 50 nxënës nga klasa 11 apo mosha 17 vjeçare dhe 50 nxënës të klasës 11 apo mosha 18 vjeçare.

Për të realizuar hulumtimin është përdorur pyetësi i standardizuar (BPAQ, 1992) i cili përbëhet nga pjesa demografike ku përfshihen gjinia, klasa, suksesi shkollor dhe kualifikimi i prindërve, në pjesën e dytë përfshihen pyetjet për nënshkallët e agresionit i cili është klasifikuar sipas shkallëve të Likertit (1 aspak sikurse unë .. 5 tërësisht sikurse unë) dhe pjesa e tretë përfshinë pyetjet për ankthin e depresionin i kategorizuar sipas shkallëve të Likertit (1 asnjëherë 5 gjatë gjithë kohës

). Analizat janë realizuar përmes programit IBM SPSS (version 25), kurse analiza e korelacionit/spearman korelacioni është përdorur për të vërtetuar hipotezën e hulumtimit.

Rezultatet empirike të analizave

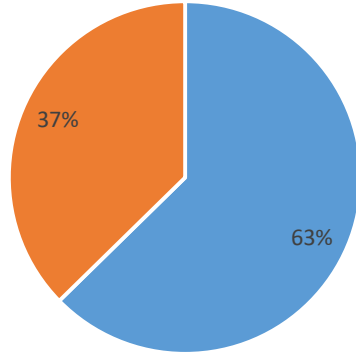
Analizat descriptive

Rezultatet tregojnë se në hulumtim kanë marrë pjesë gjithsej 150 nxënës të moshës 16-18 vjeçare, kurse në të tri kategoritë janë nga 50 nxënës. Sa i përket suksesit të tyre shkollor shohim se 4.7% kanë sukses të dobët, 7.3% kanë sukses të mjaftueshëm, 28.7% kanë sukses të mirë, 26.7% kanë sukses shumë mirë dhe 32.7% kanë sukses të shkëlqyeshëm.

Gjinia	N	%
Femër	94	62.7%
Mashkull	56	37.3%
Mosha	N	%
16 vjeç	50	33.3%
17 vjeç	50	33.3%
18 vjeç	50	33.3%
Suksesi	N	%
Dobët	7	4.7%
Mjaftueshëm	11	7.3%
Mirë	43	28.7%
Shumë mirë	40	26.7%
Shkëlqyeshëm	49	32.7%

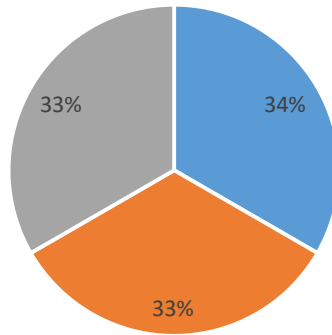
Mosha

■ Female ■ Male



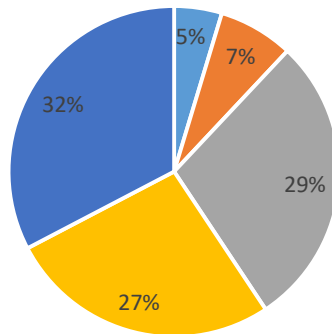
Age

■ 16 ■ 17 ■ 18



School results

■ Poorly ■ Enough ■ Good ■ Very good ■ Excellent

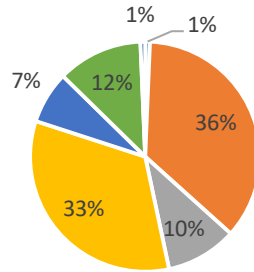


Sa i përket edukimit të prindërve shohim se edukimi i babait është më i lartë se ai i nënës sa i përket edukimit të lartë, kurse niveli ekonomik familjar tregon se shumica dërmuese e familjeve kanë nivel përfarësisht të njëjtë ekonomik.

Edukimi i nënës	N	%
Nuk ka përfunduar shkollën fillore	1	0.7%
Ka përfunduar shkollën fillore	54	36.0%
Nuk e ka përfunduar shkollën e mesme	15	10.0%
Ka përfunduar shkollën e mesme	50	33.3%
Ka filluar studimet por nuk i ka mbaruar	11	7.3%
Ka mbaruar studime universitare	18	12.0%
Prindi nuk është i gjallë	1	0.7%
Edukimi i babait	N	%
Nuk ka përfunduar shkollën fillore	1	0.7
Ka përfunduar shkollën fillore	16	10.7
Nuk e ka përfunduar shkollën e mesme	9	6.0
Ka përfunduar shkollën e mesme	66	44.0
Ka filluar studimet por nuk i ka mbaruar	18	12.0
Ka mbaruar studime universitare	40	26.7
Statusi ekonomik familjar	N	%
Ka të ardhura sikurse shumica e familjeve të tjera	108	72.0%
Ka pak më shumë të ardhura se familjet e tjera	35	23.3%
Ka shumë më shumë të ardhura se shumica e familjeve të tjera	7	4.7%

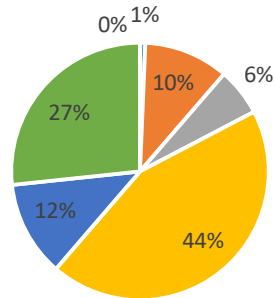
Mother education

- Has not finished elementary school
- She has finished primary school
- Has not finished high school
- Graduated from high school
- She started his studies but did not finish
- Graduated from university
- The parent is not alive



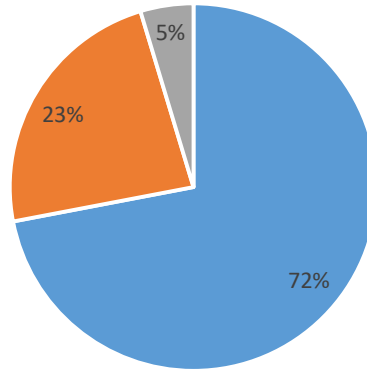
Father education

- Has not finished elementary school
- He has finished primary school
- Has not finished high school
- Graduated from high school
- He started his studies but did not finish
- Graduated from university
- The parent is not alive



Household economic status

- It has an income like most other families
- There is a little more income than other families
- There is a lot more income than most other families



Vërtetimi i hipotezës

Hipoteza 1

Nënshkallët e agresionit si agresioni verbal, armiqësia dhe zemërimi kanë ndikim në rritjen e ankthit/depresionit tek të rinjtë e moshës 15-18 vjeç.

Rezultatet tregojnë se në mes të nënshkallëve të agresionit dhe ankthit e depresionit është gjetur korelacion statistikisht i rëndësishëm, që e konfirmon se këto fenomene të agresionit janë shumë

<i>Nënshkallët e agresionit</i>	<i>Nonparametric</i>	<i>Agresion_verbal</i>	<i>Zemërimi</i>	<i>Armiqesi</i>	<i>Reagim_jo Konsistent</i>	<i>Agresioni indirekt</i>
<i>Ankthi & Depresioni</i>	<i>Spearman Correlations</i>	<i>.165*</i>	<i>.386**</i>	<i>.175*</i>	<i>.170*</i>	<i>.261**</i>
	<i>P value</i>	<i>.044</i>	<i>.000</i>	<i>.033</i>	<i>.037</i>	<i>.001</i>

me ndikim në jetën e adoleshentëve.

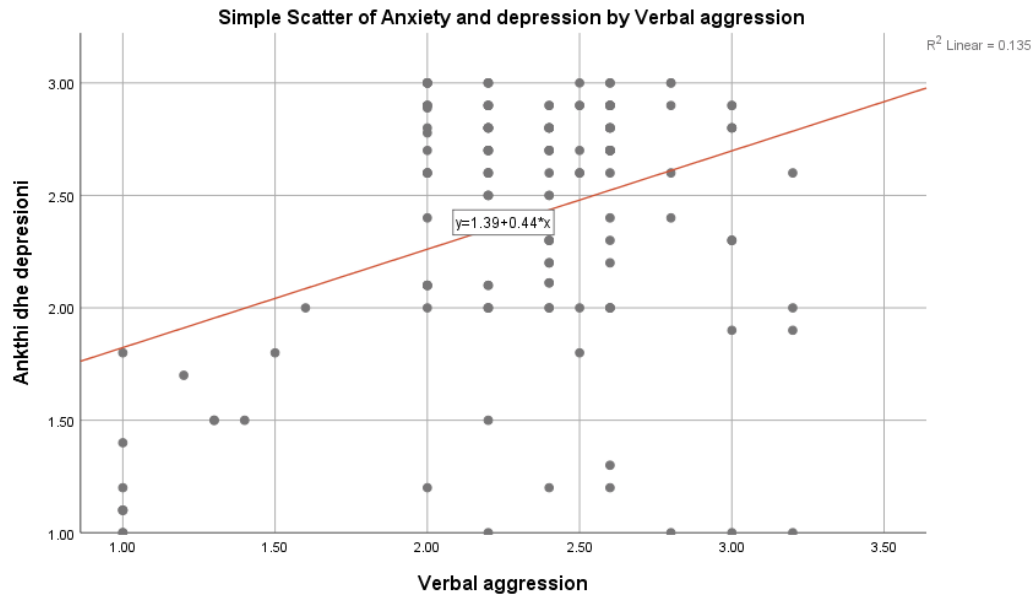
** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

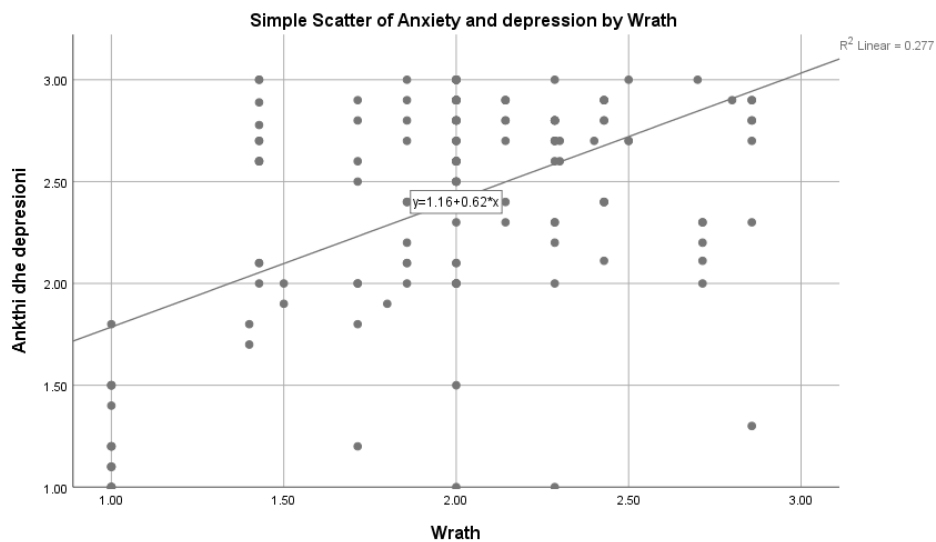
Saktësisht shohim se agresioni verbal ka një korelacion të lartë pozitiv ($\rho=.165^{**}$, $p \text{ value}=.044$) e cila është signifikante në 5% të nivelit të besueshmërisë, pra agresioni verbal si nënshkallë e agresionit ka ndikim të rëndësishëm signifikantë që i bie se ndikon në rritjen e ankthit e depresionit tek të rinjtë e moshës 16-18 vjeçare. Zemërimi si nënshkallë e agresionit ka një korelacion të lartë pozitiv ($\rho=.386^{**}$, $p \text{ value}=.000$) e cila është signifikante në 1% të nivelit të besueshmërisë, pra mund të themi se zemërimi ndikon negativisht në rritjen e ankthit dhe depresionit. Armiqësia ka një korelacion gjithashtu pozitiv ($\rho=.176^{*}$, $p \text{ value}=.033$) e cila është signifikante në 5% të nivelit të besueshmërisë dhe themi se sa më e lartë armiqësia tek adoleshentët e moshës 16-18 vjeçare, aq më e lartë do të jetë ankthi dhe depresioni i tyre. Reagimi jo-konsistent gjithashtu ka korelacion pozitiv ($\rho=.170^{*}$, $p \text{ value}=.037$) e cila është signifikante në 5% të nivelit të besueshmërisë dhe themi se sa më tepër të kenë një reagim të tillë jo-konsistent aq më shumë do

të kenë ankth e depresion. Në fund kemi agresionin indirekt që paraqet një korelacion pozitiv ($\rho = .261^{**}$, $p \text{ value} = .001$) e cila është signifikante në 1% të nivelit të besueshmërisë.

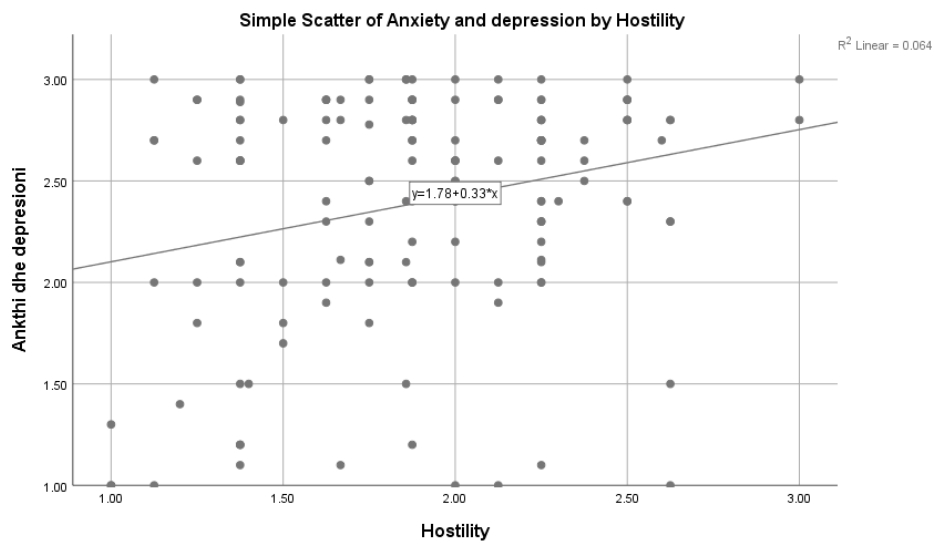
Pra, nënshkallët e agresionit si agresioni verbal, armiqtësia, zemërimi, reagimi jo-kosistent dhe agresioni indirekt, kanë ndikim në rritjen e ankthit/depresionit tek të rinjtë e moshës 16-18 vjeç.



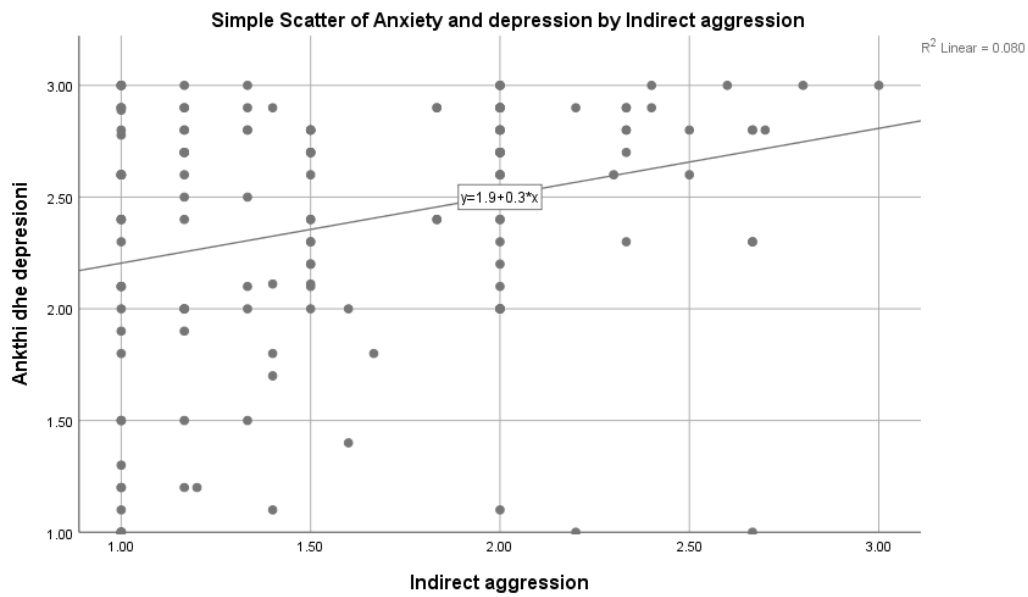
Korelacioni në mes të agresionit verbal dhe ankthit e depresionit



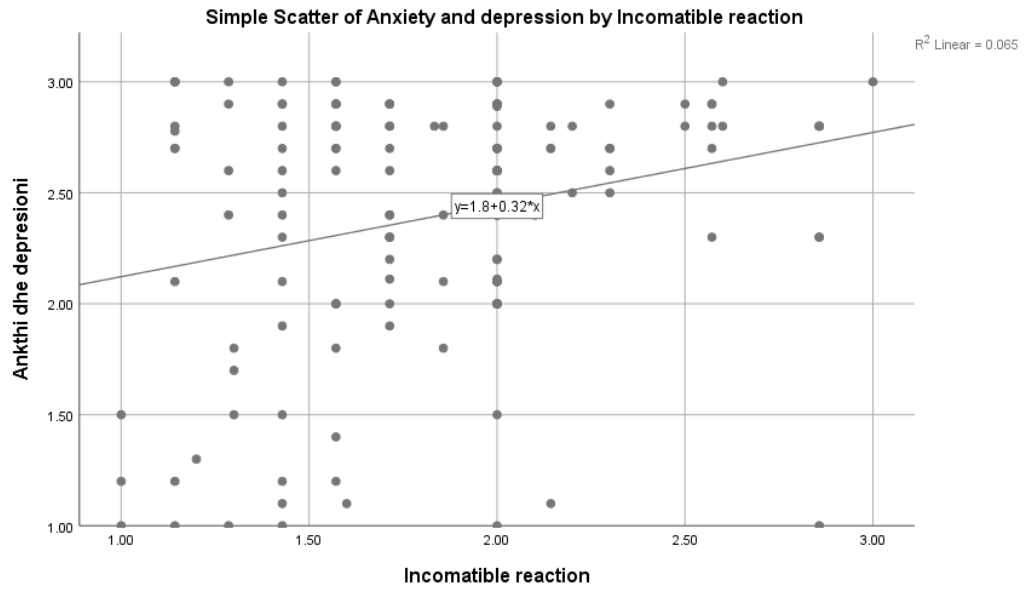
Korelacioni në mes të zemërimit dhe ankthit e depresionit



Korelacioni në mes të armiqësisë dhe ankthit e depresionit



Korelacioni në mes të agresionit indirekt dhe ankthit e depresionit



Korelacioni në mes të reagimit jo-konsistent dhe ankthit e depresionit

Përfundimet dhe rekomandimet

Rezultatet tregojnë se në hulumtim janë përfshi gjithsej 150 nxënës të moshës 16-18 vjeçare, me një nivel të ulët të suksesit shkollor, me dukim të lartë të babait dhe edukim të ulët të nënës, kurse nivel relativisht pozitiv ekonomik. Nga analiza e korelacionit është vërtetuar se nënshkallët agresionit si agresioni verbal, armiqësia, zemërimi, reagimi jo-kosistent dhe agresioni indirekt, kanë ndikim në rritjen e ankthit/depresionit tek të rinjtë e moshës 16-18 vjeç. Një hulumtim tjetër I cili është realizuar nga Sherrill et.al., (2016), individët të cilët janë veçuar me tipar të lartë të agresivitetit kanë shfaqur rritje të sjelljeve agresive, në situatat kur ka pasur provokime.

Ajo çka mund të rekomandojë për të ardhmen është se hulumtimi do të ishte mirë të zgjerohej në një territor më të gjerë në Kosovë, të përfshihen moshë të ndryshme të nxënësve/adoleshentëve, kurse duhet patjetër që çdo shkollë të ketë psikologun e shkollës i cili do të ndihmonte nxënësit e shkollave të mesme të konsullohen për problemet e tyre dhe të jenë në gjendje t'i paraprijnë problemeve. Kjo do të ishte shumë e rëndësishme pasi që shohim se nënshkallët e agresionit janë faktorë shumë të rrezikshëm që ndikojnë në ankthin dhe depresionin e nxënësve, kurse kjo paraqitet me një nivel jo të mirë të edukimi të tyre shkollor.

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Ndikimi i përdorimit të drogave/alkoolit në qëndrueshmërinë e gjendjes kognitive dhe impulsivitetit në tentim vrasje

Nderim Rizanaj

Abstrakt

Ndikimi i abuzimit me droga dhe alkool, paraqet një prej sfidave më të mëdha të shoqërisë sonë, si në aspektin global ashtu edhe në vendin tonë. Efeket negative të abuzimit të drogave dhe llojeve të ndryshme të alkoolit, ndikojnë në përkeqësimin e gjendjes psikike dhe mendore të përdoruesve, pa marrë parasysh moshën dhe gjininë e tyre. Efektet që kanë këto në qëndrueshmërinë e gjendjes kognitive dhe impulsivitetin në tentim vrasje, e kam paraqitur përmes një anketimi, i cili është realizuar me një mostër të rastësishme prej 284 respondentësh, ku mosha mesatare e pjesëmarrësve është 26 vjeç, kurse ajo minimala është 15 vjeç dhe maksimalja 57 vjeç. Rezultatet tregojnë personat që janë përdorues të drogave dhe alkoolit, kanë gjendje të paqëndrueshme dhe mundësi për tentim vrasje, në krahasim me personat që nuk janë përdorues të drogave dhe alkoolit. Kjo tregon shumë në kuptimin e zhvillimit të jetës së shëndoshë dhe për këtë duhet pasur parasysh trajtime dhe përkrahje masive ndaj shoqërisë, sidomos ato programet edukative dhe këshilluese.

Fjalët kyçe: Droga, Alkooli, Gjendja kognitive, Impulsiviteti, Tentim vrasje

Ndikimi i gjendjes shëndetësore dhe faktorët demografikë në qëndrueshmërinë e gjendjes kognitive dhe impulsivitetit në tentim vrasje

Nderim Rizanaj

Abstract

Introduction: Përmes këtij hulumtimi kam paraqitur ndikimin e gjendjes shëndetësore dhe faktorët e demogrike si gjinia, mosha dhe edukimi familjar në qëndrueshmërinë e gjendjes kognitive dhe impulsivitetin për tentim vrasje. Qëllimi i hulumtimit është krahasimi i faktorëve demografike, konkretisht dallimi në mes meshkujve dhe femrave, moshës dhe edukimit familjar në raport me gjendjen e tyre kognitive dhe impulsivitetin për vetëvrasje. Gjithashtu krahasimin në mes të personave të cilët kanë probleme shëndetësore kronike në raport me gjendjen kognitive dhe impulsivitetin në tentim vrasje.

Methods: Hulumtimi I takon tipit sasor, ndërsa u zhvillua nëpërmjet një pyetësoni online, ku morën pjesë gjithsej 284 respondentë të moshave të ndryshme në Kosovë. Pyetësoni u dërgua përmes e-mail adresave dhe formave online në rrjetet sociale. Pyetësoni është i ndërtuar përmes pyetjeve të mbyllura, ndërsa është i klasifikuar në pjesën demografike, matjen e gjendjes shëndetësore dhe në pjesën e tretë është matja e qëndrueshmërisë kognitive dhe impulsivitetit për tentim vrasje. Rezultatet e hulumtimit janë analizuar përmes programit SPSS (v.25), kurse testet statistikore të cilat vërtetuan hipotezat janë Independent Sample t-test, Anva one sample dhe korrelacioni Spearman.

Results: Rezultatet e hulumtimit tregojnë se nuk ekzistojnë dallime sinjifikante në mes meshkujve dhe femrave sa i përket impulsivitetit për tentim vrasje, por ka dallime në mes të tyre në raport me gjendjen e tyre kognitive, ku meshkujt paraqesin një gjendje më pak të favorshme, në krahasim me femrat., kurse sa i përket nivelit të edukimit familjar ku është përfshi edukimi I nënës dhe babait, shohim se nuk kemi dallime sinjifikante në mes

nivelit të edukimit familjar dhe gjendjes kongnitive dhe impulsivitetin për tentim vrasje. Sa i përket faktorit të moshës shohim se moshë ka ndikim negativ në gjendjen kongnitive, pra personat sa më të vjetër kanë një gjendje jo të favorshme kongnitive. Në analizën e fundit shohim se respondentët të cilët kanë probleme mjekësore kronike kanë ndikim sinjifikant në gjendjen e tyre kongnitive dhe impulsivitetin për tentim vrasje.

Discussion and conclusion: Në bazë të rezultateve të hulumtimit vërtetojmë se faktorët demografikë si gjinia dhe moshë kanë ndikim në gjendjen kongnitive, kurse faktori i cili është më shqetësues është gjendja e mjekësore, ku respondentët të cilët rezultojnë me sëmundje kronike kanë nivel më të keq të gjendjes kongnitive dhe impulsivitet për tentim vrasje. Rekomandimi im është që të ofrohen trajnime dhe trajtime speciale për grupe të ndryshme, në mënyrë që të evitohen tentim vrasje.

Fjalët kyçe: Gjendja shëndetësore, faktorët demografikë, gjendja kongnitive, impulsiviteti, tentim vrasje

Gjinia	N	%
Mashkull	96	33.8
Femër	188	66.2
Shkollimi i fundit	N	%
Shkollë fillore	6	2.1
Shkollë e mesme	58	20.4
Bachelor	152	53.5
Master	52	18.3
PhD	16	5.6
Vendbanimi	N	%
Urban	206	72.5
Rural	78	27.5
Kualifikimi i nënës	N	%
Shkollë fillore	116	40.8
Shkollë e mesme	92	32.4
Bachelor	62	21.8
Master	6	2.1
PhD	8	2.8
Kualifikimi i babait	N	%
Shkollë fillore	42	14.8
Shkollë e mesme	116	40.8
Bachelor	86	30.3
Master	32	11.3
PhD	8	2.8

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Mosha	284	15	57	26.65	9.140
Valid N (listwise)	284				

Sa herë në jetën tuaj jeni shtruar në spital për probleme mjekësore?	N	%
Asnjëherë	102	35.9
Rrallë	114	40.1
Ndonjëherë	54	19.0
Shpesh	10	3.5
Sa kohë më parë ishte shtrimi i fundit në spital për një problem fizik?	N	%
Para disa dite	2	0.7
Para disa javësh	6	2.1
Para disa muajsh	38	13.4
Para disa vitesh	238	83.8
A keni ndonjë problem mjekësor kronik që vazhdon të ndërhyjë në jetën tuaj?	N	%
Po	48	16.9
Jo	236	83.1
A jeni duke marrë ndonjë ilaç të përshkruar rregullisht për një problem fizik?	N	%
Po	38	13.4
Jo	246	86.6
A keni përjetuar ndonjëherë një dëmtim të rëndë në kokë?	N	%
Po	18	6.3
Jo	266	93.7

Dallimi në mes meshkujve dhe femrave

Group Statistics

	Gjinia	N	Mean	Std. Deviation	Std. Error Mean
Attidue - Scale	Mashkull	96	3.0095	0.53879	0.05499
	Femër	188	2.8153	0.60645	0.04423
Reaktive-Proaktivë pyetjet!	Mashkull	96	0.4729	0.28836	0.02943
	Femër	188	0.4388	0.23505	0.01714

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2- tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Attidue - Scale	Equal variances assumed	0.426	0.515	2.648	282	0.009	0.19419	0.07333	0.04985	0.33852
	Equal variances not assumed			2.752	212.497	0.006	0.19419	0.07057	0.05508	0.33330
Reaktive- Proaktivë pyetjet!	Equal variances assumed	4.838	0.029	1.069	282	0.286	0.03409	0.03190	- 0.09687	0.02870
	Equal variances not assumed			1.001	160.984	0.318	0.03409	0.03406	- 0.10135	0.03317

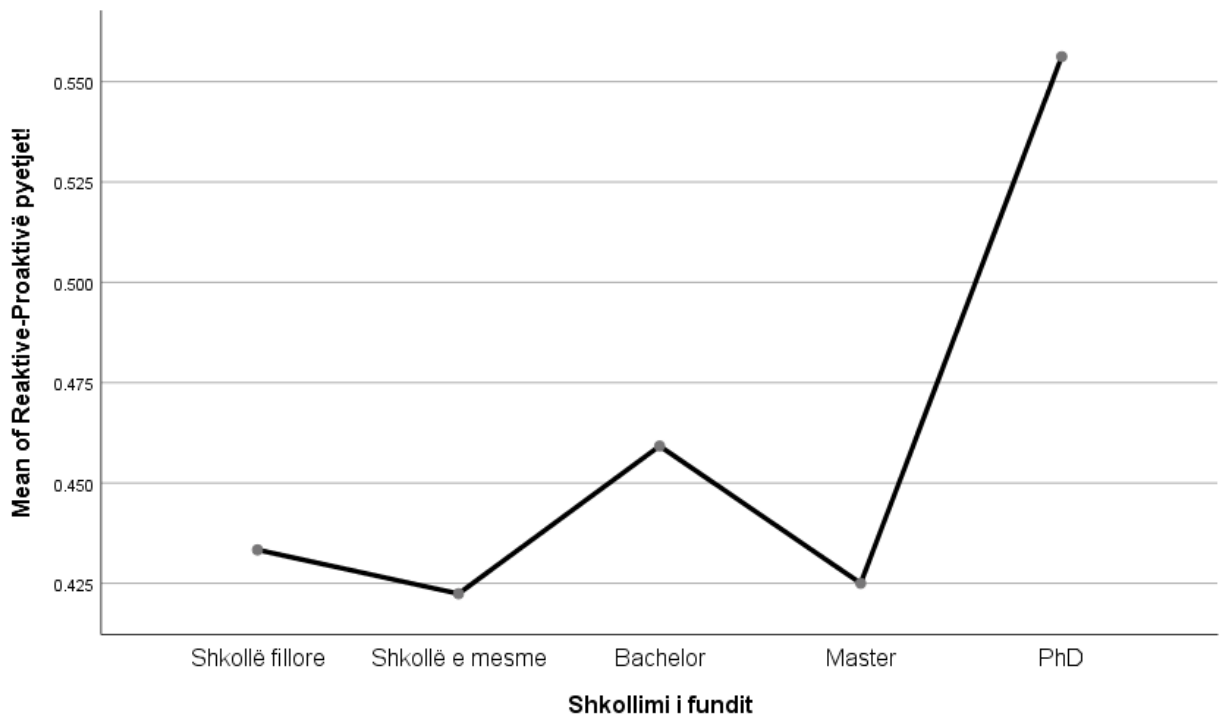
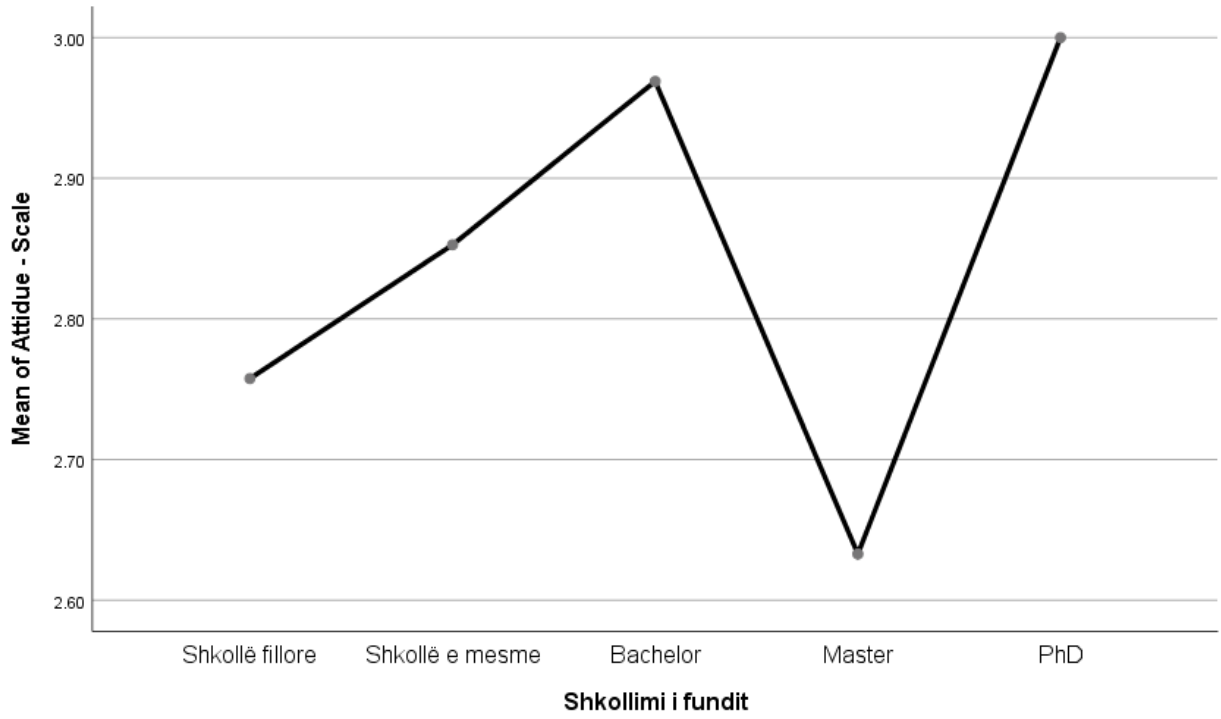
Dallimi ne mes te nivelit te edukimit

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Attidue - Scale	Shkollë fillore	6	2.7576	0.30784	0.12568	2.4345	3.0806	2.36	3.00
	Shkollë e mesme	58	2.8527	0.55254	0.07255	2.7074	2.9979	2.00	4.00
	Bachelor	152	2.9689	0.63443	0.05146	2.8672	3.0706	1.36	4.73
	Master	52	2.6329	0.48606	0.06740	2.4975	2.7682	1.64	3.73
	PhD	16	3.0000	0.46945	0.11736	2.7498	3.2502	2.27	3.82
	Total	284	2.8809	0.59071	0.03505	2.8119	2.9499	1.36	4.73
Reaktive-Proaktivë pyetjet!	Shkollë fillore	6	0.4333	0.18619	0.07601	0.2379	0.6287	0.20	0.60
	Shkollë e mesme	58	0.4224	0.22147	0.02908	0.3642	0.4806	0.00	0.85
	Bachelor	152	0.4592	0.25237	0.02047	0.4188	0.4997	0.00	1.30
	Master	52	0.4250	0.26725	0.03706	0.3506	0.4994	0.00	1.05
	PhD	16	0.5562	0.34683	0.08671	0.3714	0.7411	0.20	1.10
	Total	284	0.4504	0.25433	0.01509	0.4206	0.4801	0.00	1.30

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Attidue - Scale	Between Groups	4.741	4	1.185	3.517	0.008
	Within Groups	94.009	279	0.337		
	Total	98.750	283			
Reaktive-Proaktivë pyetjet!	Between Groups	0.272	4	0.068	1.051	0.381
	Within Groups	18.033	279	0.065		
	Total	18.305	283			



Faktori . moshe

Correlations

		Mosha	Attidue - Scale	Reaktive-Proaktivë pyetjet!
Mosha	Pearson Correlation	1	-0.168**	0.083
	Sig. (2-tailed)		0.005	0.162
	N		284	284
Attidue - Scale	Pearson Correlation		1	0.443**
	Sig. (2-tailed)			0.000
	N			284
Reaktive-Proaktivë pyetjet!	Pearson Correlation			1
	Sig. (2-tailed)			
	N			

** . Correlation is significant at the 0.01 level (2-tailed).

Problemet kronike . ndikimi

Group Statistics

		A keni ndonjë problem mjekësor kronik që vazhdon të ndërhyjë në jetën tuaj?			
		N	Mean	Std. Deviation	Std. Error Mean
Attidue - Scale	Po	48	3.1174	0.66168	0.09551
	Jo	236	2.8328	0.56471	0.03676
Reaktive-Proaktivë pyetjet!	Po	48	0.5417	0.20868	0.03012
	Jo	236	0.4318	0.25910	0.01687

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Attidue - Scale	Equal variances assumed	2.413	0.121	3.088	282	0.002	0.28460	0.09215	0.10321	0.46600
	Equal variances not assumed			2.781	61.686	0.007	0.28460	0.10234	0.08002	0.48919
Reaktive-Proaktivë pyetjet!	Equal variances assumed	3.913	0.049	2.761	282	0.006	0.10989	0.03981	0.03153	0.18824
	Equal variances not assumed			3.183	79.532	0.002	0.10989	0.03452	0.04118	0.17859

University Maribor Faculty of Health Sciences. Nderim Rizanaj receives, Certificate for active participation at the Online International Scientific Conference entitled LEARNING to Live and Work Together, Maribor, 10-th June 2021

<https://www.fzv.um.si/en/online-international-scientific-conference-%E2%80%9Clearning-live-and-work-together%E2%80%9D>

Ndikimi i përdorimit të drogave/alkoolit në qëndrueshmërinë e gjendjes kognitive dhe impulsivitetit në tentim vrasje

Nderim Rizanaj

Abstrakt

Ndikimi i abuzimit me droga dhe alkool, paraqet një prej sfidave më të mëdha të shoqërisë sonë, si në aspektin global ashtu edhe në vendin tonë. Efeket negative të abuzimit të drogave dhe llojeve të ndryshme të alkoolit, ndikojnë në përkeqësimin e gjenjes psikike dhe mendore të përdoruesve, pa marrë parasysh moshën dhe gjininë e tyre. Efektet që kanë këto në qëndrueshmërinë e gjendjes kognitive dhe impulsivitetin në tentim vrasje, e kam paraqitur përmes një anketimi, i cili është realizuar me një mostër të rastësishme prej 284 respondentësh, ku mosha mesatare e pjesëmarrësve është 26 vjeç, kurse ajo minimala është 15 vjeç dhe maksimalja 57 vjeç. Rezultatet tregojnë personat që janë përdorues të drogave dhe alkoolit, kanë gjendje të paqëndrueshme dhe mundësi për tentim vrasje, në krahasim me personat që nuk janë përdorues të drogave dhe alkoolit. Kjo tregon shumë në kuptimin e zhvillimit të jetës së shëndoshë dhe për këtë duhet pasur parasysh trajtime dhe përkrahje masive ndaj shoqërisë, sidomos ato programet edukative dhe këshilluese.

Fjalët kyçe: Droga, Alkooli, Gjendja kognitive, Impulsiviteti, Tentim vrasje

Ndikimi i gjendjes shëndetësore dhe faktorët demografikë në qëndrueshmërinë e gjendjes kognitive dhe impulsivitetit në tentim vrasje

Nderim Rizanaj

Abstract

Introduction: Përmes këtij hulumtimi kam paraqitur ndikimin e gjendjes shëndetësore dhe faktorët e demogrike si gjinia, moshë dhe edukimi familjar në qëndrueshmërinë e gjendjes kognitive dhe impulsivitetin për tentim vrasje. Qëllimi i hulumtimit është krahasimi i faktorëve demografike, konkretisht dallimi në mes meshkujve dhe femrave, moshës dhe edukimit familjar në raport me gjendjen e tyre kognitive dhe impulsivitetin për vetëvrasje. Gjithashtu krahasimin në mes të personave të cilët kanë probleme shëndetësore kronike në raport me gjendjen kognitive dhe impulsivitetin në tentim vrasje.

Methods: Hulumtimi i takon tipit sasor, ndërsa u zhvillua nëpërmjet një pyetësi online, ku morën pjesë gjithsej 284 respondentë të moshave të ndryshme në Kosovë. Pyetësi u dërgua përmes e-mail adresave dhe formave online në rrjetet sociale. Pyetësi është i ndërtuar përmes pyetjeve të mbyllura, ndërsa është i klasifikuar në pjesën demografike, matjen e gjendjes shëndetësore dhe në pjesën e tretë është matja e qëndrueshmërisë kognitive dhe impulsivitetit për tentim vrasje. Rezultatet e hulumtimit janë analizuar përmes programit SPSS (v.25), kurse testet statistikore të cilat vërtetuan hipotezat janë Independent Sample t-test, Anva one sample dhe korrelacioni Spearman.

Results: Rezultatet e hulumtimit tregojnë se nuk ekzistojnë dallime sinjifikante në mes meshkujve dhe femrave sa i përket impulsivitetit për tentim vrasje, por ka dallime në mes të tyre në raport me gjendjen e tyre kognitive, ku meshkujt paraqesin një gjendje më pak të favorshme, në krahasim me femrat., kurse sa i përket nivelit të edukimit familjar ku është përfshi edukimi i nënës dhe babait, shohim se nuk kemi dallime sinjifikante në mes nivelit të edukimit familjar dhe gjendjes kongnitive dhe impulsivitetin për tentim vrasje. Sa

i përket faktorit të moshës shohim se moshja ka ndikim negativ në gjendjen kognitive, pra personat sa më të vjetër kanë një gjendje jo të favorshme kognitive. Në analizën e fundit shohim se respondentët të cilët kanë probleme mjekësore kronike kanë ndikim sinjifikant në gjendjen e tyre kognitive dhe impulsivitetin për tentim vrasje.

Discussion and conclusion: Në bazë të rezultateve të hulumtimit vërtetojmë se faktorët demografikë si gjinia dhe moshja kanë ndikim në gjendjen kognitive, kurse faktori i cili është më shqetësues është gjendja e mjekësore, ku respondentët të cilët rezultojnë me sëmundje kronike kanë nivel më të keq të gjendjes kognitive dhe impulsivitet për tentim vrasje. Rekomandimi im është që të ofrohen trajnime dhe trajtime speciale për grupe të ndryshme, në mënyrë që të evitohen tentim vrasje.

Fjalët kyçe: Gjendja shëndetësore, faktorët demografikë, gjendja kognitive, impulsiviteti, tentim vrasje

Gjinia	N	%
Mashkull	96	33.8
Femër	188	66.2
Shkollimi i fundit	N	%
Shkollë fillore	6	2.1
Shkollë e mesme	58	20.4
Bachelor	152	53.5
Master	52	18.3
PhD	16	5.6
Vendbanimi	N	%
Urban	206	72.5
Rural	78	27.5
Kualifikimi i nënës	N	%
Shkollë fillore	116	40.8
Shkollë e mesme	92	32.4
Bachelor	62	21.8
Master	6	2.1
PhD	8	2.8
Kualifikimi i babait	N	%
Shkollë fillore	42	14.8
Shkollë e mesme	116	40.8
Bachelor	86	30.3
Master	32	11.3
PhD	8	2.8

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Mosha	284	15	57	26.65	9.140
Valid N (listwise)	284				

Sa herë në jetën tuaj jeni shtruar në spital për probleme mjekësore?	N	%
Asnjëherë	102	35.9
Rrallë	114	40.1
Ndonjëherë	54	19.0
Shpesh	10	3.5
Sa kohë më parë ishte shtrimi i fundit në spital për një problem fizik?	N	%
Para disa dite	2	0.7
Para disa javësh	6	2.1
Para disa muajsh	38	13.4
Para disa vitesh	238	83.8
A keni ndonjë problem mjekësor kronik që vazhdon të ndërhyjë në jetën tuaj?	N	%
Po	48	16.9
Jo	236	83.1
A jeni duke marrë ndonjë ilaç të përshkruar rregullisht për një problem fizik?	N	%
Po	38	13.4
Jo	246	86.6
A keni përjetuar ndonjëherë një dëmtim të rëndë në kokë?	N	%
Po	18	6.3
Jo	266	93.7

Dallimi në mes meshkujve dhe femrave

Group Statistics

	Gjinia	N	Mean	Std. Deviation	Std. Error Mean
Attidue - Scale	Mashkull	96	3.0095	0.53879	0.05499
	Femër	188	2.8153	0.60645	0.04423
Reaktive-Proaktivë pyetjet!	Mashkull	96	0.4729	0.28836	0.02943
	Femër	188	0.4388	0.23505	0.01714

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2- tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Attidue - Scale	Equal variances assumed	0.426	0.515	2.648	282	0.009	0.19419	0.07333	0.04985	0.33852
	Equal variances not assumed			2.752	212.497	0.006	0.19419	0.07057	0.05508	0.33330
Reaktive- Proaktivë pyetjet!	Equal variances assumed	4.838	0.029	1.069	282	0.286	0.03409	0.03190	- 0.09687	0.02870
	Equal variances not assumed			1.001	160.984	0.318	0.03409	0.03406	- 0.10135	0.03317

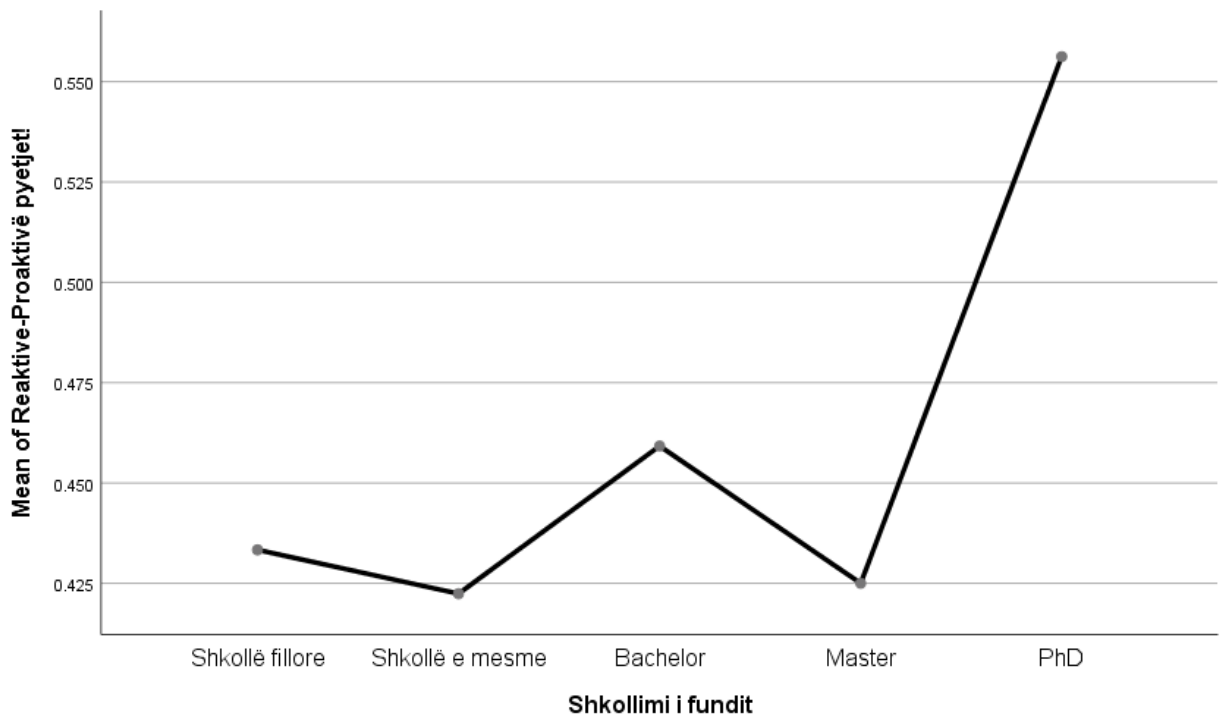
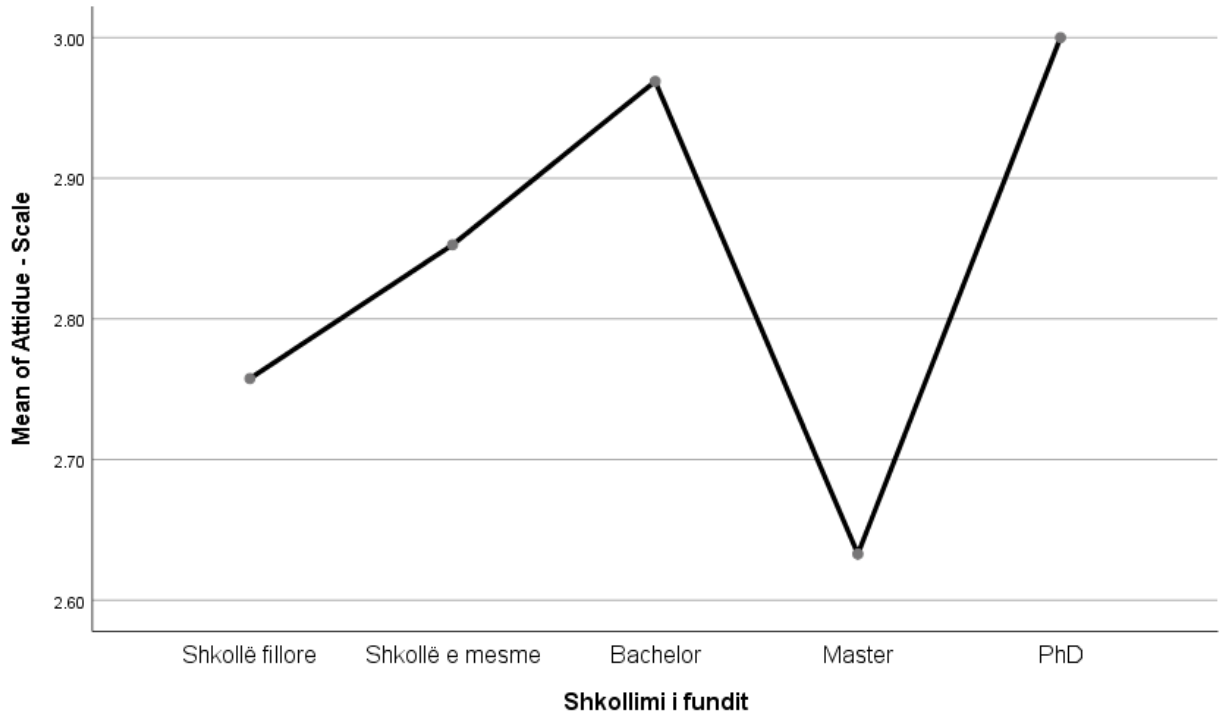
Dallimi ne mes te nivelit te edukimit

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Attidue - Scale	Shkollë fillore	6	2.7576	0.30784	0.12568	2.4345	3.0806	2.36	3.00
	Shkollë e mesme	58	2.8527	0.55254	0.07255	2.7074	2.9979	2.00	4.00
	Bachelor	152	2.9689	0.63443	0.05146	2.8672	3.0706	1.36	4.73
	Master	52	2.6329	0.48606	0.06740	2.4975	2.7682	1.64	3.73
	PhD	16	3.0000	0.46945	0.11736	2.7498	3.2502	2.27	3.82
	Total	284	2.8809	0.59071	0.03505	2.8119	2.9499	1.36	4.73
Reaktive-Proaktivë pyetjet!	Shkollë fillore	6	0.4333	0.18619	0.07601	0.2379	0.6287	0.20	0.60
	Shkollë e mesme	58	0.4224	0.22147	0.02908	0.3642	0.4806	0.00	0.85
	Bachelor	152	0.4592	0.25237	0.02047	0.4188	0.4997	0.00	1.30
	Master	52	0.4250	0.26725	0.03706	0.3506	0.4994	0.00	1.05
	PhD	16	0.5562	0.34683	0.08671	0.3714	0.7411	0.20	1.10
	Total	284	0.4504	0.25433	0.01509	0.4206	0.4801	0.00	1.30

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Attidue - Scale	Between Groups	4.741	4	1.185	3.517	0.008
	Within Groups	94.009	279	0.337		
	Total	98.750	283			
Reaktive-Proaktivë pyetjet!	Between Groups	0.272	4	0.068	1.051	0.381
	Within Groups	18.033	279	0.065		
	Total	18.305	283			



Faktori . moshe

Correlations

		Mosha	Attidue - Scale	Reaktive-Proaktivë pyetjet!
Mosha	Pearson Correlation	1	-0.168**	0.083
	Sig. (2-tailed)		0.005	0.162
	N		284	284
Attidue - Scale	Pearson Correlation		1	0.443**
	Sig. (2-tailed)			0.000
	N			284
Reaktive-Proaktivë pyetjet!	Pearson Correlation			1
	Sig. (2-tailed)			
	N			

** . Correlation is significant at the 0.01 level (2-tailed).

Problemet kronike . ndikimi

Group Statistics

		A keni ndonjë problem mjekësor kronik që vazhdon të ndërhyjë në jetën tuaj?			
		N	Mean	Std. Deviation	Std. Error Mean
Attidue - Scale	Po	48	3.1174	0.66168	0.09551
	Jo	236	2.8328	0.56471	0.03676
Reaktive-Proaktivë pyetjet!	Po	48	0.5417	0.20868	0.03012
	Jo	236	0.4318	0.25910	0.01687

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Attidue - Scale	Equal variances assumed	2.413	0.121	3.088	282	0.002	0.28460	0.09215	0.10321	0.46600
	Equal variances not assumed			2.781	61.686	0.007	0.28460	0.10234	0.08002	0.48919
Reaktive-Proaktivë pyetjet!	Equal variances assumed	3.913	0.049	2.761	282	0.006	0.10989	0.03981	0.03153	0.18824
	Equal variances not assumed			3.183	79.532	0.002	0.10989	0.03452	0.04118	0.17859

Kolegji Heimerer Certificate Nderim Rizanaj has active successfully attended the 11th International Symposium of Health Sciences on September 19, 2020 in Prishtina, Republic of Kosovo. Titulli: Efektet psikologjike të Covid 19 tek studentët

EFEKTET PSIKOLOGJIKE TË COVID 19 TEK STUDENTËT

Nderim Rizanaj

Abstrakt

Efektet psikologjike negative janë shkak kryesorë për çrregullimeve dhe dështimin në jetën studentore dhe gjatë tërë jetës në përgjithësi. Këto efekte janë të njohura, të cilat janë manifestuar gjatë pandemisë Covid19 edhe tek ne në Kosovë, saktësisht tek të rinjët, të cilët për një kohë 3 mujore, kanë qenë brenda të izoluar për shkak të pandemisë. Kjo ka ndikuar në suksesin e tyre në Universitet dhe në jetën e tyre të përditshme. Hulumtimi është realizuar me qëllim të analimit të efekteve negative emocionale në gjendjen e tyre kognitive. Për këtë hulumtimi është zhvilluar me një numër prej 200 studentësh, ku mosha mesatare e tyre ishte 22 vjeç, kurse parametrat e tjerë demografikë ishin sipas mostërs së rastit. Hulumtimi është kryer për periudhën mars-qershor 2020, për të matur këto efekte, e të cilat janë shfaqur tek studentët tanë në Kosovë.

Të dhënat janë kryer përmes programit SPSS, kurse për të vërtetuar hipotezën është përdorur korleacioni Spearman. Në bazë të rezultateve të hulumtimit, konkludojmë se eksperiencia negative emocionale gjatë periudhës së parë të pandemisë, ka pasur një ndikim sinjifikantë në rritjen e gjendjes negative kognitive të studentëve me korelacion ($r=.235^{**}$), p value=.001, si dhe eksperiencia negative emocionale e studentëve gjatë pandemisë Covid 19, ka ndikim sinjifikantë rritjen e nivelit të çrregullimeve si harresës dhe çorganizimit të jetës së tyre të përditshme me korrelacion ($r=.427^{**}$), ($r=.630^{**}$), p value=.000.

Në fund konkludojmë se periudha e valës së parë të pandemisë, se eksperiencia emocionale negative ka ndikuar në rritjen e gjendjes negative kognitive, në rritjen e harresës dhe çorganizimit të jetës së studentëve.

Fjalët kyçe: Efektet psikologjike, Covid19, Stundetët

Hyrje

Virusi Covid19 besohet se për herë të parë është vërtetuar në Wuhan të Kinës, ku ishte një gjendje jashtëzakonisht alarmante. Kjo sëmundje na përkujton virusin SARS të vitit 2003, por në të vërtetë ky virus tani kalon përmasat dhe pritshmëritë e OBSH-së. (WHO, 2003). Në anën tjetër përvojat emocionale janë të përhapura në natyrë dhe të rëndësishme dhe ndoshta edhe kritike në mjediset akademike, pasi emocioni modulon pothuajse çdo aspekt të njohjes, e sidomos gjatë kohës kur ne jemi nën presion apo kemi mungesë të aktiviteteve.

Studime të shumta kanë raportuar se proceset kognitive njerëzore ndikohen nga emocionet, përfshirë vëmendjen (Vuilleumier, 2005) të mësuarit dhe memorie (Phelps, 2004) arsyetimi (Jung, N., Wranke, C., Hamburger, K., and Knauff, M., 2014) dhe zgjidhja e problemeve (Isen, A. M., Daubman, K. A., and Nowicki, G. P., 1987). Shprehjet subjektive të përdorura në neuroshkencën afektive përfshijnë emocione, disponimi, ndjenjat, ndikon dhe drejton. Edhe pse emocioni ka kohë është studiuar, nuk ka asnjë përcaktim të vetëm. Një përmbledhje e 92 përkufizime të theksuara dhe nëntë deklaratat skeptike Emocionet përshkruajnë një grup të ndërlikuar ndërveprimesh midis variablat subjektive dhe objektive që ndërmjetësohen nga nerva dhe sistemet hormonale, të cilat mund të (a) krijojnë ndikim afektiv përvojat e valencës emocionale (kënaqësi-pakënaqësi) dhe ngjallje emocionale (aktivizim i ulët-i ulët / qetësues-zgjuet); (b) gjenerojnë procese njohëse siç janë emocionalisht të rëndësishme ndikojnë perceptues, vlerësime, proceset e etiketimit; (c) aktivizoni ndryshime të përhapura psikologjike dhe fiziologjike në kushte ngjallëse; dhe (d) motivojnë sjellje që shpesh janë por jo gjithmonë ekspresive, të drejtuara nga qëllimi dhe adaptive. (Kleinginna, P. R. Jr., and Kleinginna, A. M., 1981)

Emocionet janë proceset psikoneurale që janë me ndikim në kontrollin e fuqisë dhe modelimit të veprimeve në dinamikë fluksi i shkëmbimeve të sjelljes intensive midis kafshëve si si dhe me objekte të caktuara që janë të rëndësishme për mbijetesën. Prandaj, çdo emocion ka një "ton ndjenjash" karakteristik që është veçanërisht e rëndësishme në kodimin e vlerave të brendshme të këto ndërveprime, në varësi të mundësisë së tyre promovimi ose pengimi i mbijetesës (të dyja menjëherë Sensi "personal" dhe afatgjatë "riprodhues"). Autori (Williams, 2020) thekson se në dy korrespondenca të publikuara në psikiatrinë Lancet, ekspertët kanë tërëqur vëmendjen te popullsia e pacientëve që mund të kenë nevojë për ndërhyrje dhe mbështetje psikologjike. Ai

theksoi rolin e shërbimeve këshilluese psikologjike të bazuara në WeChat, duke përfshirë terapitë për depresion, ankth dhe pagjumësi, si dhe programe inteligjence artificiale si Tree Holes Rescue, e cila monitoron rrezikun e vetëvrasjeve duke analizuar mesazhe. Në përgjithësi, epidemia e COVID-19 ka nënvizuar boshllëqet e mundshme në shërbimet e shëndetit mendor gjatë urgjencave, ndërsa teston gjithashtu aftësinë e punonjësve të kujdesit shëndetësor dhe sistemeve mjekësore, gjë që është dëshmuar edhe në vendin tonë.

Vlen të përmendet se përkundër problemeve të zakonshme të shëndetit mendor dhe çrregullime që gjenden në mesin e pacientëve dhe punonjësve shëndetësorë në mjedise të tilla, shumica e profesionistëve shëndetësorë që punojnë në njësi izolimi dhe spitale dhe mos marrja e asnjë trajnimi për ofrimin e kujdesit për shëndetin mendor, ndikojnë në një situatë të papërshtatshme, e cila pasohet me katastrofa të cilat dalin jashtë kontrollit. (Xiang, 2020).

Autori (Barbisch, 2015) përshkruani se si mbyllja "shkaktoi ndjenjën e histerisë kolektive, duke e çuar stafin në masa të dëshpëruara". Frika duket me siguri si pasojë e masës karantinë. Ankthi brenda Wuhan pritet edhe pa qenë në karantinë. Gjatë shpërthimit të sëmundjes, ankthi i komunitetit mund të rritet pas vdekjes së parë, raportim i shtuar në media dhe një përshkallëzim numri i çështjeve të reja. Kështu, karantina masive ka të ngjarë të ngjallë ankth në thelb, për arsye të shumta, e në anën tjetër ankthi i ngritur mund të ketë gjithashtu implikimet e goditura për masat e tjera shëndetësore.

Qëllimi i hulumtimit

Hulumtimi ka për qëllim të analizojë efektet psikologjike të cilat janë shkaktuar nga pandemia e fundit Covid19, gjatë karantinës në periudhën mars – qershor 2020, ku janë përfshirë studentët e nivelit bachelor dhe master.

Pyetja e hulumtimit

1. Cili është niveli ndikimit të eksperiencës negative emocionale në gjendjen kognitive të studentëve ?

1.1. Çfarë ndikimi do të kishte eksperiencia emocionale negative në rritjen e çrregullimeve si harresa dhe çorganizimi në jetën e përditshme të studentëve ?

Hipotezat e hulumtimit

H01. Eksperiencia negative emocionale gjatë periudhës së parë të pandemisë, ka ndikim në rritjen e gjendjes negative kognitive të studentëve.

H02. Eksperiencia negative emocionale e studentëve gjatë pandemisë Covid 19, ka ndikim në rritjen e nivelit të çrregullimeve si harresës dhe ç'organizimit të jetës së tyre të përditshme.

Metodologjia e hulumtimit

Hulumtimi i takon tipit sasior, kurse është realizuar përmes pyetësorit online, i cili është dërguar tek studentët përmes email adresës. Studim rasti janë marrë 200 studentë të nivelit bachelor dhe master, kurse gjinia, mosha dhe statusi i tyre martesor ka qenë sipas mostrës së rastit.

Pyetësori është formuluar duke u bazuar në formularët e organizuar nga OBSH-ja, ku janë shkoqitur pjesët të cilat ndihmojnë në matjen e ndikimit të Covid19 dhe efektet që ka në gjendjen emocionale dhe kognitive të studentëve. Në pjesën e parë të pyetësorit janë vendosur pyetjet demografike dhe informacione mbi Covid19, në pjesën e dytë është analizuar eksperiencia emocionale e studentëve dhe në pjesën e tretë eksperiencia kognitive.

Analizat janë analizuar përmes programit SPSS, kurse përmes metodës së korelacion Spearman është bërë vërtetimi i hipotezave të hulumtimit.

Rezultatet empirike të analizave

Në këtë pjesë janë të paraqitura rezultatet e analizave të hulumtimit, ku fillimisht është paraqitur analiza demografike dhe përshkruese, pastaj janë paraqitur rezultatet e hipotezave të hulumtimit .

Analizat demografike

Në hulumtim kanë marrë pjesë gjithsej 200 studentë, ku prej tyre 34 ishin meshkuj apo 17% dhe 166 femra apo 83%. Nga vendbanimet urbane janë gjithsej 119 studentë apo 59.5% dhe 81 të tjerë nga ato rurale apo 40.5%. Të pamartuar janë 183 prej tyre apo 91.5%, kurse të martuar ishin 17 studentë apo 8.5%, kurse mosha mesatare e studentëve pjesëmarrës në hulumtim është 22.33 vjeç, me një devijim të lartë prej 5.90 vjeç. Mesatarisht jetojnë 5.32 persona në një shtëpi/banesë, kurse nën moshën 18 vjeçare mesatarisht janë 1.14 persona në familje. Rezultatet tregojnë se 99.5% e studentëve kanë dëgjuar për Covid19, kurse vetëm një nuk ka kthyer përgjigje, e po ashtu edhe tek dëgjimi i lajmeve kemi 99.5%.

Gjinia	N	%
Mashkull	34	17.0%
Femër	166	83.0%
Vendbanimi	N	%
Urban	119	59.5%
Rural	81	40.5%
Statusi martesor	N	%
I/e pamartuar	183	91.5%
I/e martuar	17	8.5%
Sa persona jetoni në një shtëpi/banesë?	Mesatarja	Devijimi standard
	5.32	1.59
Sa persona nën moshën 18 vjeçare jetojnë në një banesë me ju ?	Mesatarja	Devijimi standard
	1.14	0.94
Mosha	Mesatarja	Devijimi standard
	22.33	5.90
A keni dëgjuar për Coronaviursin?	N	%
Po	199	99.5%
N/A	1	0.5%
A keni dëgjuar apo pa ndonjë lajm për Coronavirusin ?	N	%
Po	199	99.5
N/A	1	0.5

Sa i përket opinionit të studentëve mbi rëndësinë e informacioneve dhe ndikimin e tyre, shohim se shumica e studentëve shprehen se e kanë të vështirë se cilin informacion ta klasifikojnë si të vërtetë, kurse një përqindje prej më shumë se 30% shprehen se lajmet si nga njerëzit, televizioni apo rrjetet sociale, kanë ndikuar negativisht në sjelljet e tyre të përditshme. Sa i përket vlerësimit të saktësisë së lajmeve nga studentët, shohim se ajo kalon nivelin mesatar prej 5.97, me një devijim të lartë prej 2.48 pikësh.

	Aspak nuk pajtohem		Nuk pajtohem		Neutral		Pajtohem		Plotësisht pajtohem	
	Count	Row N %	Count	Row N %	Count	Row N %	Count	Row N %	Count	Row N %
Është shumë e vështirë të vendosi se cili informacion është i vërtetë apo i rrejshëm.	22	11.0 %	18	9.0%	60	30.0 %	53	26.5%	47	23.5 %
Lajmet që kam dëgjuar nga njerëzit për Coronavirusin, kanë ndikuar negativisht në sjelljet e mia të përditshme.	74	37.0 %	38	19.0 %	27	13.5 %	49	24.5%	12	6.0%
Lajmet në programet televizive kanë ndikuar negativisht në sjelljet e mia të përditshme.	73	36.5 %	33	16.5 %	44	22.0 %	29	14.5%	21	10.5 %
Lajmet në rrjetet sociale kanë ndikuar negativisht në sjelljet e mia të përditshme.	86	43.0 %	14	7.0%	48	24.0 %	35	17.5%	17	8.5%
	N	Minimum	Maximum		Mean		Std. Deviation			
Vlerësoni prej 1 deri në 10 saktësinë e lajmeve gjatë kohës së pandemisë	197		0		10		5.97			2.487

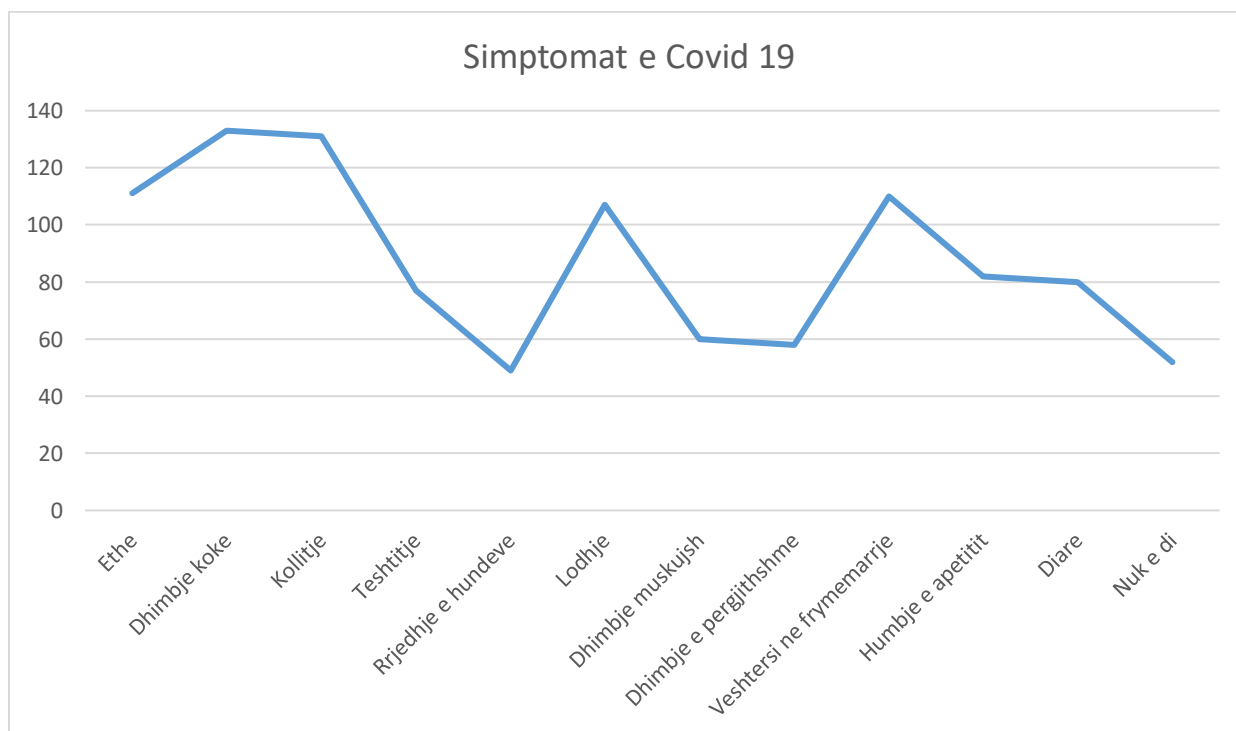
Njohuritë e studentëve për simptomat e Covid 19

Rezultatet e hulumtimit tregojnë se studentët kanë informacione në lidhje me simptomat e Covid19, duke u bazuar në tabelën e mëposhtme shohim se simptoma që më së shumti paraqet këtë sëmundje është dhimbja e kokës me 12.7% të rasteve, pastaj kollitja me 12.5%, ethet me 10.6%, vështirësi në frymëmarrje me 10.5% dhe lodhja me 10.2%, kurse simptomat e tjera kanë përqindje më të ulëta.

Sim Frequencies

		Responses	
		N	Percent
SIm ^a	Ethe	111	10.6%
	Dhimbje koke	133	12.7%
	Kollitje	131	12.5%
	Teshtitje	77	7.3%
	Rrjedhje e hundeve	49	4.7%
	Lodhje	107	10.2%
	Dhimbje muskujsh	60	5.7%
	Dhimbje e pergjithshme	58	5.5%
	Veshtersi ne frymemarrje	110	10.5%
	Humbje e apetitit	82	7.8%
	Diare	80	7.6%
	Nuk e di	52	5.0%
Total		1,050	100.0%

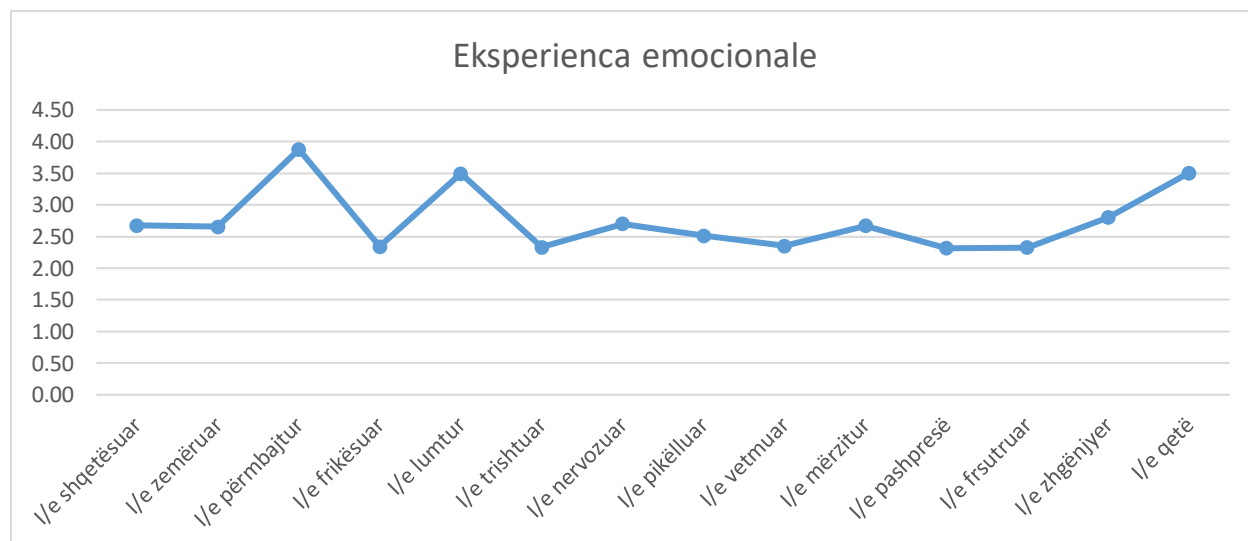
a. Dichotomy group tabulated at value 1.



Ekspierienca emocionale e studentëve

Rezultatet e hulumtimit tregojnë se tek ekspierienca emocionale, shohim se studentët në shumicën e rasteve shprehen në një përqindje mesatarisht të lartë të shqetësuar, ku mbi 30% thonë se janë të shqetësuar, afërsisht në përqindje janë të zemëruar, kurse mbi 20% e tyre shprehen se janë të frikësuar. Edhe të frikësuar keni një përqindje mbi 20% e cila është shprehur se ndihen të tillë, kurse të trishtuar, nevrozuar, pikëlluar, vetmuar, të mërzitur kanë një përqindje më të lartë se 20% , ku studentët e shohin veten jo mirë në këto aspekte, gjë që është negative dhe ndikon në përditshmërinë e tyre. Një përqindje e studentëve shprehen se ndjehen edhe të pashpresë, të frustruar dhe të zhgënjyer, kruse në anën tjetër kanë treguar se janë të përmbajtur, të lumtur deri diku dhe të qetë.

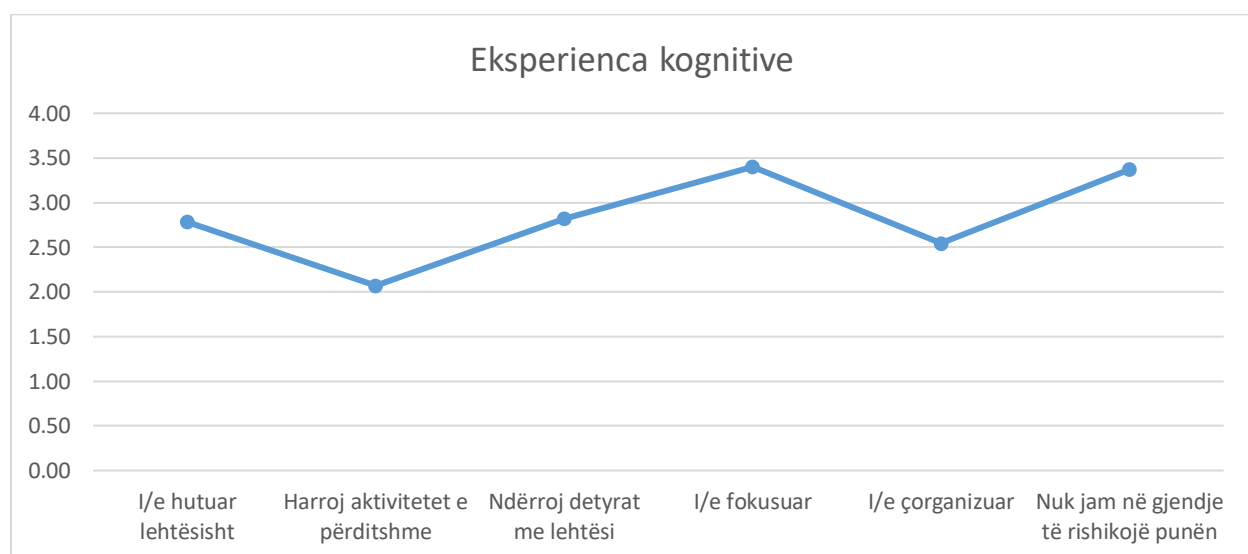
	Aspak e vërtetë		Pak e vërtetë		As e vërtetë, as e pavërtetë		E vërtetë		Plotësisht e vërtetë	
	N	%	N	%	N	%	N	%	N	%
I/e shqetësuar	60	30.0%	26	13.0%	54	27.0%	39	19.5%	21	10.5%
I/e zemëruar	54	27.0%	31	15.5%	57	28.5%	46	23.0%	12	6.0%
I/e përmbajtur	4	2.0%	17	8.5%	62	31.0%	34	17.0%	83	41.5%
I/e frikësuar	71	35.5%	50	25.0%	38	19.0%	22	11.0%	19	9.5%
I/e lumtur	15	7.5%	18	9.0%	71	35.5%	45	22.5%	51	25.5%
I/e trishtuar	81	40.5%	17	8.5%	68	34.0%	23	11.5%	11	5.5%
I/e nevrozuar	44	22.0%	38	19.0%	66	33.0%	38	19.0%	14	7.0%
I/e pikëlluar	54	27.0%	50	25.0%	52	26.0%	27	13.5%	17	8.5%
I/e vetmuar	94	47.0%	20	10.0%	35	17.5%	24	12.0%	27	13.5%
I/e mërzitur	55	27.5%	29	14.5%	61	30.5%	37	18.5%	18	9.0%
I/e pashpresë	81	40.5%	38	19.0%	38	19.0%	23	11.5%	20	10.0%
I/e fsutruar	63	31.5%	49	24.5%	56	28.0%	24	12.0%	8	4.0%
I/e zhgënjyer	53	26.5%	26	13.0%	60	30.0%	30	15.0%	31	15.5%
I/e qetë	15	7.5%	37	18.5%	40	20.0%	48	24.0%	60	30.0%



Eksperienca kognitive e studentëve

Eksperienca kognitive tregon gjendjen e tyre, e cila vjen si rezultat i gjendjes emocionale të studentëve gjatë periudhës së karantinës në valën e parë të virusit Covid19. Rezultatet tregojnë se kemi një përqindje tepër të lartë të të hutuarit të studentëve, ku mbi 50% janë shprehur se kjo ka ndodhë ndonjëherë, kurse mbi 15% thonë se kjo u ka ndodhë shpesh apo shumë shpesh. Sa i përket harresës, është një gjendje pak më të ulët, mirëpo shqetësuese pasi që mbi 25% shprehen se kjo u ndodhë ndonjëherë, kurse mbi 13% shprehen se kjo u ndodhë shpesh apo shumë shpesh. Ndërrimi i detyrave me lehtësi u ndodhë shumicë dërmuese të studentëve, kurse çorganizimi është një faktor i cili ndikon në jetën e përditshme të studentëve.

	Asnjëherë		Rrallë		Ndonjëherë		Shumë shpesh		Shumë shpesh	
	Count	Row N %	Count	Row N %	Count	Row N %	Count	Row N %	Count	Row N %
I/e hutuar lehtësisht	24	12.0%	39	19.5%	106	53.0%	18	9.0%	13	6.5%
Harroj aktivitetet e përditshme	103	51.5%	17	8.5%	53	26.5%	17	8.5%	10	5.0%
Ndërroj detyrat me lehtësi	35	17.5%	48	24.0%	63	31.5%	26	13.0%	28	14.0%
I/e çorganizuar	70	35.0%	25	12.5%	61	30.5%	14	7.0%	30	15.0%
Nuk janë në gjendje të rishikojë punën nga fillimi	19	9.5%	15	7.5%	87	43.5%	31	15.5%	48	24.0%



Vërtetimi i hipotezave

Në këtë pjesë është paraqitur vërtetimi i hipotezave, ku në të dy rastet është përdorur korelacioni Spearman, i cili na mundëson të vërtetojmë ndërlidhjen në mes të eksperiencës negative emocionale dhe gjendjes kognitive tek studentët, si dhe ndikimi i eksperiencës emocionale negative në çrregullimet si harresa dhe çorganizimi i jetës së përditshme të studentëve.

H01. *Eksperiencia negative emocionale gjatë periudhës së parë të pandemisë, ka ndikim në rritjen e gjendjes negative kognitive të studentëve.*

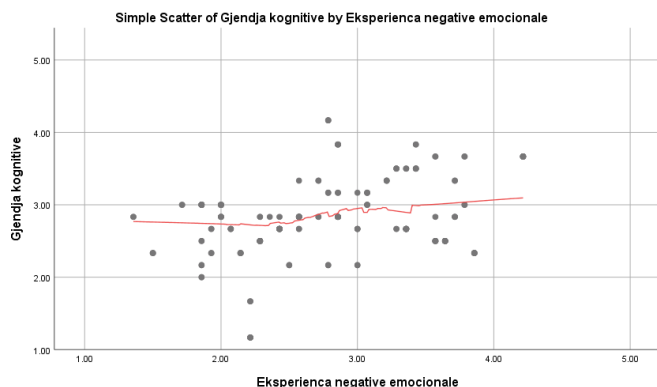
Për të vërtetuar hipotezën e më sipërme kam përdorur korelacionin Spearman, i cili na mungësojë të vërtetojmë ndikimin dhe ndërlidhjen e eksperiencës negative emocionale tek gjendja kognitive e studentëve. Variabël e pavarur në këtë rast është eksperiencia negative emocionale, kurse ajo e varur është gjendja kognitive e studentëve.

Correlations

		Eksperiencia	
		negative emocionale	Gjendja kognitive
Spearman's rho	Eksperiencia negative emocionale	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	200
	Gjendja kognitive	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	

** . Correlation is significant at the 0.01 level (2-tailed).

Në bazë të rezultateve të mësipërme shohim se kemi një korelacion të lartë ($r=.235^{**}$) që nënkupton se eksperiencia negative emocionale ka ndikim në rritjen e gjendjes kognitive, p value=.001, e cila është sinjifikante në 1% të nivelit të besueshmërisë. Me këtë rast unë konkludojë se eksperiencia negative emocionale gjatë periudhës së parë të pandemisë, ka ndikim sinjifikantë në rritjen e gjendjes negative kognitive të studentëve, p value= 0.001.



H02. Eksperienca negative emocionale e studentëve gjatë pandemisë Covid 19, ndikon në rritjen e nivelit të çrregullimeve si harresës dhe çorganizimit të jetës së tyre të përditshme.

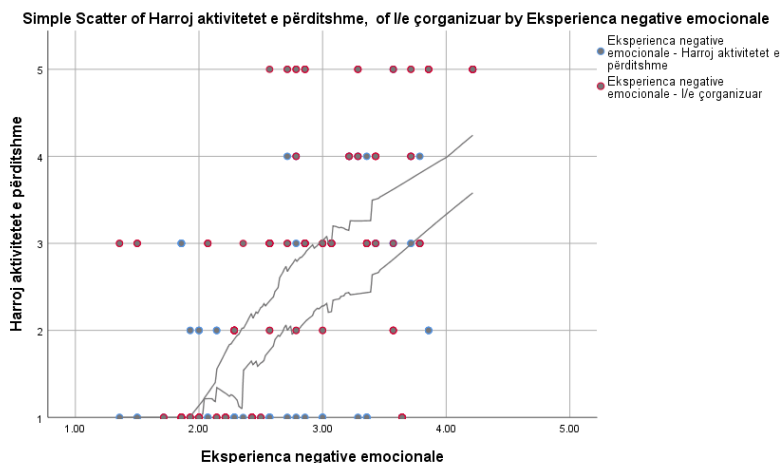
Hipotezën e mësipërme e kam vërtetuar përmes korelacionit Spearman, ku variabël e pavarur është eksperienca negative e studentëve, kurse variabla të varura janë harresa dhe çorganizimi i studentëve gjatë periudhës së valës së parë të pandemisë Covid19.

Correlations

		Eksperienca negative emocionale		
			Harresa	Ç'organizimi
Spearman's rho	Eksperienca negative emocionale	Correlation Coefficient	1.000	0.630**
		Sig. (2-tailed)	.	0.000
		N	200	200
	Harresa	Correlation Coefficient	1.000	0.544**
		Sig. (2-tailed)	.	0.000
		N	200	200
	Ç'organizimi	Correlation Coefficient		1.000
		Sig. (2-tailed)		.
		N		200

** . Correlation is significant at the 0.01 level (2-tailed).

Në bazë të rezultateve të mësipërme shohim se në mes të eksperiencës negative emocionale dhe harresës kemi korelacion të lartë ($r=.427^{**}$) dhe me ç'organizimin ($r=.630^{**}$), që nënkuptojmë se eksperienca negative emocionale e studentëve ka ndikim në rritjen e harresës dhe ç'organizimit të jetës së tyre të përditshme, e të cilat janë sinjifikante në 1% të nivelit të besueshmërisë, p valu=.000. Pra, pranojmë hipotezën dhe themi se eksperienca negative emocionale e studentëve gjatë pandemisë Covid 19, ndikon në rritjen e nivelit të çrregullimeve si harresës dhe çorganizimit të jetës së tyre të përditshme.



Përfundime dhe rekomandime

Në hulumtim morën pjesë gjithsej 200 studentë, ndërsa mosha mesatare e tyre ishte 22.23 vjeç. Studentët ishin të njoftuar me gjendjen aktuale me Covid19 dhe kishin informacione në lidhje me këtë sëmundje, si në aspektin e ndikimit të saj në çrregullimin e jetës së përditshme, por edhe në format e shfaqjes së saj. Shumica dërmuese e studentëve vinin nga vendbanimet urbane, kurse raporti gjinor i pjesëmarrjes në hulumtim ishte 83% femra dhe 17% meshkuj. Ajo që u vërejtë dhe do të duhej të ishte shqetësim për të gjithë, janë lajmet të cilat transmetohen nga mediat televizive dhe ato të shkruara, ku studentët shprehen se shpeshherë ndihen se këto lajme ndikojnë negativisht tek ta, e kanë të vështirë t'i dallojnë lajmet e vërteta prej atyre të pavërteta, e kjo besoj që është një qështje tepër me rëndësi me të cilën duhet të mirren autoritetet.

Ata e vlerësojnë saktësinë e lajmeve me mesatare prej 5.97, një vlerësim tepër i ulët e që tregon se gjendja në këtë fushë është shumë shqetësuese. Studentët janë të njoftuar edhe me simptomat e Covid19, gjë që është pozitive pasi që ata janë të njofuar me këtë aspekt, e që mund të ndikojnë në ruajtjen e shëndetit dhe marrjen e masave me kohë, që të mos përhapet virusi më tepër. Rezultatet e hulumtimit tregojnë se studentët kanë pësuar ndikimet negative emocionale gjatë pandemisë, saktësisht gjatë fazës së parë të pandemisë në periudhën mars-qershor 2020, ku këto aspekte negative ata i kanë shprehur përmes këtij pyetësori, duke na treguar se gjendja e tyre emocionale ka pasur ndryshime të mëdha, në aspektin negativ.

Nga rezultatet e hipotezave është vërtetuar se eksperiencia negative emocionale gjatë periudhës së parë të pandemisë, ka pasur një ndikim sinjifikantë në rritjen e gjendjes negative kognitive të studentëve $p \text{ value} = .001$, kurse në rastin e dytë është vërtetuar se eksperiencia negative emocionale e studentëve gjatë pandemisë Covid 19, ka ndikim sinjifikantë rritjen e nivelit të çrregullimeve si harresës dhe çorganizimit të jetës së tyre të përditshme me $p \text{ value} = .000$

Në bazë të rezultateve të hulumtimit, mund të konkludojmë se Qeveria e Kosovës duhet të ndërmarrë disa hapa, në mënyrë që të jetë në gjendje të kontrollojë gjendjen pandemike dhe të ndihmojë të rinjtë që të kalojnë këtë periudhë me minimum ndikime negative emocionale. Për këtë duhet të mirren parasysh këto rekomandime:

1. Të krijohet një portal i cili i informon të gjithë qytetarët e Kosovës me gjendjen reale të Covid19, ndërsa ky portal duhet të jetë i qasshëm 24 orë në ditë dhe të ofrohet edhe përmes televizionit dhe rrjeteve sociale.
2. Të kontrollohen portalet të cilat publikojnë lajme jo të sakta në lidhje me gjendjen pandemike, në mënyrë që ndikimi negativ të minimizohet.
3. Të ofrohen trajtime psiko-sociale pa pagesë, përmes telefonit apo platformave të ndryshme, në formë të shkruar apo edhe direkt me psikologë.
4. Të zbatohen masat e kërkuara nga OBSH-ja dhe të ofrohet ndihma ndaj të gjithë të rinjëve të cilët shfaqin çrregullime psikologjike.

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Article

Direct Cross-Coupling of Alcohols with *O*-Nucleophiles Mediated by *N*-Iodosuccinimide as a Precatalyst under Mild Reaction Conditions

Njomza Ajvazi ^{1,2,*}  and Stojan Stavber ^{1,3} ¹ Jožef Stefan International Postgraduate School, Jamova 39, 1000 Ljubljana, Slovenia; stojan.stavber@ijs.si² Alma Mater Europaea Campus College "REZONANCA", 10000 Pristina, Kosovo³ Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

* Correspondence: njomza.ajvazi@rezonanca-rks.com; Tel.: +383-44-258-553

Abstract: We report *N*-iodosuccinimide as the most efficient and selective precatalyst among the *N*-halosuccinimides for dehydrative *O*-alkylation reactions between various alcohols under high-substrate concentration reaction conditions. The protocol is non-metal, one-pot, selective, and easily scalable, with excellent yields; enhancing the green chemical profiles of these transformations.

Keywords: alcohols; *N*-iodosuccinimide; cross-coupling; etherification; green chemistry



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1. Introduction

The C–O bond construction is one of the significant implements in organic synthesis since it provides access to the production of cosmetics, fragrances, pharmaceuticals, and dyestuffs [1]. Since alcohols are inexpensive and could effectively serve as alkylating agents, their direct cross-coupling with *O*-nucleophiles could be a very desirable strategy for avoiding an additional synthetic step for coupling reactions.

Activation of alcohols for nucleophilic substitution using a substoichiometric amount of different catalysts, such as metal ions: [RuCp(o-EtOdppe)](OTs), CuBr₂, NaAuCl₄, Brønsted, or Lewis acids (hypervalent [bis(trifluoroacetoxy)iodo]benzene catalyst has been found to function as a Lewis acid) or other supporters such as organohalides or molecular iodine in the presence of solvents has been touched on by some reviews [2–10] and recently developed reports [11–14]. However, the requirement for toxic and costly reagents, environmentally inappropriate solvents, multiple synthetic steps, and high temperatures make such a synthetic protocol less desirable from a sustainability perspective. Therefore, these disadvantages have challenged chemists to invent and develop novel methods for direct dehydrative C–O coupling, using alcohols as electrophiles under environmentally benign conditions.

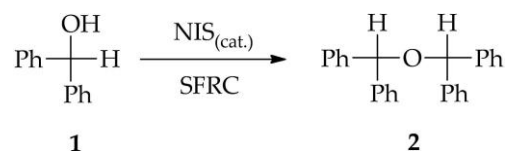
We previously reported the role of *N*-halosuccinimides (NXSs) (chloro, bromo, or iodo) as the mediator for the direct conversion of a hydroxyl group, forming new C–C or C-heteroatom bonds. *N*-iodosuccinimide (NIS) was the most efficient and selective mediator among the NXSs [15].

With our continuous research work on improving and developing greener protocols [16–19], we now report the expanding role of NIS as a commercially available, metal-free, and easy-to-handle precatalyst for direct dehydrative *O*-alkylation reactions between various alcohols under high-substrate concentration reaction conditions (HCRC), and leading to a practical, efficient, selective, and easily scalable method.

A high-concentration reaction condition in our case is defined as a concentration where less than 0.2 mL of solvent/mmol of substrate and reagent was used.

2. Results and Discussion

We began our investigations by using diphenylmethanol **1** with NIS as the precatalyst under solvent-free reaction conditions (SFRC), where the dimerization process was observed (Scheme 1), resulting in the formation of symmetric ether **2** (entry 1, Table 1). For the transformation of diphenylmethanol **1** without NIS, in the presence of MeOH **3** under HCRC, no conversion of the starting material was observed [15].



Scheme 1. The conversion of diphenylmethanol **1** mediated by NIS under SFRC.

Table 1. The effect of the loading of NIS on the conversion of diphenylmethanol **1** under SFRC ^a.

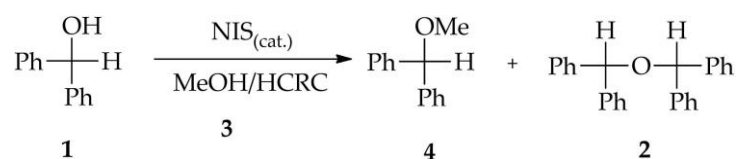
Entry	NIS (mol %)	Conv. ^b (%) of 1	Yield ^c (%) 2
1	0.5	79	78 ^d
2	1	90	89 ^d
3	2	100	100

^a Reaction conditions: diphenylmethanol **1** (0.5 mmol), 70–75 °C, 3.5 h. ^{b,c} Determined by ¹H NMR spectroscopy. ^d Benzophenone 1%.

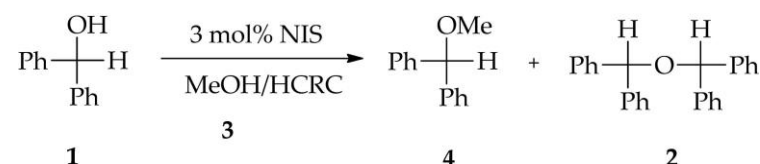
The effect of NIS loading for the reaction of diphenylmethanol **1** in the absence of a nucleophile source under SFRC was examined, and the results are presented in Table 1, and in Supplementary Materials To increase the yield of the product, different concentrations of the precatalyst were used. Employing 0.5 mol% of the precatalyst, the formation of the corresponding product **2** with good yield was observed, accompanied by a trace of oxidized alcohol (entry 1). By increasing the precatalyst loading to 1 mol%, we observed the high conversion of the starting material **1** into the corresponding product **2**, accompanied by a trace of oxidized alcohol (entry 2). We found that for the quantitative conversion of the starting material **1** to the dimeric ether **2**, 2 mol% of the precatalyst was effective (entry 3).

Furthermore, we continued our investigations by heating diphenylmethanol **1** with methanol (MeOH) **3** mediated by NIS under HCRC, affording the corresponding ether **4** in excellent yield [15]. (Scheme 1).

In searching for the optimal reaction conditions, different parameters, such as loading of the NIS as the mediator and temperature for the reaction of diphenylmethanol **1** with MeOH **3** under HCRC (Schemes 2 and 3), were first examined, and the results are presented in the Tables 2 and 3 and in Supplementary Materials. To determine the effectiveness of the NIS as the mediator, different concentrations of the precatalyst were employed. In the reaction of diphenylmethanol **1** with MeOH **3** using 0.5 mol% of the precatalyst, the formation of the corresponding product **4** in good yield and accompanied by a small amount of symmetric ether **2** was observed (entry 1). By increasing the precatalyst loading to 1 mol%, quantitative conversion of the starting material **1** into the corresponding product **4** was noticed, accompanied by a small amount of dimeric ether **2** (entry 2). Similar results were achieved when the precatalyst loading was increased up to 2 mol% (entry 3). An improvement of 100% yield, without forming the dimeric ether was attained by increasing the precatalyst loading up to 3 mol% (entry 4).



Scheme 2. The conversion of diphenylmethanol **1** in the presence of MeOH **3** mediated by NIS under HCRC.



Scheme 3. The conversion of diphenylmethanol **1** in the presence of MeOH **3** mediated by NIS under HCRC.

Table 2. The effect of the loading of NIS on the conversion of diphenylmethanol **1** with MeOH **3** under HCRC ^a.

Entry	NIS (mol %)	Conv. ^b (%) of 1	Relative Distribution ^c (%)	
			4	2
1	0.5	83	78	5
2	1	100	94	6
3	2	100	95	5
4	3	100	100	/

^a Reaction conditions: diphenylmethanol **1** (0.5 mmol), MeOH **3** (1 mmol), 70–75 °C, 6 h. ^b Determined by ¹H NMR spectroscopy. ^c Yields calculated relative to alcohol **1**.

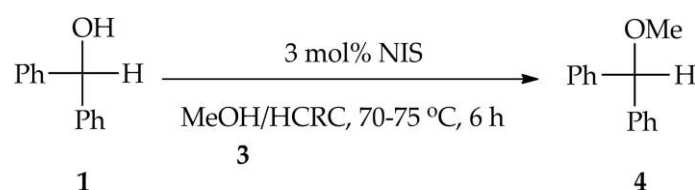
Table 3. The catalytic effect of NIS on the conversion of diphenylmethanol **1** with MeOH **3** based on the temperature under HCRC ^a.

Entry	T(°C)	Conv. ^b (%) of 1	Relative Distribution ^c (%)	
			4	2
1	rt	/	/	/
2	40–45	26	23	3
3	50–55	61	56	5
4	60–65	86	81	3 ^d
5	70–75	100	100	/

^a Reaction conditions: diphenylmethanol **1** (0.5 mmol), MeOH **3** (1 mmol), 6 h. ^b Determined by ¹H NMR spectroscopy. ^c Yields calculated relative to alcohol **1**. ^d Benzophenone 2%. rt—room temperature.

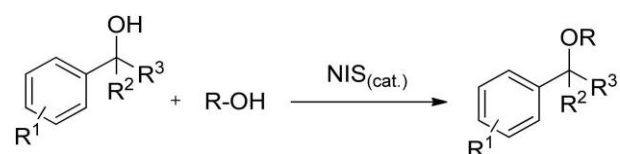
The effect of temperature on the course of the reaction was examined by the reaction of diphenylmethanol **1** with MeOH **3** mediated by NIS under HCRC. When the reaction was carried out at room temperature, no transformation took place (entry 1). When the reaction was performed at 40–45 °C, the desired product **4** in low yield and a small amount of dimeric ether **2** were obtained (entry 2). An increase of reaction temperature up to 50–55 °C provided the corresponding product **4** in moderate yield, accompanied by a small amount of symmetric ether **2** (entry 3). Further optimization revealed that the reaction could be performed smoothly by raising the temperature to 60–65 °C. An improvement by 100% yield without forming the symmetric ether was attained by increasing the reaction temperature to 70–75 °C.

Screening on precatalyst loading and reaction temperature showed that 3 mol% NIS, 70–75 °C, and 6 h were the best conditions ensuring complete conversion of **1** into the corresponding ether **4** (Scheme 4).



Scheme 4. Optimal reaction conditions for the conversion of diphenylmethanol **1** into the (methoxymethylene)dibenzene **4** mediated by NIS under HCRC.

Encouraged by these promising results, we applied the obtained optimal reaction conditions to direct dehydrative *O*-alkylation reactions between different alcohols mediated by NIS under HCRC (Scheme 5), and the results are collected in Table 4.

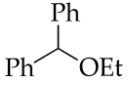
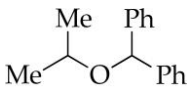
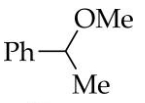
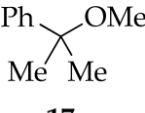
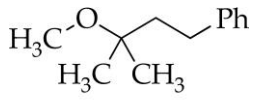
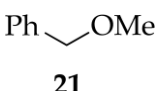
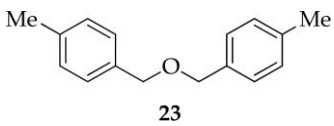
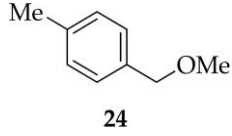
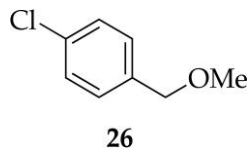
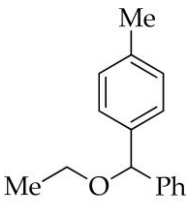


Scheme 5. Transformation of benzyl and tertiary alkyl alcohols with alkyl alcohols mediated by NIS under SFRC, under HCRC or in solution.

Table 4. Transformation of benzyl and tertiary alkyl alcohols with alkyl alcohols mediated by NIS under SFRC, under HCRC or in solution ^a.

Entry	R ¹ , R ² , R ³	R-OH, Time (h)	Product	Conversion ^b (%) (Yield %)
1 ^g	R ¹ = R ² = H, R ³ = Ph 1	/ (3.5)	 2	100 “(99) [15]”
2	R ¹ = R ² = H, R ³ = Ph 1	MeOH 3 (6)	 4	100
3 ^g	R ¹ = Me, R ² = H, R ³ = Ph 5	/ (4.5)	 6	100 (99)
4	R ¹ = Me, R ² = H, R ³ = Ph 5	MeOH 3 (6)	 7	100 ^d (90)
5	R ¹ = Cl, R ² = H, R ³ = Ph 8	MeOH 3 (24)	 9	100 (99)

Table 4. Cont.

Entry	R ¹ , R ² , R ³	R-OH, Time (h)	Product	Conversion ^b (%) (Yield q%)
6	R ¹ = R ² = H, R ³ = Ph 1	EtOH 10 (24)		100 ^d (89)
7	R ¹ = R ² = H, R ³ = Ph 1	<i>i</i> PrOH 12 (24)		100 ^d (88)
8	R ¹ = R ² = H, R ³ = Me 14	MeOH 3 (24)		67 ^e “(61) ^f [15]”
9	R ¹ = H, R ² = R ³ = Me 16	MeOH 3 (24)		93 “(90) [15]”
10	C ₆ H ₅ (CH ₂) ₂ C(CH ₃) ₂ 18	MeOH 3 (24)		74 “(64) [15]”
11	R ¹ = R ² = R ³ = H 20	MeOH 3 (24)		2 ^e [15] -
12 ^g	R ¹ = Me, R ² = R ³ = H 22	/ (24)		100 (99)
13	R ¹ = Me, R ² = R ³ = H 22	MeOH 3 (24)		10 ^d
14	R ¹ = Cl, R ² = R ³ = H 25	MeOH 3 (24)		-
15	R ¹ = Me, R ² = H, R ³ = Ph 5	TMSOEt 27 (24)		100 ^e “(89) [15]”

^a Reaction conditions: alcohol (0.5 mmol), NIS (2–10 mol %), MeOH, EtOH, *i*PrOH, or TMSOEt (0.55 mmol–1 mL), 50–85 °C, 3.5–24 h.^b Determined by ¹H NMR spectroscopy. ^c Isolated yields. ^d Oxidized alcohol and dimeric ether 4–6%. ^e Oxidized alcohol 2–9%. ^f Specific rotation [α] = +15°. ^g Reaction was performed under SFRC.

Effective transformation with MeOH **3**, mediated by NIS, was observed in the reaction with additional derivatives of diphenylmethanol bearing EDG or EWG on the aromatic rings affording the corresponding products in excellent yields. We checked the reaction of phenyl(*p*-tolyl)methanol **5** with NIS as the mediator, in the absence or in the presence of MeOH, wherein in both cases we established the quantitative formation of the dimeric ether **6** (entry 3, Table 4) or the corresponding ether **7** (entry 4, Table 4). Etherification of (4-chlorophenyl)(phenyl)methanol **8** with MeOH **3**, mediated by NIS under HCRC, provided the corresponding product **9** in excellent yield (entry 5, Table 4).

Furthermore, we performed the reaction of diphenylmethanol **1** catalyzed by NIS, in the presence of EtOH **10**, under HCRC, which efficiently and successfully resulted in the formation of the corresponding ether **11**, accompanied by a small amount of dimeric ether **2** and oxidized alcohol (entry 6, Table 4). The transformation of diphenylmethanol **1** catalyzed by NIS in isopropyl solution (*i*-PrOH) **12**, provided the corresponding ether **13** in high yield, accompanied by a small amount of oxidized alcohol (entry 7, Table 4).

The effective and selective transformation was observed in the reaction with tertiary benzyl and alkyl alcohols. 2-Phenylpropan-2-ol **16** was readily mediated by NIS under HCRC to afford quantitative yields of target ether **17** in methanol solution [15] (entry 9, Table 4). α,α -dimethylbenzenepropanol **18** catalyzed by NIS, in the presence of MeOH **3** under HCRC was successfully converted into their corresponding ether **19** [15] (entry 10, Table 4).

In the case of 1,1-diphenylethanol in the presence of a substoichiometric amount of NIS (0.5 mol% NIS, 70–75 °C, 24 h) under SFRC, 1,1-diphenylethene in the quantitative yield was observed, while in the presence of MeOH, EtOH, and *i*-PrOH increasing the amount of NIS up to 5 mol%, under HCRC, the formation of the corresponding alkene in high yield, accompanied by the formation of benzophenone (12–30%) was observed. We further investigated reactions of primary benzyl alcohols bearing EWG and EDG on the aromatic ring with MeOH under HCRC. In the case of primary unsubstituted benzyl alcohol **20**, in the presence of MeOH, mediated by NIS, and under HCRC, only a trace amount of benzaldehyde was observed [15] (entry 11, Table 4). The transformation of 4-methylbenzyl alcohol **22** catalyzed by NIS in the absence of MeOH, under SFRC, was efficiently and selectively converted into the dimeric ether **23** (entry 12, Table 4). Etherification of 4-methylbenzyl alcohol **22** with MeOH under HCRC or in solution, using NIS as the precatalyst was not efficient (entry 13, Table 4). 4-chlorobenzyl alcohol **25** in the presence of MeOH, mediated by NIS was unable to undergo conversion (entry 14, Table 4).

Furthermore, we performed the reaction of phenyl(*p*-tolyl)methanol **5** with NIS as the mediator in the presence of ethoxytrimethylsilane (TMSOEt) **26** as the nucleophile source, where the ethoxy functional group was introduced efficiently into the organic molecule **27** [15], (entry 15, Table 4).

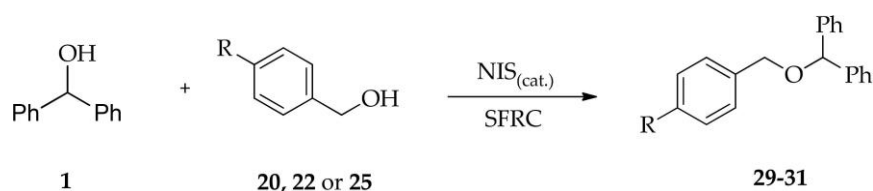
Additionally, to verify the synthetic value of the reported procedure, synthesis of (methoxymethylene)dibenzene **4** was accomplished at 10 mmol scale with high yield.

It was reported that the I–N bond of NIS as a precatalyst was activated by its reaction with the addition of alcohol. Consequently, it seems possible that transient halogen bonding could be necessary for the catalytic activity of NIS. Based on all the presented results, a potential explanation indicates the formation of HOI from the decomposition of NIS as the precatalyst by its reaction with the addition of alcohol [20–23]. The halogen bonding adducts are not the activated species. Instead, halonium (X^+) transfer will generate the intermediate resulting succinimide anion and HOI, promoting the etherification reaction.

HOI decomposes, forming I_2 and HIO_3 . Iodine is well known for forming HOI and HI in aqueous reaction media, providing the regeneration of HOI for further activity as a catalyst. It could be seen that the formation of water through the etherification reaction as the only by-product of the reaction could accelerate the reaction. The assumption that NIS was a precatalyst providing HOI, I_2 , and protons during the system, which could correspond to nucleophilic substitution acceleration, was indicated to be reasonable [15,18]. To get insight into the precatalyst's thermal stability, thermal gravimetric analysis (TGA)

on the NIS was accomplished. It was observed that degradation of the NIS did not occur at 25–200 °C [15].

To further extend this etherification protocol's scope, we studied the impact of NIS as the precatalyst for direct etherification of diphenylmethanol **1** with primary benzyl alcohols bearing an electron-withdrawing, as well as electron-donating, substituent on the aromatic ring under SFRC (Scheme 6). The results are collected in Table 5. Direct etherification of diphenylmethanol **1** with unsubstituted benzyl alcohol **20** was carried out using NIS as the precatalyst under SFRC, leading to the corresponding ether **29** in high yield. In contrast, a small amount of oxidized alcohol and aldehyde were detected as side products (entry 1, Table 5). In the case of the reaction of diphenylmethanol **1** with 4-methylbenzyl alcohol **22** using NIS as the precatalyst under SFRC, the formation of the corresponding ether **30** in good yield, with a small amount of oxidized alcohol and dimeric ether **2** as side products (entry 2, Table 5), was detected. In the case of the reaction of diphenylmethanol **1** with 4-chlorobenzyl alcohol **25**, mediated by NIS under SFRC, we observed the quantitative conversion of the starting material **1** into the corresponding ether **31**, accompanied by a small amount of oxidized alcohol (entry 3, Table 5).



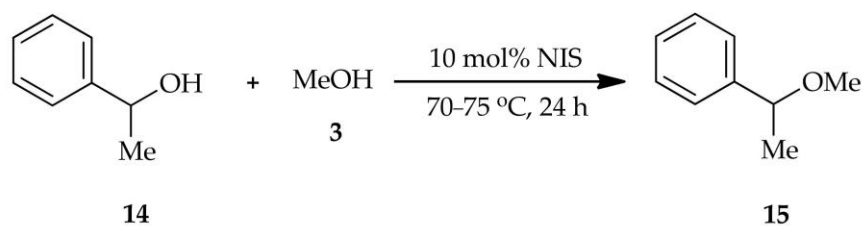
Scheme 6. Direct etherification of diphenylmethanol **1** with primary benzyl alcohols **20**, **22**, and **25**, mediated by NIS under SFRC.

Table 5. Direct etherification of diphenylmethanol **1** with primary benzyl alcohols **20**, **22**, and **25**, mediated by NIS under SFRC ^a.

Entry.	R	Conversion. ^b (%) of 1 (Yield ^c %)
1	H	100 ^d (88)
2	4-Me	96 ^e (73) ^d
3	4-Cl	100 ^d (90)

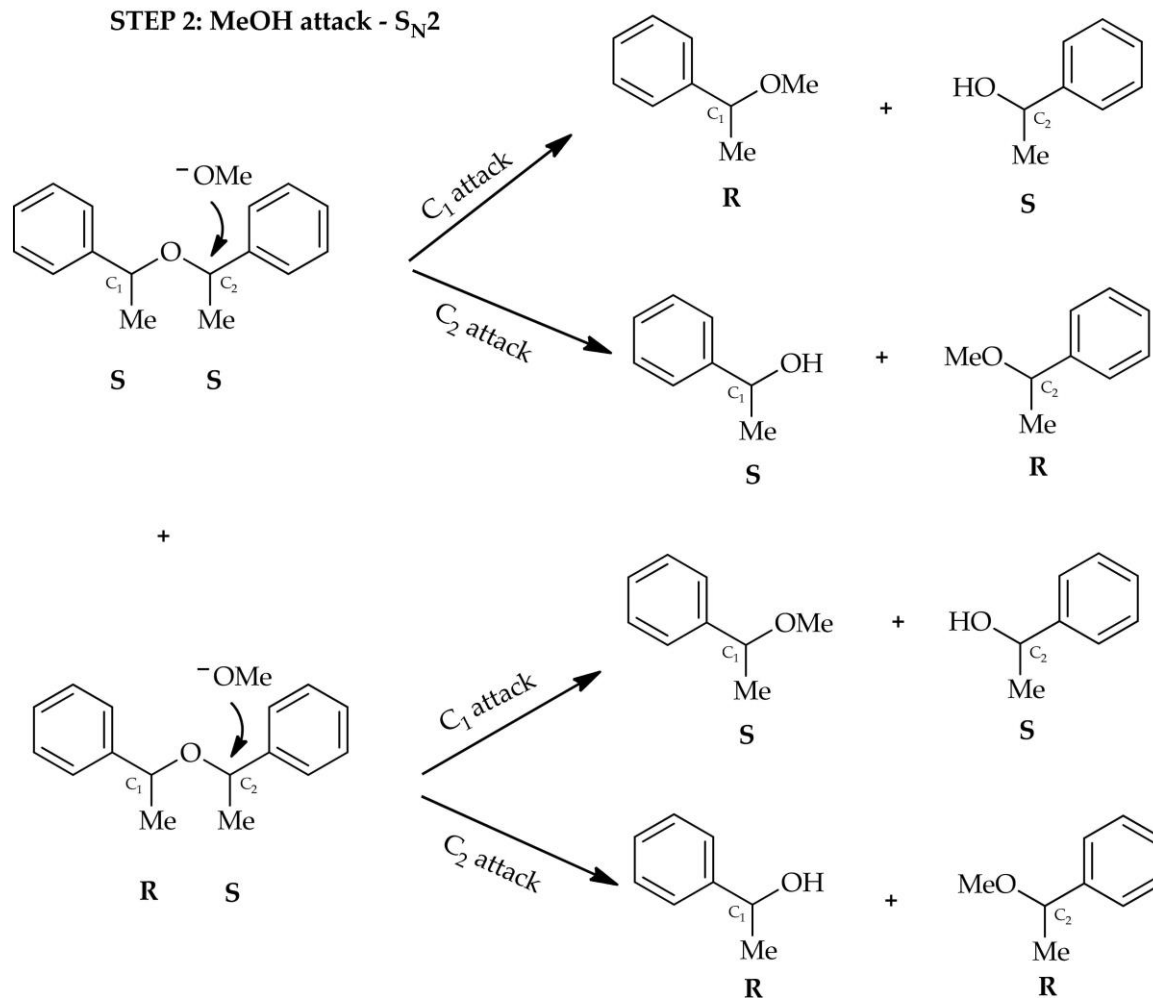
^a Reaction conditions: Diphenylmethanol **1** (0.5 mmol), primary benzyl alcohols **20**, **22**, and **25** (0.65 mmol), NIS (6–10 mol%), 70–75 °C, 24 h. ^b Determined by ¹H NMR spectroscopy. ^c Yields calculated relative to alcohol **1**. Values in parentheses are isolated yields. ^d Oxidized alcohol and aldehyde 6–10%. ^e Oxidized alcohol and dimeric ether 18%.

We previously presented the stereochemical pathway of the etherification between (S)-(-)-1-phenylethanol **14** and MeOH **3**, mediated by NIS, affording the corresponding ether **15** in moderate yield (entry 8, Table 4) and accompanied by a small amount of oxidized alcohol. The specific rotation of pure product **15** provided the value $[\alpha] = +15^\circ$, disclosing that we are not dealing with totally S_N1 or S_N2 cases but with the combination of both. It could be seen that the dimerization process is the S_N1 and the final etherification is the S_N2 mechanism, (Scheme 7).



STEP 1: Ether formation - S_N1

STEP 2: MeOH attack - S_N2



Scheme 7. Plausible reaction mechanism.

3. Materials and Methods

Chemicals used for synthetic methods were provided from commercial resources (Sigma Aldrich, St. Louis, MO, USA; Merck, Darmstadt, Germany; Fluka, Seelze, Germany). Reactions were observed by thin-layer chromatography (mobile phase: dichloromethane/hexane 9:1) with silica gel coated plates (Silica gel/TLC cards; DC-Alufolien-Kieselgel, Sigma Aldrich, St. Louis, MO, USA), and detected by UV (Camag, Muttensz, Switzerland) lamp (254 nm). Column chromatography (CC) was performed using silica gel Kieselgel 60 (Fluka, Sigma-Aldrich, St. Louis, MO, USA, particle size: 0.063–0.200 mm). Using a Varian INOVA300 NMR, Ljubljana, Slovenia instrument, ¹H and ¹³C NMR spectra were recorded using CDCl₃ as the solvent with SiMe₄ (TMS) as an internal reference. Melting points were measured using Buchi-Melting Point M-560 equipment, BUCHI Switzerland.

General procedure for etherification of alcohols mediated by NIS on half mmol scale: A mixture of benzyl alcohol (0.5 mmol), and *N*-iodosuccinimide as a mediator (3–10 mol%), which had been powdered in a mortar in the case of solid-state reactants, was placed in a 4 mL screw-capped vial, followed by adding liquid component alkyl alcohol (1 mmol-1 mL) and heated to 70–75 °C for 6 h–24 h. TLC detected the progress of the reaction mixture. After cooling down to room temperature the mixture was diluted with ethyl acetate (15 mL), washed thoroughly with Na₂S₂O₃ (2 × 3 mL), NaHCO₃ (2 × 3 mL), and distilled water (2 × 5 mL), and dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure, and the crude reaction mixture obtained was determined by ¹H NMR.

The scale-up procedure for the synthesis of (Methoxymethylene)dibenzene **4** with MeOH **3**, mediated by NIS: A mixture of diphenylmethanol **1** (10 mmol, 2.2425 g), 3 mol% NIS (67.5 mg, 0.3 mol), which had been previously powdered in a mortar, was transferred to a 20 mL screw-capped glass scintillation vial, MeOH **3** (20 mmol, (800 µL) was added, and heated at 70–75 °C for 6 h. TLC followed the progress of the reaction mixture. Upon completion of the reaction, the mixture was cooled to room temperature. Finally, the crude reaction mixture was purified by column chromatography to obtain a pure product in excellent yield (colorless oil, 2.1725 g, and 90%).

4. Conclusions

In conclusion, we have presented an efficient, selective, one-pot, metal-free methodology for direct C–O bond formation from readily available alcohols, using NIS as a metal-free and easy-to-handle precatalyst under HCRC. In the case of 1,1-diphenylethanol mediated by NIS under SFRC, dehydration resulting in 1,1-diphenylethene was observed, while phenyl-substituted primary and secondary alcohols under the same conditions gave dimeric ethers. Phenyl-substituted primary, secondary, and tertiary alcohols under HCRC gave alkyl ethers, while under the same conditions 1,1-diphenylethanol gave 1,1-diphenylethene. The large scale synthesis of methoxymethylene)dibenzene **4** was performed, with excellent yield. Moreover, etherification could also be achieved by cross coupling two different benzyl alcohols and mediated by NIS as the precatalyst under SFRC.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/catal11070858/s1>, General Information, Optimisation of Reaction Conditions, Scheme S1. The conversion of diphenylmethanol **1** mediated by NIS under SFRC, Figure S1: The effect of loading of NIS on the conversion of diphenylmethanol **1** under SFRC, Scheme S2. The conversion of diphenylmethanol **1** in the presence of MeOH **3** mediated by NIS under HCRC, Figure S2: The effect of loading of NIS on the conversion of diphenylmethanol **1** with MeOH **3** under HCRC, Scheme S3. The conversion of diphenylmethanol **1** in the presence of MeOH **3** mediated by NIS under HCRC, Figure S3: The catalytic effect of NIS on the conversion of diphenylmethanol **1** with MeOH **3** based on temperature, under HCRC, Characterization Data of Isolated Final Products, Measurements of Specific Rotation, ¹H NMR and ¹³C NMR Spectra of Isolated Final Products, Figure S4. ¹H NMR and ¹³C NMR spectra for (methoxymethylene)dibenzene (**4**), Figure S5: ¹H NMR and ¹³C NMR spectra for () 4,4'-(oxybis(phenylmethylene))bis(methylbenzene) (**6**), Figure S6: ¹H NMR and ¹³C NMR spectra for () 1-(methoxy(phenyl)methyl)-4-methylbenzene (**7**), Figure S7: ¹H NMR and ¹³C NMR spectra for 1-chloro-4-(methoxy(phenyl)methyl)benzene (**9**), Figure S8: ¹H NMR and ¹³C NMR spectra for (ethoxymethylene)dibenzene (**11**), Figure S9: ¹H NMR and ¹³C NMR spectra for isopropoxydiphenylmethane (**13**), Figure S10: ¹H NMR and ¹³C NMR spectra for 4,4'-(oxybis(methylene))bis(methylbenzene) (**23**), Figure S11: ¹H NMR and ¹³C NMR spectra for ((benzyloxy)methylene)dibenzene (**29**), Figure S12: ¹H NMR and ¹³C NMR spectra for (((4-methylbenzyl)oxy)methylene)dibenzene (**30**), Figure S13: ¹H NMR and ¹³C NMR spectra for (((4-chlorobenzyl)oxy)methylene)dibenzene (**31**), Figure S14: Thermal Gravimetric (TG) analysis of the NIS. References [24–33] are cited in the Supplementary Materials.

Author Contributions: Conceptualization, S.S.; formal analysis, N.A. and S.S.; Investigation, N.A. and S.S.; methodology, N.A. and S.S.; writing—original draft, N.A. and S.S.; writing—review and editing, N.A. and S.S. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available in the Supplementary Materials.

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Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Article

Direct Cross-Coupling of Alcohols with O-Nucleophiles Mediated by N-Iodosuccinimide as a Precatalyst under Mild Reaction Conditions

Njomza Ajvazi ^{1,2,*}  and Stojan Stavber ^{1,3} ¹ Jožef Stefan International Postgraduate School, Jamova 39, 1000 Ljubljana, Slovenia; stojan.stavber@ijs.si² Alma Mater Europaea Campus College "REZONANCA", 10000 Pristina, Kosovo³ Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

* Correspondence: njomza.ajvazi@rezonanca-rks.com; Tel.: +383-44-258-553

Abstract: We report *N*-iodosuccinimide as the most efficient and selective precatalyst among the *N*-halosuccinimides for dehydrative *O*-alkylation reactions between various alcohols under high-substrate concentration reaction conditions. The protocol is non-metal, one-pot, selective, and easily scalable, with excellent yields; enhancing the green chemical profiles of these transformations.

Keywords: alcohols; *N*-iodosuccinimide; cross-coupling; etherification; green chemistry



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1. Introduction

The C–O bond construction is one of the significant implements in organic synthesis since it provides access to the production of cosmetics, fragrances, pharmaceuticals, and dyestuffs [1]. Since alcohols are inexpensive and could effectively serve as alkylating agents, their direct cross-coupling with *O*-nucleophiles could be a very desirable strategy for avoiding an additional synthetic step for coupling reactions.

Activation of alcohols for nucleophilic substitution using a substoichiometric amount of different catalysts, such as metal ions: [RuCp(o-EtOdppe)](OTs), CuBr₂, NaAuCl₄, Brønsted, or Lewis acids (hypervalent [bis(trifluoroacetoxy)iodo]benzene catalyst has been found to function as a Lewis acid) or other supporters such as organohalides or molecular iodine in the presence of solvents has been touched on by some reviews [2–10] and recently developed reports [11–14]. However, the requirement for toxic and costly reagents, environmentally inappropriate solvents, multiple synthetic steps, and high temperatures make such a synthetic protocol less desirable from a sustainability perspective. Therefore, these disadvantages have challenged chemists to invent and develop novel methods for direct dehydrative C–O coupling, using alcohols as electrophiles under environmentally benign conditions.

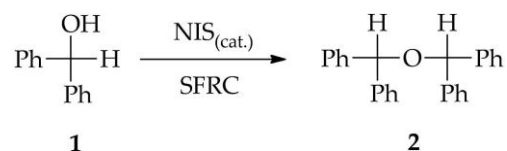
We previously reported the role of *N*-halosuccinimides (NXSs) (chloro, bromo, or iodo) as the mediator for the direct conversion of a hydroxyl group, forming new C–C or C-heteroatom bonds. *N*-iodosuccinimide (NIS) was the most efficient and selective mediator among the NXSs [15].

With our continuous research work on improving and developing greener protocols [16–19], we now report the expanding role of NIS as a commercially available, metal-free, and easy-to-handle precatalyst for direct dehydrative *O*-alkylation reactions between various alcohols under high-substrate concentration reaction conditions (HCRC), and leading to a practical, efficient, selective, and easily scalable method.

A high-concentration reaction condition in our case is defined as a concentration where less than 0.2 mL of solvent/mmol of substrate and reagent was used.

2. Results and Discussion

We began our investigations by using diphenylmethanol **1** with NIS as the precatalyst under solvent-free reaction conditions (SFRC), where the dimerization process was observed (Scheme 1), resulting in the formation of symmetric ether **2** (entry 1, Table 1). For the transformation of diphenylmethanol **1** without NIS, in the presence of MeOH **3** under HCRC, no conversion of the starting material was observed [15].



Scheme 1. The conversion of diphenylmethanol **1** mediated by NIS under SFRC.

Table 1. The effect of the loading of NIS on the conversion of diphenylmethanol **1** under SFRC ^a.

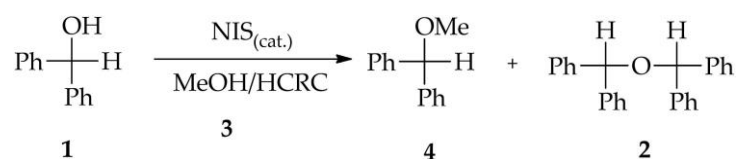
Entry	NIS (mol %)	Conv. ^b (%) of 1	Yield ^c (%) 2
1	0.5	79	78 ^d
2	1	90	89 ^d
3	2	100	100

^a Reaction conditions: diphenylmethanol **1** (0.5 mmol), 70–75 °C, 3.5 h. ^{b,c} Determined by ¹H NMR spectroscopy. ^d Benzophenone 1%.

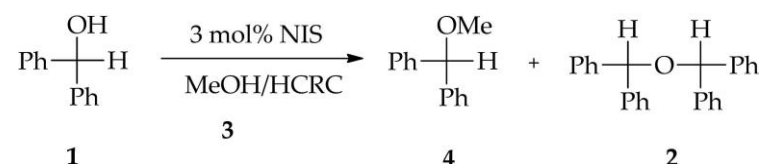
The effect of NIS loading for the reaction of diphenylmethanol **1** in the absence of a nucleophile source under SFRC was examined, and the results are presented in Table 1, and in Supplementary Materials To increase the yield of the product, different concentrations of the precatalyst were used. Employing 0.5 mol% of the precatalyst, the formation of the corresponding product **2** with good yield was observed, accompanied by a trace of oxidized alcohol (entry 1). By increasing the precatalyst loading to 1 mol%, we observed the high conversion of the starting material **1** into the corresponding product **2**, accompanied by a trace of oxidized alcohol (entry 2). We found that for the quantitative conversion of the starting material **1** to the dimeric ether **2**, 2 mol% of the precatalyst was effective (entry 3).

Furthermore, we continued our investigations by heating diphenylmethanol **1** with methanol (MeOH) **3** mediated by NIS under HCRC, affording the corresponding ether **4** in excellent yield [15]. (Scheme 1).

In searching for the optimal reaction conditions, different parameters, such as loading of the NIS as the mediator and temperature for the reaction of diphenylmethanol **1** with MeOH **3** under HCRC (Schemes 2 and 3), were first examined, and the results are presented in the Tables 2 and 3 and in Supplementary Materials. To determine the effectiveness of the NIS as the mediator, different concentrations of the precatalyst were employed. In the reaction of diphenylmethanol **1** with MeOH **3** using 0.5 mol% of the precatalyst, the formation of the corresponding product **4** in good yield and accompanied by a small amount of symmetric ether **2** was observed (entry 1). By increasing the precatalyst loading to 1 mol%, quantitative conversion of the starting material **1** into the corresponding product **4** was noticed, accompanied by a small amount of dimeric ether **2** (entry 2). Similar results were achieved when the precatalyst loading was increased up to 2 mol% (entry 3). An improvement of 100% yield, without forming the dimeric ether was attained by increasing the precatalyst loading up to 3 mol% (entry 4).



Scheme 2. The conversion of diphenylmethanol **1** in the presence of MeOH **3** mediated by NIS under HCRC.



Scheme 3. The conversion of diphenylmethanol **1** in the presence of MeOH **3** mediated by NIS under HCRC.

Table 2. The effect of the loading of NIS on the conversion of diphenylmethanol **1** with MeOH **3** under HCRC ^a.

Entry	NIS (mol %)	Conv. ^b (%) of 1	Relative Distribution ^c (%)	
			4	2
1	0.5	83	78	5
2	1	100	94	6
3	2	100	95	5
4	3	100	100	/

^a Reaction conditions: diphenylmethanol **1** (0.5 mmol), MeOH **3** (1 mmol), 70–75 °C, 6 h. ^b Determined by ¹H NMR spectroscopy. ^c Yields calculated relative to alcohol **1**.

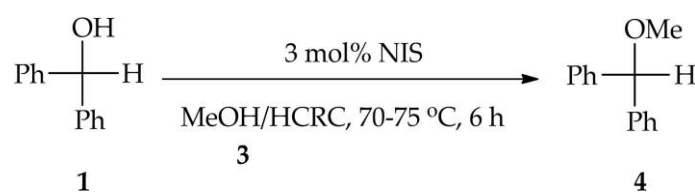
Table 3. The catalytic effect of NIS on the conversion of diphenylmethanol **1** with MeOH **3** based on the temperature under HCRC ^a.

Entry	T(°C)	Conv. ^b (%) of 1	Relative Distribution ^c (%)	
			4	2
1	rt	/	/	/
2	40–45	26	23	3
3	50–55	61	56	5
4	60–65	86	81	3 ^d
5	70–75	100	100	/

^a Reaction conditions: diphenylmethanol **1** (0.5 mmol), MeOH **3** (1 mmol), 6 h. ^b Determined by ¹H NMR spectroscopy. ^c Yields calculated relative to alcohol **1**. ^d Benzophenone 2%. rt—room temperature.

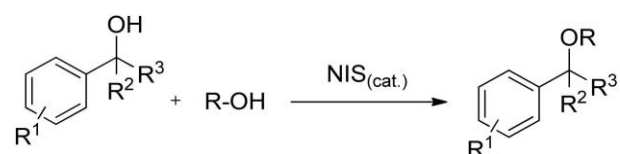
The effect of temperature on the course of the reaction was examined by the reaction of diphenylmethanol **1** with MeOH **3** mediated by NIS under HCRC. When the reaction was carried out at room temperature, no transformation took place (entry 1). When the reaction was performed at 40–45 °C, the desired product **4** in low yield and a small amount of dimeric ether **2** were obtained (entry 2). An increase of reaction temperature up to 50–55 °C provided the corresponding product **4** in moderate yield, accompanied by a small amount of symmetric ether **2** (entry 3). Further optimization revealed that the reaction could be performed smoothly by raising the temperature to 60–65 °C. An improvement by 100% yield without forming the symmetric ether was attained by increasing the reaction temperature to 70–75 °C.

Screening on precatalyst loading and reaction temperature showed that 3 mol% NIS, 70–75 °C, and 6 h were the best conditions ensuring complete conversion of **1** into the corresponding ether **4** (Scheme 4).



Scheme 4. Optimal reaction conditions for the conversion of diphenylmethanol **1** into the (methoxymethylene)dibenzene **4** mediated by NIS under HCRC.

Encouraged by these promising results, we applied the obtained optimal reaction conditions to direct dehydrative *O*-alkylation reactions between different alcohols mediated by NIS under HCRC (Scheme 5), and the results are collected in Table 4.

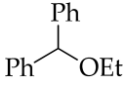
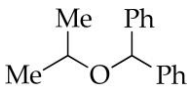
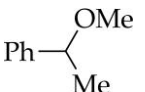
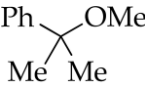
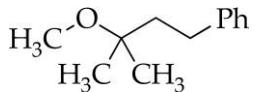
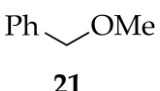
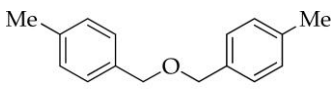
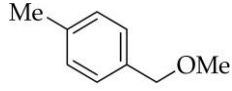
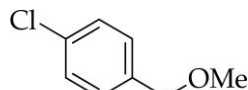
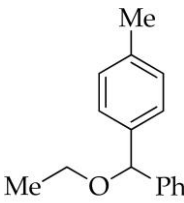


Scheme 5. Transformation of benzyl and tertiary alkyl alcohols with alkyl alcohols mediated by NIS under SFRC, under HCRC or in solution.

Table 4. Transformation of benzyl and tertiary alkyl alcohols with alkyl alcohols mediated by NIS under SFRC, under HCRC or in solution ^a.

Entry	R ¹ , R ² , R ³	R-OH, Time (h)	Product	Conversion ^b (%) (Yield %)
1 ^g	R ¹ = R ² = H, R ³ = Ph 1	/ (3.5)	 2	100 “(99) [15]”
2	R ¹ = R ² = H, R ³ = Ph 1	MeOH 3 (6)	 4	100
3 ^g	R ¹ = Me, R ² = H, R ³ = Ph 5	/ (4.5)	 6	100 (99)
4	R ¹ = Me, R ² = H, R ³ = Ph 5	MeOH 3 (6)	 7	100 ^d (90)
5	R ¹ = Cl, R ² = H, R ³ = Ph 8	MeOH 3 (24)	 9	100 (99)

Table 4. Cont.

Entry	R ¹ , R ² , R ³	R-OH, Time (h)	Product	Conversion ^b (%) (Yield q%)
6	R ¹ = R ² = H, R ³ = Ph 1	EtOH 10 (24)	 11	100 ^d (89)
7	R ¹ = R ² = H, R ³ = Ph 1	<i>i</i> PrOH 12 (24)	 13	100 ^d (88)
8	R ¹ = R ² = H, R ³ = Me 14	MeOH 3 (24)	 15	67 ^e “(61) ^f [15]”
9	R ¹ = H, R ² = R ³ = Me 16	MeOH 3 (24)	 17	93 “(90) [15]”
10	C ₆ H ₅ (CH ₂) ₂ C(CH ₃) ₂ 18	MeOH 3 (24)	 19	74 “(64) [15]”
11	R ¹ = R ² = R ³ = H 20	MeOH 3 (24)	 21	2 ^e [15] -
12 ^g	R ¹ = Me, R ² = R ³ = H 22	/ (24)	 23	100 (99)
13	R ¹ = Me, R ² = R ³ = H 22	MeOH 3 (24)	 24	10 ^d
14	R ¹ = Cl, R ² = R ³ = H 25	MeOH 3 (24)	 26	-
15	R ¹ = Me, R ² = H, R ³ = Ph 5	TMSOEt 27 (24)	 28	100 ^e “(89) [15]”

^a Reaction conditions: alcohol (0.5 mmol), NIS (2–10 mol %), MeOH, EtOH, *i*PrOH, or TMSOEt (0.55 mmol–1 mL), 50–85 °C, 3.5–24 h.^b Determined by ¹H NMR spectroscopy. ^c Isolated yields. ^d Oxidized alcohol and dimeric ether 4–6%. ^e Oxidized alcohol 2–9%. ^f Specific rotation [α] = +15°. ^g Reactions was performed under SFRC.

Effective transformation with MeOH **3**, mediated by NIS, was observed in the reaction with additional derivatives of diphenylmethanol bearing EDG or EWG on the aromatic rings affording the corresponding products in excellent yields. We checked the reaction of phenyl(*p*-tolyl)methanol **5** with NIS as the mediator, in the absence or in the presence of MeOH, wherein in both cases we established the quantitative formation of the dimeric ether **6** (entry 3, Table 4) or the corresponding ether **7** (entry 4, Table 4). Etherification of (4-chlorophenyl)(phenyl)methanol **8** with MeOH **3**, mediated by NIS under HCRC, provided the corresponding product **9** in excellent yield (entry 5, Table 4).

Furthermore, we performed the reaction of diphenylmethanol **1** catalyzed by NIS, in the presence of EtOH **10**, under HCRC, which efficiently and successfully resulted in the formation of the corresponding ether **11**, accompanied by a small amount of dimeric ether **2** and oxidized alcohol (entry 6, Table 4). The transformation of diphenylmethanol **1** catalyzed by NIS in isopropyl solution (*i*-PrOH) **12**, provided the corresponding ether **13** in high yield, accompanied by a small amount of oxidized alcohol (entry 7, Table 4).

The effective and selective transformation was observed in the reaction with tertiary benzyl and alkyl alcohols. 2-Phenylpropan-2-ol **16** was readily mediated by NIS under HCRC to afford quantitative yields of target ether **17** in methanol solution [15] (entry 9, Table 4). α,α -dimethylbenzenepropanol **18** catalyzed by NIS, in the presence of MeOH **3** under HCRC was successfully converted into their corresponding ether **19** [15] (entry 10, Table 4).

In the case of 1,1-diphenylethanol in the presence of a substoichiometric amount of NIS (0.5 mol% NIS, 70–75 °C, 24 h) under SFRC, 1,1-diphenylethene in the quantitative yield was observed, while in the presence of MeOH, EtOH, and *i*-PrOH increasing the amount of NIS up to 5 mol%, under HCRC, the formation of the corresponding alkene in high yield, accompanied by the formation of benzophenone (12–30%) was observed. We further investigated reactions of primary benzyl alcohols bearing EWG and EDG on the aromatic ring with MeOH under HCRC. In the case of primary unsubstituted benzyl alcohol **20**, in the presence of MeOH, mediated by NIS, and under HCRC, only a trace amount of benzaldehyde was observed [15] (entry 11, Table 4). The transformation of 4-methylbenzyl alcohol **22** catalyzed by NIS in the absence of MeOH, under SFRC, was efficiently and selectively converted into the dimeric ether **23** (entry 12, Table 4). Etherification of 4-methylbenzyl alcohol **22** with MeOH under HCRC or in solution, using NIS as the precatalyst was not efficient (entry 13, Table 4). 4-chlorobenzyl alcohol **25** in the presence of MeOH, mediated by NIS was unable to undergo conversion (entry 14, Table 4).

Furthermore, we performed the reaction of phenyl(*p*-tolyl)methanol **5** with NIS as the mediator in the presence of ethoxytrimethylsilane (TMSOEt) **26** as the nucleophile source, where the ethoxy functional group was introduced efficiently into the organic molecule **27** [15], (entry 15, Table 4).

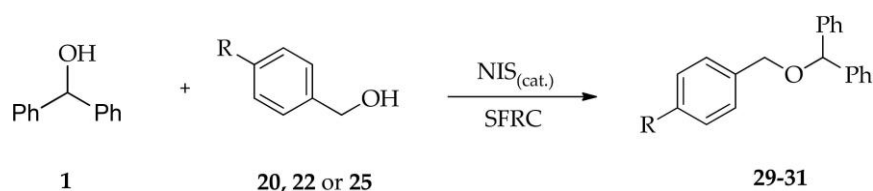
Additionally, to verify the synthetic value of the reported procedure, synthesis of (methoxymethylene)dibenzene **4** was accomplished at 10 mmol scale with high yield.

It was reported that the I–N bond of NIS as a precatalyst was activated by its reaction with the addition of alcohol. Consequently, it seems possible that transient halogen bonding could be necessary for the catalytic activity of NIS. Based on all the presented results, a potential explanation indicates the formation of HOI from the decomposition of NIS as the precatalyst by its reaction with the addition of alcohol [20–23]. The halogen bonding adducts are not the activated species. Instead, halonium (X^+) transfer will generate the intermediate resulting succinimide anion and HOI, promoting the etherification reaction.

HOI decomposes, forming I_2 and HIO_3 . Iodine is well known for forming HOI and HI in aqueous reaction media, providing the regeneration of HOI for further activity as a catalyst. It could be seen that the formation of water through the etherification reaction as the only by-product of the reaction could accelerate the reaction. The assumption that NIS was a precatalyst providing HOI, I_2 , and protons during the system, which could correspond to nucleophilic substitution acceleration, was indicated to be reasonable [15,18]. To get insight into the precatalyst's thermal stability, thermal gravimetric analysis (TGA)

on the NIS was accomplished. It was observed that degradation of the NIS did not occur at 25–200 °C [15].

To further extend this etherification protocol's scope, we studied the impact of NIS as the precatalyst for direct etherification of diphenylmethanol **1** with primary benzyl alcohols bearing an electron-withdrawing, as well as electron-donating, substituent on the aromatic ring under SFRC (Scheme 6). The results are collected in Table 5. Direct etherification of diphenylmethanol **1** with unsubstituted benzyl alcohol **20** was carried out using NIS as the precatalyst under SFRC, leading to the corresponding ether **29** in high yield. In contrast, a small amount of oxidized alcohol and aldehyde were detected as side products (entry 1, Table 5). In the case of the reaction of diphenylmethanol **1** with 4-methylbenzyl alcohol **22** using NIS as the precatalyst under SFRC, the formation of the corresponding ether **30** in good yield, with a small amount of oxidized alcohol and dimeric ether **2** as side products (entry 2, Table 5), was detected. In the case of the reaction of diphenylmethanol **1** with 4-chlorobenzyl alcohol **25**, mediated by NIS under SFRC, we observed the quantitative conversion of the starting material **1** into the corresponding ether **31**, accompanied by a small amount of oxidized alcohol (entry 3, Table 5).



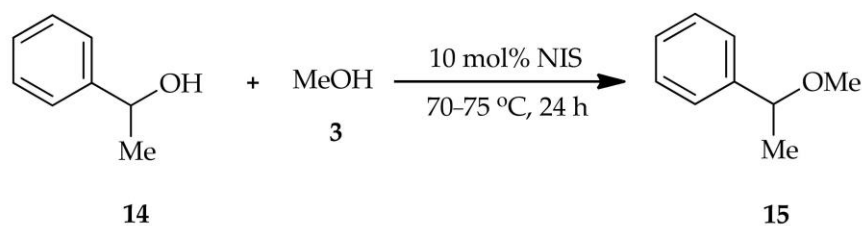
Scheme 6. Direct etherification of diphenylmethanol **1** with primary benzyl alcohols **20**, **22**, and **25**, mediated by NIS under SFRC.

Table 5. Direct etherification of diphenylmethanol **1** with primary benzyl alcohols **20**, **22**, and **25**, mediated by NIS under SFRC ^a.

Entry.	R	Conversion. ^b (%) of 1 (Yield %)
1	H	100 ^d (88)
2	4-Me	96 ^e (73) ^d
3	4-Cl	100 ^d (90)

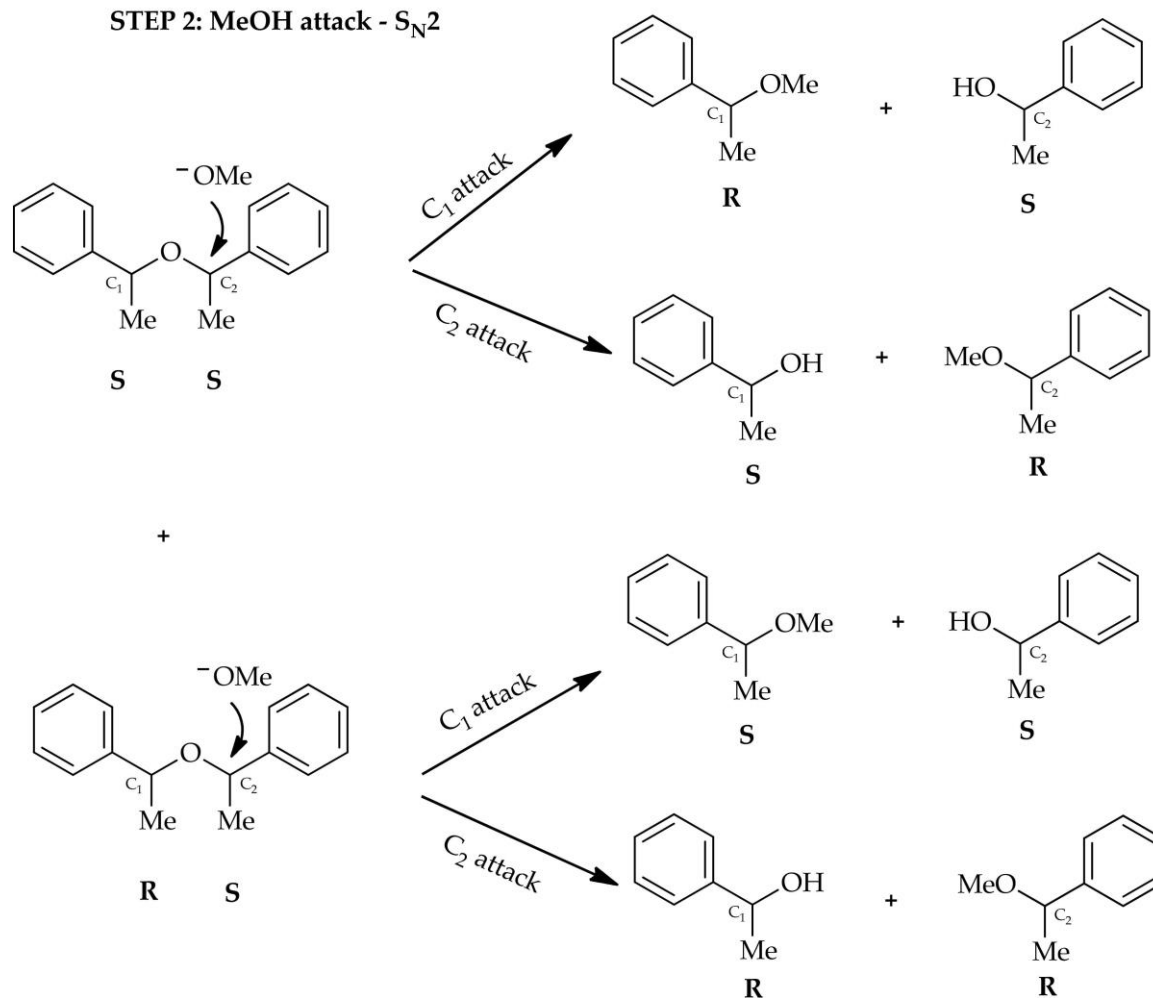
^a Reaction conditions: Diphenylmethanol **1** (0.5 mmol), primary benzyl alcohols **20**, **22**, and **25** (0.65 mmol), NIS (6–10 mol%), 70–75 °C, 24 h. ^b Determined by ¹H NMR spectroscopy. ^c Yields calculated relative to alcohol **1**. Values in parentheses are isolated yields. ^d Oxidized alcohol and aldehyde 6–10%. ^e Oxidized alcohol and dimeric ether 18%.

We previously presented the stereochemical pathway of the etherification between (S)-(-)-1-phenylethanol **14** and MeOH **3**, mediated by NIS, affording the corresponding ether **15** in moderate yield (entry 8, Table 4) and accompanied by a small amount of oxidized alcohol. The specific rotation of pure product **15** provided the value $[\alpha] = +15^\circ$, disclosing that we are not dealing with totally S_N1 or S_N2 cases but with the combination of both. It could be seen that the dimerization process is the S_N1 and the final etherification is the S_N2 mechanism, (Scheme 7).



STEP 1: Ether formation - S_N1

STEP 2: MeOH attack - S_N2



Scheme 7. Plausible reaction mechanism.

3. Materials and Methods

Chemicals used for synthetic methods were provided from commercial resources (Sigma Aldrich, St. Louis, MO, USA; Merck, Darmstadt, Germany; Fluka, Seelze, Germany). Reactions were observed by thin-layer chromatography (mobile phase: dichloromethane/hexane 9:1) with silica gel coated plates (Silica gel/TLC cards; DC-Alufolien-Kieselgel, Sigma Aldrich, St. Louis, MO, USA), and detected by UV (Camag, Muttensz, Switzerland) lamp (254 nm). Column chromatography (CC) was performed using silica gel Kieselgel 60 (Fluka, Sigma-Aldrich, St. Louis, MO, USA, particle size: 0.063–0.200 mm). Using a Varian INOVA300 NMR, Ljubljana, Slovenia instrument, ¹H and ¹³C NMR spectra were recorded using CDCl₃ as the solvent with SiMe₄ (TMS) as an internal reference. Melting points were measured using Buchi-Melting Point M-560 equipment, BUCHI Switzerland.

General procedure for etherification of alcohols mediated by NIS on half mmol scale: A mixture of benzyl alcohol (0.5 mmol), and *N*-iodosuccinimide as a mediator (3–10 mol%), which had been powdered in a mortar in the case of solid-state reactants, was placed in a 4 mL screw-capped vial, followed by adding liquid component alkyl alcohol (1 mmol-1 mL) and heated to 70–75 °C for 6 h–24 h. TLC detected the progress of the reaction mixture. After cooling down to room temperature the mixture was diluted with ethyl acetate (15 mL), washed thoroughly with Na₂S₂O₃ (2 × 3 mL), NaHCO₃ (2 × 3 mL), and distilled water (2 × 5 mL), and dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure, and the crude reaction mixture obtained was determined by ¹H NMR.

The scale-up procedure for the synthesis of (Methoxymethylene)dibenzene **4** with MeOH **3**, mediated by NIS: A mixture of diphenylmethanol **1** (10 mmol, 2.2425 g), 3 mol% NIS (67.5 mg, 0.3 mol), which had been previously powdered in a mortar, was transferred to a 20 mL screw-capped glass scintillation vial, MeOH **3** (20 mmol, (800 μL) was added, and heated at 70–75 °C for 6 h. TLC followed the progress of the reaction mixture. Upon completion of the reaction, the mixture was cooled to room temperature. Finally, the crude reaction mixture was purified by column chromatography to obtain a pure product in excellent yield (colorless oil, 2.1725 g, and 90%).

4. Conclusions

In conclusion, we have presented an efficient, selective, one-pot, metal-free methodology for direct C–O bond formation from readily available alcohols, using NIS as a metal-free and easy-to-handle precatalyst under HCRC. In the case of 1,1-diphenylethanol mediated by NIS under SFRC, dehydration resulting in 1,1-diphenylethene was observed, while phenyl-substituted primary and secondary alcohols under the same conditions gave dimeric ethers. Phenyl-substituted primary, secondary, and tertiary alcohols under HCRC gave alkyl ethers, while under the same conditions 1,1-diphenylethanol gave 1,1-diphenylethene. The large scale synthesis of methoxymethylene)dibenzene **4** was performed, with excellent yield. Moreover, etherification could also be achieved by cross coupling two different benzyl alcohols and mediated by NIS as the precatalyst under SFRC.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/catal11070858/s1>, General Information, Optimisation of Reaction Conditions, Scheme S1. The conversion of diphenylmethanol **1** mediated by NIS under SFRC, Figure S1: The effect of loading of NIS on the conversion of diphenylmethanol **1** under SFRC, Scheme S2. The conversion of diphenylmethanol **1** in the presence of MeOH **3** mediated by NIS under HCRC, Figure S2: The effect of loading of NIS on the conversion of diphenylmethanol **1** with MeOH **3** under HCRC, Scheme S3. The conversion of diphenylmethanol **1** in the presence of MeOH **3** mediated by NIS under HCRC, Figure S3: The catalytic effect of NIS on the conversion of diphenylmethanol **1** with MeOH **3** based on temperature, under HCRC, Characterization Data of Isolated Final Products, Measurements of Specific Rotation, ¹H NMR and ¹³C NMR Spectra of Isolated Final Products, Figure S4. ¹H NMR and ¹³C NMR spectra for (methoxymethylene)dibenzene (**4**), Figure S5: ¹H NMR and ¹³C NMR spectra for () 4,4'-(oxybis(phenylmethylene))bis(methylbenzene) (**6**), Figure S6: ¹H NMR and ¹³C NMR spectra for () 1-(methoxy(phenyl)methyl)-4-methylbenzene (**7**), Figure S7: ¹H NMR and ¹³C NMR spectra for 1-chloro-4-(methoxy(phenyl)methyl)benzene (**9**), Figure S8: ¹H NMR and ¹³C NMR spectra for (ethoxymethylene)dibenzene (**11**), Figure S9: ¹H NMR and ¹³C NMR spectra for isopropoxydiphenylmethane (**13**), Figure S10: ¹H NMR and ¹³C NMR spectra for 4,4'-(oxybis(methylene))bis(methylbenzene) (**23**), Figure S11: ¹H NMR and ¹³C NMR spectra for ((benzyloxy)methylene)dibenzene (**29**), Figure S12: ¹H NMR and ¹³C NMR spectra for (((4-methylbenzyl)oxy)methylene)dibenzene (**30**), Figure S13: ¹H NMR and ¹³C NMR spectra for (((4-chlorobenzyl)oxy)methylene)dibenzene (**31**), Figure S14: Thermal Gravimetric (TG) analysis of the NIS. References [24–33] are cited in the Supplementary Materials.

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Article

N-Iodosuccinimide as a Precatalyst for Direct Cross-Coupling of Alcohols with C-Nucleophiles under Solvent-Free Reaction Conditions

Njomza Ajvazi ^{1,*}  and Stojan Stavber ^{1,2} ¹ Jožef Stefan International Postgraduate School, Jamova 39, 1000 Ljubljana, Slovenia; stojan.stavber@ijs.si² Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

* Correspondence: njomza.ajvazi@rezonanca-rks.com; Tel.: +383-44-258-553

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Abstract: C–C bond formation is one of the most important implements in synthetic organic chemistry. In pursuit of effective synthetic routes functioning under greener pathways to achieve direct C–C bond formation, we report *N*-iodosuccinimide (NIS) as the most effective precatalyst among the *N*-halosuccinimides (NXSs) for the direct cross-coupling of benzyl alcohols with C-nucleophiles under solvent-free reaction conditions (SFRC). The protocol is metal-free, and air- and water-tolerant, providing a large-scale synthesis with almost quantitative yields.

Keywords: alcohols; *N*-iodosuccinimide; C–C coupling; solvent-free reactions; green chemistry

1. Introduction

C–C bond formation is one of the most significant approaches for the synthesis of complex organic compounds such as pharmaceuticals and agrochemicals [1]. Alcohols are readily available and one of the most abundant organic compounds. Their employment for direct cross-coupling reactions with other partners is highly desirable, producing water as the only by-product, making the protocol environmentally benign. Since hydroxyl moieties are a weak leaving group, often an additional activation is unavoidable [2].

The hydroxyl group's activation employing a substoichiometric amount of Brønsted acids, metal ions, Lewis/Brønsted acid combinations, or other promoters has been shown by several excellent reviews [1,3–9] and recent advanced related reports [10–14]. Nevertheless, the necessity of hazardous or costly reagents, environmentally unfriendly solvents, multiple-step synthesis, a high amount of the catalyst, or high temperatures make such a synthetic procedure less preferable than the green chemical standpoint. Therefore, planning organic reactions and processes following the principles of green chemistry [15] is one of the main challenges in organic synthesis. As a result, solvent-free synthetic methods have attracted significant interest not only in laboratory synthesis but also in the chemical industry due to their simplicity and cost-efficiency. With this in mind, it is becoming essential to develop efficient, selective, and environmentally benign catalytic systems for direct dehydrative C–C coupling.

Recently, we presented the introduction of *N*-halosuccinimides (NXSs) (chloro, bromo, and iodo), as the catalyst for the transformation of organic compounds bearing the hydroxyl functional group in one pot, constructing new carbon–carbon or carbon–heteroatom bonds [16]. *N*-iodosuccinimide (NIS) in substoichiometric amounts was favored as the most effective and selective catalyst among the NXSs. In continuation of our research on developing greener synthetic transformation [17–19] herein, we reported the expanding role of NIS as a non-metal, commercially available, and eco-friendly precatalyst for direct dehydrative C–C coupling between various benzyl alcohols and different type of

electron-rich compounds including: β -diketones, phenyl substituted alkene, tertiary benzyl alcohols bearing a vicinal hydrogen atom as alkene precursors, and heteroatom benzocyclenes selectively and efficiently into the corresponding products, under solvent-free reaction conditions.

2. Results and Discussion

The coupling of diphenylmethanol **1** and 1,3-diphenylpropane-1,3-dione **2** was chosen as a model reaction to employ NIS as the catalyst in direct cross-coupling of β -diketones and alcohols and to study the effects of different reaction conditions. Initially, the impact of different solvents on the conversion of **1** with **2** was studied, where it seemed to be inconvenient for the conversion of **1** with **2**, while solvent-free reaction conditions were critical for quantitative transformation, and the results are shown in the Supplementary Materials (Table S1).

In search of optimal reaction conditions, different parameters, including the loading of the NIS as the catalyst, reaction time, reaction temperature for the reaction of diphenylmethanol **1** with **2** under solvent-free conditions, and the reactivity of alcohols, were first studied, and the results are given in the Supplementary Materials (Tables S2–S5). It could be seen that under the mentioned optimal reaction conditions, the reaction successfully proceeded only in the case of secondary benzyl alcohol **1** (entry 1, Table 1). In contrast, no reaction occurred with unsubstituted benzyl alcohol **5** as the type of primary alcohol, due to lower reactivity, and trityl alcohol **6** as the type of tertiary alcohol, which could be explained by the steric effect [16].

By further investigation of the crucial reaction parameters, such as the structure of the β -dicarbonyl compound, the structure of alcohol, and loading of the catalyst, showed us the real value of this methodology. Thus, the effect of the structure of β -diketone on the course of the reaction with **1** as the most reactive benzyl alcohol derivative was studied. We selected benzoylacetone as a further candidate where the addition of NIS as the catalyst was observed to promote effectively and selectively the conversion of **1** with benzoylacetone **7** under solvent-free reaction conditions (SFRC) into the corresponding product **8** (entry 5, Table 1).

To increase the yield of the corresponding product **8**, different concentrations of the catalyst and variations of reaction temperatures were applied, and the results are shown in the Supplementary Materials (Tables S6 and S7). In the absence of a precatalyst, no reaction occurred.

Given the mentioned experience, it seemed that acetylacetone could be the next instructive substrate for realizing the effect of the structure of a β -dicarbonyl compound to the course of the reaction, where the addition of NIS as the catalyst was found to provide efficient and selective transformation of **1** with acetylacetone **9** under SFRC into the corresponding product **10** (entry 7, Table 1). To increase the yield of the corresponding product **10**, different concentrations of the catalyst were applied, and the results are presented in the Supplementary Materials (Table S8). In the absence of a catalyst, no reaction took place.

According to these results, the most reactive of β -dicarbonyl compounds was 1,3-diphenylpropane-1,3-dione **2**, optimal loading of NIS as the catalyst was 1 mol%, reaction temperature was 70–75 °C, and reaction time was 24 h. Moreover, during the optimization of reaction conditions, we found that varying the temperature from 70–75 °C to 100–105 °C for the same reaction shortened the reaction time (6 h) and provided excellent yield.

We believed that the reaction's key point was the degree of enolization of β -diketone on one side, and stability of the carbocationic intermediate formed from the alcohol on another side. A higher degree of enolization (pK_E) caused more potent and particular transformation into the desired coupling between β -dicarbonyl and alcohol, and a more stabilized carbocationic intermediate caused more efficient and selective product formation.

Through solvation, stability and reactivity of enolates can be controlled. Enolates are more stable in polar protic solvents, such as water, than in polar aprotic solvents, such as dimethyl sulfoxide [20].

Encouraged by these promising results, we checked the scope of the reaction system by applying the obtained optimal reaction conditions on cross-coupling reactions of β -dicarbonyl compounds with

different alcohols catalyzed by NIS under SFRC. The results of reactions carried out under SFRC are collected in Table 1.

Table 1. Reactions of β -dicarbonyl compounds with different alcohols catalyzed by *N*-iodosuccinimide (NIS) under solvent-free reaction conditions (SFRC)^a.

Entry		R ¹ , R ² , R ³	NIS (Mol%)	Yield ^b (%)
1		R ¹ =R ² =H, R ³ =Ph	4	100 (99)
2		R ¹ =Me, R ² =H, R ³ =Ph	11	100 (99)
3		R ¹ =Cl, R ² =H, R ³ =Ph	13	100 (98)
4		R ¹ =OMe, R ² =R ³ =H	15	100 (98)
5		R ¹ =R ² =H, R ³ =Ph	1	100 (98)
6		R ¹ =Me, R ² =H, R ³ =Ph	11	100 (99)
7		R ¹ =R ² =H, R ³ =Ph	1	100 (98)
8		R ¹ =Me, R ² =H, R ³ =Ph	11	100 (99)
9		R ¹ =R ² =H, R ³ =Ph	1	100 (99)

^a Reaction conditions: alcohols (0.5 mmol), diketones (0.5 mmol), NIS (1–10 mol%), 70–75 °C, 23–24 h. ^b Determined from ¹H NMR spectra of isolated crude reaction mixtures; yield calculated relative to alcohol; values in parentheses are isolated yields.

Effective transformation with β -diketone **2** was observed in the reaction with additional classes of secondary benzyl alcohols, including derivatives of diphenylmethanol bearing electron-donating or

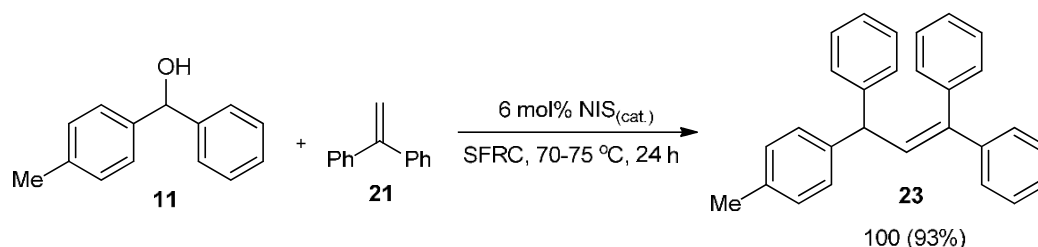
electron-withdrawing substituents on the aromatic rings **11** and **13**, and established the quantitative formation of the corresponding products **12** and **14** (entries 2 and 3, Table 1).

Furthermore, we checked the reaction of the primary benzyl alcohol bearing electron-donating group with **2**. 4-methoxybenzyl alcohol **15** was efficiently and selectively converted into the 2-(4-methoxybenzyl)-1,3-diphenylpropane-1,3-dione **16** (entry 4, Table 1). We further investigated the corresponding reactions of alcohols with benzoylacetone **7**. In the case of the reaction of primary benzyl alcohol **5** with **7** in the presence of NIS as the catalyst under solvent-free conditions, no transformation took place. Effective and selective transformation with **7** was observed in the reaction with phenyl(*p*-tolyl)methanol **11**, providing the quantitative formation of the corresponding product **17** (entry 6, Table 1). No transformation was observed with sterically bulkier triphenylmethanol **6** with **7** catalyzed by NIS under SFRC.

Moreover, we investigated the corresponding reactions of alcohols with acetylacetone **9**. In the case of the reaction of unsubstituted benzyl alcohol **5** with **9** catalyzed by NIS under SFRC, no transformation took place. Effective transformation of phenyl(*p*-tolyl)methanol **11** with **9** was observed and established the quantitative formation of the corresponding product **18** (entry 8, Table 1). No transformation was observed in the case of triphenylmethanol **6** with **9** catalyzed by NIS under SFRC. Additionally, the effective transformation was obtained between 1,3-bis(4-methoxyphenyl)propane-1,3-dione **19** and **1**, furnishing the respective product **20** in excellent yield (entry 9, Table 1).

To establish the synthetic value of the reported methodology, the following reaction was performed on the 10 mmol scales. Coupling of **1** with **2** under the above-mentioned optimal reaction conditions afforded the corresponding product **4** in quantitative yield (99%).

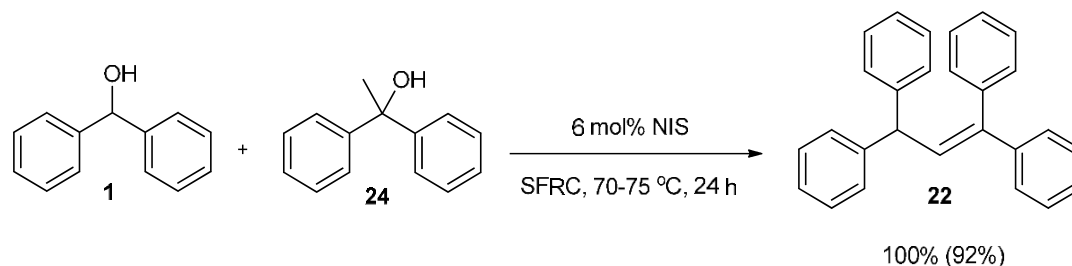
Inspired by these results, we investigated NIS's efficiency as the catalyst for the direct coupling of phenyl substituted alkene with secondary benzyl alcohol under solvent-free conditions. NIS, as an effective catalyst, was observed to promote the direct coupling of 1,1 diphenylethene **21** with phenyl(*p*-tolyl) methanol **11** under SFRC, producing the corresponding substituted alkene **23** in nearly quantitative yield (Scheme 1).



Scheme 1. Reaction of phenyl(*p*-tolyl)methanol **11** with 1,1 diphenylethene **21** catalyzed by NIS under SFRC.

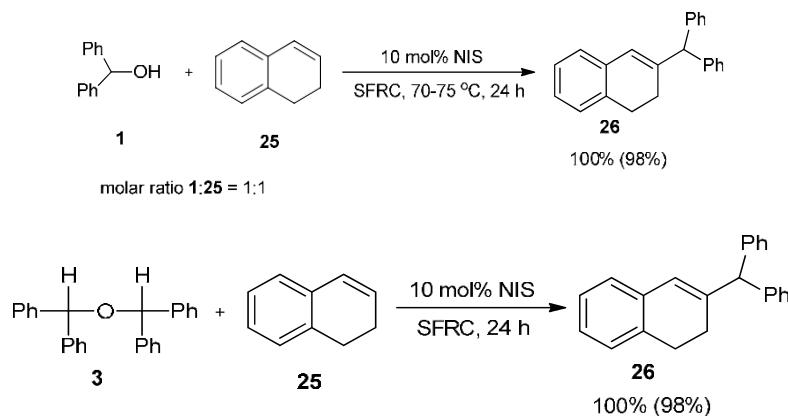
It is well-known that alkenes can be readily achieved by dehydration of tertiary alcohols bearing a vicinal hydrogen atom. Inspired by this finding, we performed the reaction between 1,1-diphenylethanol **24** and **1** catalyzed by NIS under SFRC. We observed that the direct coupling of two alcohols, **1** and **24**, forming the corresponding product **22** as the major product took place, accompanied by a trace amount of side products identified as benzophenone and dimeric ether **3**, (Scheme 2).

Electron-rich benzocyclenes with a general structure (X=NAc, O) were chosen as potential partners for the NIS-promoted reactions with benzyl alcohols under SFRC. The reaction of diphenylmethanol **1** with 1,2-dihydronaphthalene **25** catalyzed by NIS under SFRC performed the corresponding substituted alkene **26**, accompanied by a small amount of dimeric ether **3**, was observed. Different parameters such as the loading of the NIS as the catalyst and the reaction temperature for the reaction of **1** with 1,2-dihydronaphthalene **25** under SFRC were first studied, and the results are shown in the Supplementary Materials (Tables S8 and S9). Moreover, during the optimization of reaction conditions, we found that equimolar of **1** and **25** provided the best result in terms of efficiency and selectivity.



Scheme 2. Direct cross-coupling of diphenylmethanol **1** and 1,1-diphenylethanol **24** catalyzed by NIS under SFRC.

To verify the presumption that the symmetric ether **3** could be the intermediate of the cross-coupling [21], we independently performed the reaction between symmetric ether **3** as a source of benzylic cation, which was synthesized from **1** catalyzed by NIS (2 mol%) at 70–75 °C, for 3.5 h under SFRC. Then, alkylation of the dimeric ether **3** by dihydronaphthalene **25** took place, providing the corresponding product **26** in quantitative yield. Another possible pathway might be a direct reaction of carbocation derived from alcohol **1** and followed by nucleophile displacement (Scheme 3).



Scheme 3. Control reactions.

According to the results of the control reaction shown in Scheme 3, a potential explanation suggests a decomposition of the precatalyst NIS to form HOI in alcoholic reaction media, which could be responsible for NIS's mediation activity [22–25]. The X_2 forms HOI and HI in aqueous media, providing the regeneration of HOI for the following catalysis. Therefore, it was reasonable to assume that the water produced as the only by-product of the process could act as a supporting factor in the acceleration of the reaction, since no conversion was observed in the reaction between diphenylmethanol **1** and 1,3-diphenylpropane-1,3-dione **2** catalyzed by NIS in aqueous media. The presumption that NIS was actually a precatalyst forming HOI, I_2 , and protons during the process, which might catalyze nucleophilic substitutions, showed to be reasonable [16,26].

The generality of this methodology for C–C hetero coupling approaches with heteroatom benzocycles **27** and **29** with **1**, catalyzed by NIS under SFRC, was further examined. The results of the reactions carried out under SFRC are collected in Table 2. NIS catalyzed the direct coupling of **1** with an *N*-based heteroarene such as 1-acetylidole **23** under SFRC, affording C-3 alkylated product in high yield (**28**, entry 1, Table 2). The direct coupling of **1** with an *O*-containing heteroarene such as 2,3-benzofuran **29** underwent smooth coupling to provide the C-2 alkylated product in good yield (**30**, entry 2, Table 2). It could be seen that the electrophilic substitution in 1-acetylidole **27** took place at C-3 and not at C-2. This orientation could be explained by comparing the stability of the carbocation resulting from the electrophilic attack at C-2, which was less favorable than the carbocation from

the electrophilic attack at C-3. The electrophilic substitution at C-2 in benzofuran **29** could also be explained in the same way.

Table 2. Reactions of diphenylmethanol **1** with heteroarenes **27–29** catalyzed by NIS under SFRC ^a.

Reaction scheme: Diphenylmethanol (**1**) reacts with heteroarenes (**27–29**) in the presence of 6–10 mol% NIS under SFRC to form products (**28–30**).

	1	27–29		28–30	
Entry	X		Product 28–30		Yield (%)^b
1	NAc	27		28	91
2	O	29		30	61

^a Reaction conditions: diphenylmethanol **1** (0.5 mmol), heteroarenes **27–29** (0.4 mmol), 70–75 °C, 24 h. ^b Isolated products; yields calculated relative to heteroatom benzocycles.

To gain clearer insight into NIS's thermal stability, thermal gravimetric analysis (TGA) on the precatalyst was carried out. It was observed that the degradation did not happen to the precatalyst at temperatures 25–200 °C [16].

3. Materials and Methods

All starting materials and NIS were commercially available and used without further purification (Merck, Darmstadt, Germany; Sigma Aldrich, St. Louis, MO, USA). Reactions were performed in 4 mL screw-capped vials. All reactions were observed by thin-layer chromatography (TLC, mobile phase: dichloromethane/hexane 9:1) (with silica gel/TLC cards; DC-Alufolien-Kieselgel, Sigma-Aldrich, St. Louis, MO, USA) and visualized by UV lamp (254 nm, Camag, Muttenz, Switzerland). ¹H and ¹³C NMR spectra were measured on Varian INOVA 300 NMR instrument, using a solution of CDCl₃ as the solvent with SiMe₄ as an internal reference standard. Melting points were measured by Buchi 535 equipment. Purification of a few products was performed by column chromatography (CC) using silica gel 60 (particle size: 0.063–0.200 mm).

General procedure for new C–C bond construction through β-diketone or electron-rich C=C bonds in organic molecule catalyzed by NIS on half mmol scale:

The mixture of benzyl alcohol (0.5 mmol), β-diketone, or alkene (0.5 mmol) and NIS (1–10 mol%), after being powdered in a mortar in the case of solid-state reactants, was transferred to a 4 mL screw-capped vial and heated at 70–75 °C for 24 h. TLC monitored the progress of the reaction mixture. The crude reaction mixture was cooled down to room temperature and diluted with a mixture of (3 × 5 mL EtOAc), (2 × 3 mL saturated Na₂S₂O₃), (2 × 3 mL saturated NaHCO₃), and (2 × 5 mL distilled water). The organic phase was dried Na₂SO₄, and the organic solvent was removed under reduced pressure, leaving the resultant residue.

The scaled-up procedure for the synthesis of 2-benzhydryl-1,3-diphenylpropane-1,3-dione **4** catalyzed by NIS:

The mixture of β-diketone **2** (10 mmol, 1.8423 g), diphenylmethanol **1** (10 mmol, 2.2425 g), and NIS (1 mol%, 0.1 mol, 22.5 mg), which was powdered in a mortar, was transferred to a 20 mL screw-capped glass scintillation vial and heated at 70–75 °C for 24 h. TLC followed the progress of the reaction

mixture. Upon completion of the reaction, the mixture was cooled to room temperature. Finally, the crude reaction mixture was washed with hot water and filtered by vacuum filtration to obtain a pure product in almost quantitative yield (white solid, m.p. 220–223 °C, 3.9 g, 99%).

4. Conclusions

In summary, simple, efficient, selective, and easily scalable methodology for C–C bond formation through the direct cross-coupling of various benzyl alcohols with β -diketones, heteroatom benzocyclohexenes, phenyl substituted alkenes, or tertiary benzyl alcohols bearing a vicinal hydrogen atom as alkene precursors employing NIS, an environmentally friendly and metal-free precatalyst, under SFRC was developed. The reactivity of β -dicarbonyl compounds was found to be the function of their enolisability, whereas primary benzyl alcohol targets needed strong activation of the ring; a comprehensive range of secondary benzyl alcohols gave efficient coupling, whereas tertiary benzyl alcohols without vicinal hydrogen atoms were found as inconvenient targets. Benzylation of the position C-2 was observed in the case of 1,2-dihydronaphthalene **25** and 2,3-benzofuran **29**, whereas C-3 was benzylated in 1-acetylimidazole **27**.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4344/10/8/850/s1>, detailed experimental data, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra of isolated final products.

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Article

N-Iodosuccinimide as a Precatalyst for Direct Cross-Coupling of Alcohols with C-Nucleophiles under Solvent-Free Reaction Conditions

Njomza Ajvazi ^{1,*}  and Stojan Stavber ^{1,2} ¹ Jožef Stefan International Postgraduate School, Jamova 39, 1000 Ljubljana, Slovenia; stojan.stavber@ijs.si² Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

* Correspondence: njomza.ajvazi@rezonanca-rks.com; Tel.: +383-44-258-553

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Abstract: C–C bond formation is one of the most important implements in synthetic organic chemistry. In pursuit of effective synthetic routes functioning under greener pathways to achieve direct C–C bond formation, we report *N*-iodosuccinimide (NIS) as the most effective precatalyst among the *N*-halosuccinimides (NXSs) for the direct cross-coupling of benzyl alcohols with C-nucleophiles under solvent-free reaction conditions (SFRC). The protocol is metal-free, and air- and water-tolerant, providing a large-scale synthesis with almost quantitative yields.

Keywords: alcohols; *N*-iodosuccinimide; C–C coupling; solvent-free reactions; green chemistry

1. Introduction

C–C bond formation is one of the most significant approaches for the synthesis of complex organic compounds such as pharmaceuticals and agrochemicals [1]. Alcohols are readily available and one of the most abundant organic compounds. Their employment for direct cross-coupling reactions with other partners is highly desirable, producing water as the only by-product, making the protocol environmentally benign. Since hydroxyl moieties are a weak leaving group, often an additional activation is unavoidable [2].

The hydroxyl group's activation employing a substoichiometric amount of Brønsted acids, metal ions, Lewis/Brønsted acid combinations, or other promoters has been shown by several excellent reviews [1,3–9] and recent advanced related reports [10–14]. Nevertheless, the necessity of hazardous or costly reagents, environmentally unfriendly solvents, multiple-step synthesis, a high amount of the catalyst, or high temperatures make such a synthetic procedure less preferable than the green chemical standpoint. Therefore, planning organic reactions and processes following the principles of green chemistry [15] is one of the main challenges in organic synthesis. As a result, solvent-free synthetic methods have attracted significant interest not only in laboratory synthesis but also in the chemical industry due to their simplicity and cost-efficiency. With this in mind, it is becoming essential to develop efficient, selective, and environmentally benign catalytic systems for direct dehydrative C–C coupling.

Recently, we presented the introduction of *N*-halosuccinimides (NXSs) (chloro, bromo, and iodo), as the catalyst for the transformation of organic compounds bearing the hydroxyl functional group in one pot, constructing new carbon–carbon or carbon–heteroatom bonds [16]. *N*-iodosuccinimide (NIS) in substoichiometric amounts was favored as the most effective and selective catalyst among the NXSs. In continuation of our research on developing greener synthetic transformation [17–19] herein, we reported the expanding role of NIS as a non-metal, commercially available, and eco-friendly precatalyst for direct dehydrative C–C coupling between various benzyl alcohols and different type of

electron-rich compounds including: β -diketones, phenyl substituted alkene, tertiary benzyl alcohols bearing a vicinal hydrogen atom as alkene precursors, and heteroatom benzocyclenes selectively and efficiently into the corresponding products, under solvent-free reaction conditions.

2. Results and Discussion

The coupling of diphenylmethanol **1** and 1,3-diphenylpropane-1,3-dione **2** was chosen as a model reaction to employ NIS as the catalyst in direct cross-coupling of β -diketones and alcohols and to study the effects of different reaction conditions. Initially, the impact of different solvents on the conversion of **1** with **2** was studied, where it seemed to be inconvenient for the conversion of **1** with **2**, while solvent-free reaction conditions were critical for quantitative transformation, and the results are shown in the Supplementary Materials (Table S1).

In search of optimal reaction conditions, different parameters, including the loading of the NIS as the catalyst, reaction time, reaction temperature for the reaction of diphenylmethanol **1** with **2** under solvent-free conditions, and the reactivity of alcohols, were first studied, and the results are given in the Supplementary Materials (Tables S2–S5). It could be seen that under the mentioned optimal reaction conditions, the reaction successfully proceeded only in the case of secondary benzyl alcohol **1** (entry 1, Table 1). In contrast, no reaction occurred with unsubstituted benzyl alcohol **5** as the type of primary alcohol, due to lower reactivity, and trityl alcohol **6** as the type of tertiary alcohol, which could be explained by the steric effect [16].

By further investigation of the crucial reaction parameters, such as the structure of the β -dicarbonyl compound, the structure of alcohol, and loading of the catalyst, showed us the real value of this methodology. Thus, the effect of the structure of β -diketone on the course of the reaction with **1** as the most reactive benzyl alcohol derivative was studied. We selected benzoylacetone as a further candidate where the addition of NIS as the catalyst was observed to promote effectively and selectively the conversion of **1** with benzoylacetone **7** under solvent-free reaction conditions (SFRC) into the corresponding product **8** (entry 5, Table 1).

To increase the yield of the corresponding product **8**, different concentrations of the catalyst and variations of reaction temperatures were applied, and the results are shown in the Supplementary Materials (Tables S6 and S7). In the absence of a precatalyst, no reaction occurred.

Given the mentioned experience, it seemed that acetylacetone could be the next instructive substrate for realizing the effect of the structure of a β -dicarbonyl compound to the course of the reaction, where the addition of NIS as the catalyst was found to provide efficient and selective transformation of **1** with acetylacetone **9** under SFRC into the corresponding product **10** (entry 7, Table 1). To increase the yield of the corresponding product **10**, different concentrations of the catalyst were applied, and the results are presented in the Supplementary Materials (Table S8). In the absence of a catalyst, no reaction took place.

According to these results, the most reactive of β -dicarbonyl compounds was 1,3-diphenylpropane-1,3-dione **2**, optimal loading of NIS as the catalyst was 1 mol%, reaction temperature was 70–75 °C, and reaction time was 24 h. Moreover, during the optimization of reaction conditions, we found that varying the temperature from 70–75 °C to 100–105 °C for the same reaction shortened the reaction time (6 h) and provided excellent yield.

We believed that the reaction's key point was the degree of enolization of β -diketone on one side, and stability of the carbocationic intermediate formed from the alcohol on another side. A higher degree of enolization (pK_E) caused more potent and particular transformation into the desired coupling between β -dicarbonyl and alcohol, and a more stabilized carbocationic intermediate caused more efficient and selective product formation.

Through solvation, stability and reactivity of enolates can be controlled. Enolates are more stable in polar protic solvents, such as water, than in polar aprotic solvents, such as dimethyl sulfoxide [20].

Encouraged by these promising results, we checked the scope of the reaction system by applying the obtained optimal reaction conditions on cross-coupling reactions of β -dicarbonyl compounds with

different alcohols catalyzed by NIS under SFRC. The results of reactions carried out under SFRC are collected in Table 1.

Table 1. Reactions of β -dicarbonyl compounds with different alcohols catalyzed by *N*-iodosuccinimide (NIS) under solvent-free reaction conditions (SFRC) ^a.

Entry		R ¹ , R ² , R ³	NIS (Mol%)	Yield ^b (%)
1		R ¹ =R ² =H, R ³ =Ph	4	100 (99)
2		R ¹ =Me, R ² =H, R ³ =Ph	11	100 (99)
3		R ¹ =Cl, R ² =H, R ³ =Ph	13	100 (98)
4		R ¹ =OMe, R ² =R ³ =H	15	100 (98)
5		R ¹ =R ² =H, R ³ =Ph	1	100 (98)
6		R ¹ =Me, R ² =H, R ³ =Ph	11	100 (99)
7		R ¹ =R ² =H, R ³ =Ph	1	100 (98)
8		R ¹ =Me, R ² =H, R ³ =Ph	11	100 (99)
9		R ¹ =R ² =H, R ³ =Ph	1	100 (99)

^a Reaction conditions: alcohols (0.5 mmol), diketones (0.5 mmol), NIS (1–10 mol%), 70–75 °C, 23–24 h. ^b Determined from ¹H NMR spectra of isolated crude reaction mixtures; yield calculated relative to alcohol; values in parentheses are isolated yields.

Effective transformation with β -diketone **2** was observed in the reaction with additional classes of secondary benzyl alcohols, including derivatives of diphenylmethanol bearing electron-donating or

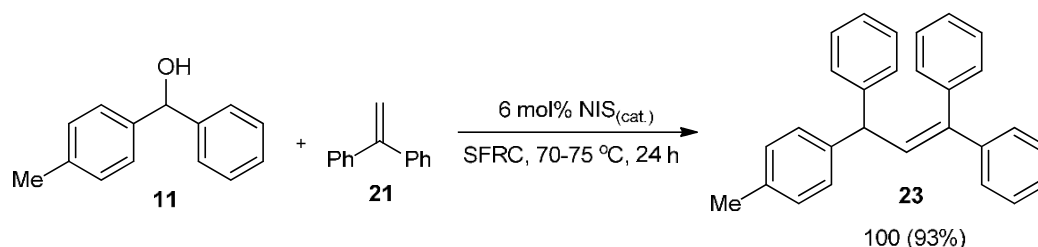
electron-withdrawing substituents on the aromatic rings **11** and **13**, and established the quantitative formation of the corresponding products **12** and **14** (entries 2 and 3, Table 1).

Furthermore, we checked the reaction of the primary benzyl alcohol bearing electron-donating group with **2**. 4-methoxybenzyl alcohol **15** was efficiently and selectively converted into the 2-(4-methoxybenzyl)-1,3-diphenylpropane-1,3-dione **16** (entry 4, Table 1). We further investigated the corresponding reactions of alcohols with benzoylacetone **7**. In the case of the reaction of primary benzyl alcohol **5** with **7** in the presence of NIS as the catalyst under solvent-free conditions, no transformation took place. Effective and selective transformation with **7** was observed in the reaction with phenyl(*p*-tolyl)methanol **11**, providing the quantitative formation of the corresponding product **17** (entry 6, Table 1). No transformation was observed with sterically bulkier triphenylmethanol **6** with **7** catalyzed by NIS under SFRC.

Moreover, we investigated the corresponding reactions of alcohols with acetylacetone **9**. In the case of the reaction of unsubstituted benzyl alcohol **5** with **9** catalyzed by NIS under SFRC, no transformation took place. Effective transformation of phenyl(*p*-tolyl)methanol **11** with **9** was observed and established the quantitative formation of the corresponding product **18** (entry 8, Table 1). No transformation was observed in the case of triphenylmethanol **6** with **9** catalyzed by NIS under SFRC. Additionally, the effective transformation was obtained between 1,3-bis(4-methoxyphenyl)propane-1,3-dione **19** and **1**, furnishing the respective product **20** in excellent yield (entry 9, Table 1).

To establish the synthetic value of the reported methodology, the following reaction was performed on the 10 mmol scales. Coupling of **1** with **2** under the above-mentioned optimal reaction conditions afforded the corresponding product **4** in quantitative yield (99%).

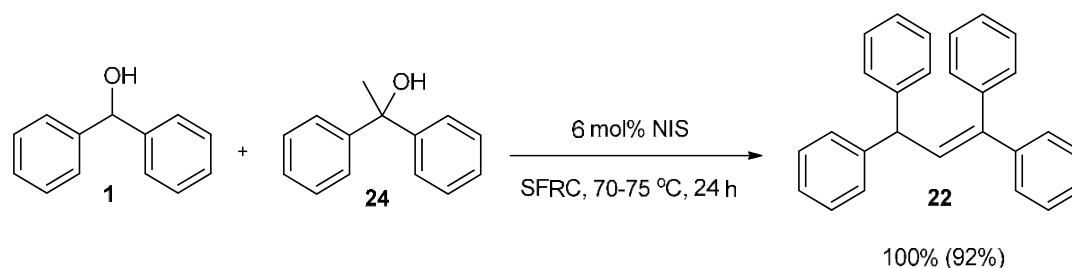
Inspired by these results, we investigated NIS's efficiency as the catalyst for the direct coupling of phenyl substituted alkene with secondary benzyl alcohol under solvent-free conditions. NIS, as an effective catalyst, was observed to promote the direct coupling of 1,1 diphenylethene **21** with phenyl(*p*-tolyl) methanol **11** under SFRC, producing the corresponding substituted alkene **23** in nearly quantitative yield (Scheme 1).



Scheme 1. Reaction of phenyl(*p*-tolyl)methanol **11** with 1,1 diphenylethene **21** catalyzed by NIS under SFRC.

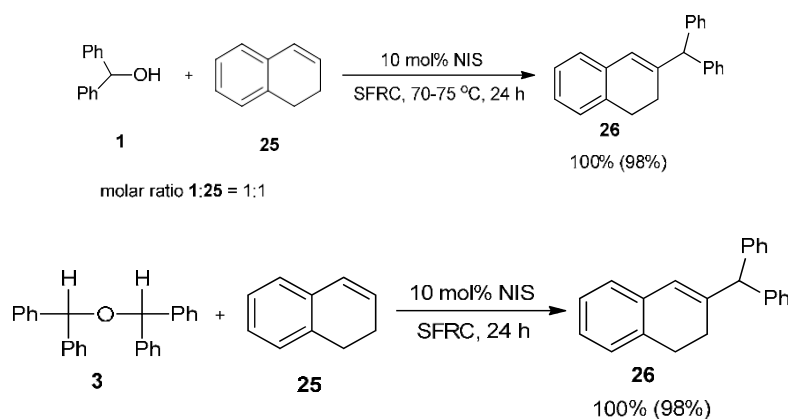
It is well-known that alkenes can be readily achieved by dehydration of tertiary alcohols bearing a vicinal hydrogen atom. Inspired by this finding, we performed the reaction between 1,1-diphenylethanol **24** and **1** catalyzed by NIS under SFRC. We observed that the direct coupling of two alcohols, **1** and **24**, forming the corresponding product **22** as the major product took place, accompanied by a trace amount of side products identified as benzophenone and dimeric ether **3**, (Scheme 2).

Electron-rich benzocyclenes with a general structure (X=NAc, O) were chosen as potential partners for the NIS-promoted reactions with benzyl alcohols under SFRC. The reaction of diphenylmethanol **1** with 1,2-dihydronaphthalene **25** catalyzed by NIS under SFRC performed the corresponding substituted alkene **26**, accompanied by a small amount of dimeric ether **3**, was observed. Different parameters such as the loading of the NIS as the catalyst and the reaction temperature for the reaction of **1** with 1,2-dihydronaphthalene **25** under SFRC were first studied, and the results are shown in the Supplementary Materials (Tables S8 and S9). Moreover, during the optimization of reaction conditions, we found that equimolar of **1** and **25** provided the best result in terms of efficiency and selectivity.



Scheme 2. Direct cross-coupling of diphenylmethanol **1** and 1,1-diphenylethanol **24** catalyzed by NIS under SFRC.

To verify the presumption that the symmetric ether **3** could be the intermediate of the cross-coupling [21], we independently performed the reaction between symmetric ether **3** as a source of benzylic cation, which was synthesized from **1** catalyzed by NIS (2 mol%) at 70–75 °C, for 3.5 h under SFRC. Then, alkylation of the dimeric ether **3** by dihydronaphthalene **25** took place, providing the corresponding product **26** in quantitative yield. Another possible pathway might be a direct reaction of carbocation derived from alcohol **1** and followed by nucleophile displacement (Scheme 3).



Scheme 3. Control reactions.

According to the results of the control reaction shown in Scheme 3, a potential explanation suggests a decomposition of the precatalyst NIS to form HOI in alcoholic reaction media, which could be responsible for NIS's mediation activity [22–25]. The X_2 forms HOI and HI in aqueous media, providing the regeneration of HOI for the following catalysis. Therefore, it was reasonable to assume that the water produced as the only by-product of the process could act as a supporting factor in the acceleration of the reaction, since no conversion was observed in the reaction between diphenylmethanol **1** and 1,3-diphenylpropane-1,3-dione **2** catalyzed by NIS in aqueous media. The presumption that NIS was actually a precatalyst forming HOI, I_2 , and protons during the process, which might catalyze nucleophilic substitutions, showed to be reasonable [16,26].

The generality of this methodology for C–C hetero coupling approaches with heteroatom benzocyclenes **27** and **29** with **1**, catalyzed by NIS under SFRC, was further examined. The results of the reactions carried out under SFRC are collected in Table 2. NIS catalyzed the direct coupling of **1** with an *N*-based heteroarene such as 1-acetylidole **23** under SFRC, affording C-3 alkylated product in high yield (**28**, entry 1, Table 2). The direct coupling of **1** with an *O*-containing heteroarene such as 2,3-benzofuran **29** underwent smooth coupling to provide the C-2 alkylated product in good yield (**30**, entry 2, Table 2). It could be seen that the electrophilic substitution in 1-acetylidole **27** took place at C-3 and not at C-2. This orientation could be explained by comparing the stability of the carbocation resulting from the electrophilic attack at C-2, which was less favorable than the carbocation from

the electrophilic attack at C-3. The electrophilic substitution at C-2 in benzofuran **29** could also be explained in the same way.

Table 2. Reactions of diphenylmethanol **1** with heteroarenes **27–29** catalyzed by NIS under SFRC ^a.

Reaction scheme: Diphenylmethanol (**1**) reacts with heteroarenes (**27–29**) in the presence of 6–10 mol% NIS under SFRC to yield products (**28–30**).

	1	27–29		28–30	
Entry	X		Product	Yield (%) ^b	
1	NAc	27		28	91
2	O	29		30	61

^a Reaction conditions: diphenylmethanol **1** (0.5 mmol), heteroarenes **27–29** (0.4 mmol), 70–75 °C, 24 h. ^b Isolated products; yields calculated relative to heteroatom benzocycles.

To gain clearer insight into NIS's thermal stability, thermal gravimetric analysis (TGA) on the precatalyst was carried out. It was observed that the degradation did not happen to the precatalyst at temperatures 25–200 °C [16].

3. Materials and Methods

All starting materials and NIS were commercially available and used without further purification (Merck, Darmstadt, Germany; Sigma Aldrich, St. Louis, MO, USA). Reactions were performed in 4 mL screw-capped vials. All reactions were observed by thin-layer chromatography (TLC, mobile phase: dichloromethane/hexane 9:1) (with silica gel/TLC cards; DC-Alufolien-Kieselgel, Sigma-Aldrich, St. Louis, MO, USA) and visualized by UV lamp (254 nm, Camag, Muttenz, Switzerland). ¹H and ¹³C NMR spectra were measured on Varian INOVA 300 NMR instrument, using a solution of CDCl₃ as the solvent with SiMe₄ as an internal reference standard. Melting points were measured by Buchi 535 equipment. Purification of a few products was performed by column chromatography (CC) using silica gel 60 (particle size: 0.063–0.200 mm).

General procedure for new C–C bond construction through β-diketone or electron-rich C=C bonds in organic molecule catalyzed by NIS on half mmol scale:

The mixture of benzyl alcohol (0.5 mmol), β-diketone, or alkene (0.5 mmol) and NIS (1–10 mol%), after being powdered in a mortar in the case of solid-state reactants, was transferred to a 4 mL screw-capped vial and heated at 70–75 °C for 24 h. TLC monitored the progress of the reaction mixture. The crude reaction mixture was cooled down to room temperature and diluted with a mixture of (3 × 5 mL EtOAc), (2 × 3 mL saturated Na₂S₂O₃), (2 × 3 mL saturated NaHCO₃), and (2 × 5 mL distilled water). The organic phase was dried Na₂SO₄, and the organic solvent was removed under reduced pressure, leaving the resultant residue.

The scaled-up procedure for the synthesis of 2-benzhydryl-1,3-diphenylpropane-1,3-dione **4** catalyzed by NIS:

The mixture of β-diketone **2** (10 mmol, 1.8423 g), diphenylmethanol **1** (10 mmol, 2.2425 g), and NIS (1 mol%, 0.1 mol, 22.5 mg), which was powdered in a mortar, was transferred to a 20 mL screw-capped glass scintillation vial and heated at 70–75 °C for 24 h. TLC followed the progress of the reaction

mixture. Upon completion of the reaction, the mixture was cooled to room temperature. Finally, the crude reaction mixture was washed with hot water and filtered by vacuum filtration to obtain a pure product in almost quantitative yield (white solid, m.p. 220–223 °C, 3.9 g, 99%).

4. Conclusions

In summary, simple, efficient, selective, and easily scalable methodology for C–C bond formation through the direct cross-coupling of various benzyl alcohols with β -diketones, heteroatom benzocyclenes, phenyl substituted alkenes, or tertiary benzyl alcohols bearing a vicinal hydrogen atom as alkene precursors employing NIS, an environmentally friendly and metal-free precatalyst, under SFRC was developed. The reactivity of β -dicarbonyl compounds was found to be the function of their enolisability, whereas primary benzyl alcohol targets needed strong activation of the ring; a comprehensive range of secondary benzyl alcohols gave efficient coupling, whereas tertiary benzyl alcohols without vicinal hydrogen atoms were found as inconvenient targets. Benzylation of the position C-2 was observed in the case of 1,2-dihydronaphthalene **25** and 2,3-benzofuran **29**, whereas C-3 was benzylated in 1-acetylintole **27**.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4344/10/8/850/s1>, detailed experimental data, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra of isolated final products.

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Article

N-Halosuccinimides as Precatalysts for C-, N-, O-, and X-Nucleophilic Substitution Reactions of Alcohols under Mild Reaction Conditions

Njomza Ajvazi ^{1,*}  and Stojan Stavber ^{1,2} ¹ Jožef Stefan International Postgraduate School, Jamova 39, 1000 Ljubljana, Slovenia; stojan.stavber@ijs.si² Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

* Correspondence: njomza.ajvazi@rezonanca-rks.com; Tel.: +383 44 258 553

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Abstract: *N*-halosuccinimides (chloro, bromo, and iodo, respectively) were introduced, tested, and applied as efficient and non-metal precatalysts for C-, N-, O-, and X-nucleophilic substitution reactions of alcohols under solvent-free reaction conditions (SFRC) or under high substrate concentration reaction conditions (HCRC) efficiently and selectively, into the corresponding products.

Keywords: alcohols; *N*-halosuccinimides; C–C or C–heteroatom bond formation; solvent-free; green chemistry

1. Introduction

The development of protocols for the transformation of organic compounds following the principles of green chemistry [1] is currently one of the main trends in organic synthesis. In accordance with the principle of atom economy, effective catalytic approaches, replacement of volatile or toxic organic compounds used as solvents with safer reaction media is one of the main challenges in organic synthesis.

Since alcohols are readily available and inexpensive alkylating agents, their nucleophilic substitution is an important and attractive process used in the synthesis of organic compounds, which offers a potential impact on the environment, resulting in water as the only side product of the reaction.

In order to apply the direct transformation of a hydroxyl group often an additional activation is inescapable [2].

Many related transformations, including the use of Brønsted and Lewis acids, metal ions, or other supporters in a substoichiometric amount, have been reported by several reviews [3–13] and recent related advanced reports [14–18]. However, the requirement of toxic or expensive reagents, environmentally undesirable solvents, a high concentration of the mediator, prolonged reaction time, or high temperature make such a method less attractive from the green chemical aspect. Accordingly, it was essential to design environmentally friendly synthetic protocols for the C–C and C–heteroatom bond formation. A group of organic compounds bearing an active *N*-halogen bond, *N*-halosuccinimides (NXSs) (chloro, bromo, and iodo), are an inexpensive, commercially available, easy-to-handle, and metal-free compounds, employed for oxidation, hydroxyhalogenation or halogenation reactions [19,20]. NXSs have attracted significant interest as mediators for comprehensive organic transformations [21–28]. However, the use of NXSs as the mediator for the direct transformation of alcohols forming C–C or C–heteroatom bonds has not been discovered so far.

In our continuous research on developing greener synthetic routes [29–31], we wish to report herein the introduction of NXSs as a non-metal substoichiometric mediator for the comprehensive transformations of alcohols bearing newly C–C or C–heteroatom bonds, under SFRC or under HCRC.

2. Results and Discussion

Initially, diphenylmethanol **1** has been chosen as the model substrate to investigate the efficiency of *N*-halosuccinimides as a mediator of the process and to find the best reaction conditions for alcohol transformation (Table 1). As can be seen from Table 1 the transformation of diphenylmethanol **1** in the presence of methyl alcohol **2** in the absence of any of the NXs, no reaction has occurred (entry 1, Table 1). The transformation of diphenylmethanol **1** mediated by *N*-halosuccinimides in the presence of MeOH **2** under HCRC gave ether **3** (entries 2–4), while in the absence of MeOH under SFRC, dimerization has been observed, affording the dimeric ether **4**, (entry 5, Table 1). Under the typical reaction conditions in the dark or the presence of radical scavenger, the quantitative conversion of starting material **1** into the corresponding product **3** was established (entries 6–7, Table 1).

Table 1. Optimal reaction conditions for the highest conversion of diphenylmethanol **1** into the (methoxymethylene)dibenzene **3** in the presence of NXs as mediators under HCRC ^a.

Entry	NXS	Mol %	Reaction Conditions	Conversion ^b (%) of 1	Relative Distribution ^b (%)	
					3	4
1	-	-	MeOH (1mmol), 70–75 °C, 6 h	-	-	-
2	NIS	3	MeOH (1mmol), 70–75 °C, 6 h	100	100	-
3	NCS	3	MeOH (1mmol), 70–75 °C, 6 h	100	92	5 ^c
4	NBS	3	MeOH (1mmol), 70–75 °C, 6 h	100	92	5 ^c
5	NIS	2	70–75 °C, 3.5 h	100	-	100
6	NIS	2	In the dark, MeOH (1 mmol), 70–75 °C, 6 h	100	100	-
7	NIS	2	TEMPO (10 mol %), MeOH (1 mmol), 70–75 °C, 6 h	100	100	-

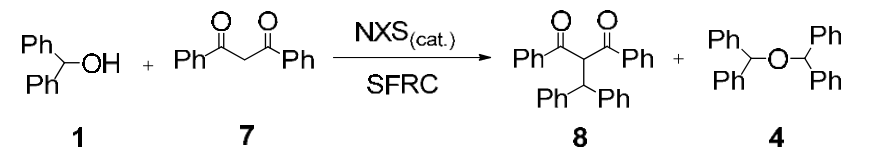
^a Reaction conditions: diphenylmethanol **1** (0.5 mmol). ^b Determined from ¹H NMR spectra of isolated crude reaction mixtures. ^c Benzophenone 3%.

Furthermore, we investigated the role of *N*-halosuccinimides as the mediators for the direct C–C bond formation by direct coupling reactions of secondary benzyl alcohol with various types of electron-rich compounds, including 1,3-dicarbonyl compounds or electron-rich alkene under SFRC. We have examined the reaction of diphenylmethanol **1** with dibenzoylmethane **7** under SFRC, and results are summarized in Table 2. The transformation of diphenylmethanol **1** in the presence of dibenzoylmethane **7** under SFRC in the absence of any of the NXs, no reaction has occurred (entry 1, Table 2). In the presence of *N*-chlorosuccinimide (NCS) as the mediator, we did not observe any conversion of the diphenylmethanol **1** (entry 2), while in the presence of *N*-bromosuccinimide (NBS) a low conversion of the diphenylmethanol **1** into the dimeric ether **4** was observed (entry 3). Moreover, in the presence of *N*-iodosuccinimide (NIS) as the mediator, the coupling of benzyl moiety with **7** took place, providing efficiently and selectively the corresponding product **8** with the new C–C bond formed between the benzyl carbon atom and C-2 carbon of dicarbonyl target **7** (entries 4 and 5). Under the typical reaction conditions in the dark or the presence of radical scavenger, the efficient and selective transformation of diphenylmethanol **1** into the corresponding product **8** was observed (entries 6–7, Table 2).

In order to support the assumption that the dimeric ether **4** might be the intermediate of this dehydrative coupling [32], few control reactions were performed (Scheme 1). Under the typical conditions when diphenylmethanol **1** was efficiently and selectively converted into dimeric ether **4**, it was applied as a starting material in the reaction with 1,1-diphenylethene **9** providing the

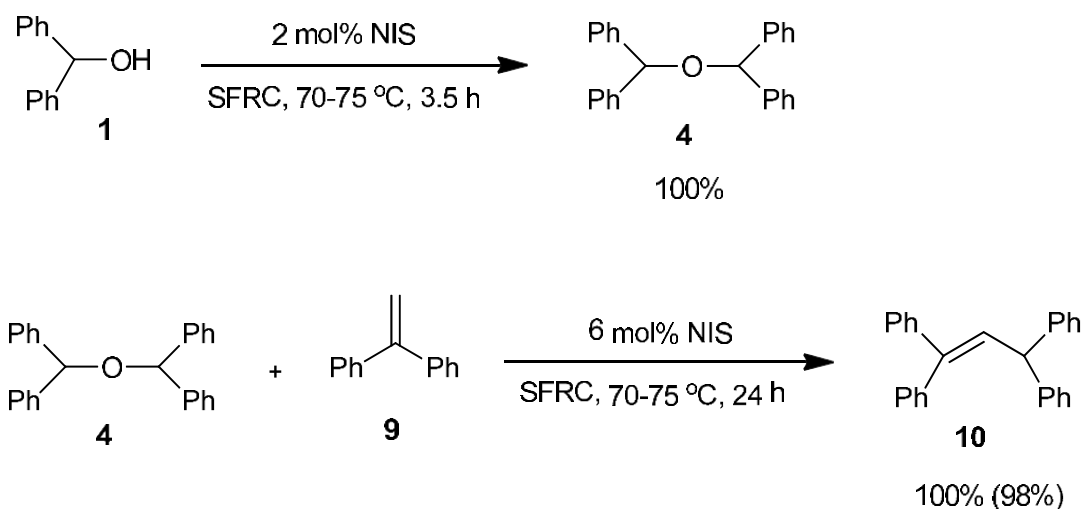
corresponding substituted alkene **10** in quantitative yield. With **9** providing the corresponding substituted alkene **10** in quantitative yield.

Table 2. The effect of substoichiometric amounts of NXS on conversion of diphenylmethanol **1** with dibenzoylmethane **7** under SFRC ^a.



Entry	NXS	Mol %	Conversion ^b (%) of 1	Relative Distribution ^b (%)	
				8	4
1	-	-	0	-	-
2	NCS	3	-	-	-
3	NBS	3	4	-	4
4	NIS	3	100	100	-
5	NIS	1	100	100	-
6 ^c	NIS	1	100	100	-
7 ^d	NIS	1	100	100	-

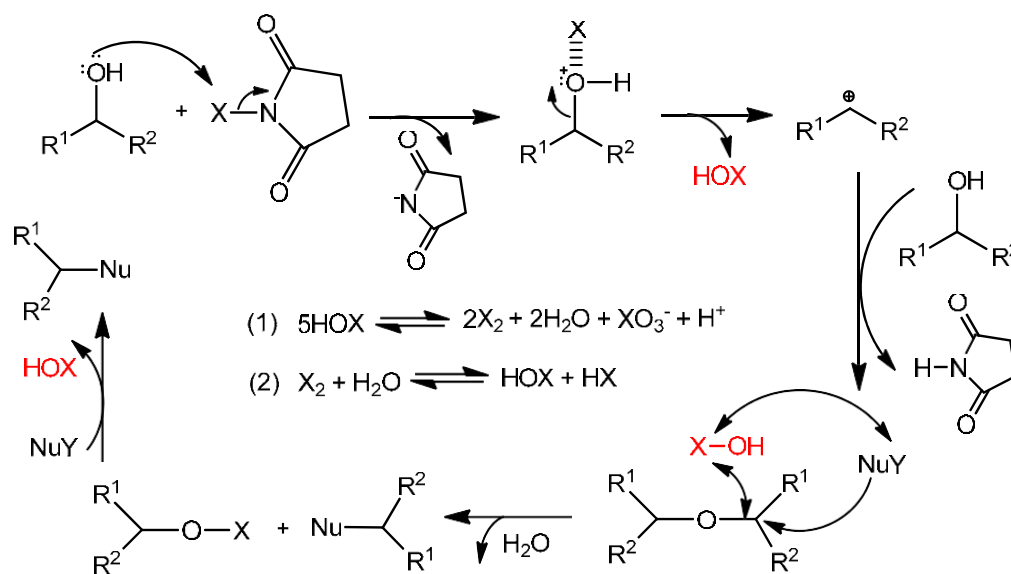
^a Reaction conditions: diphenylmethanol **1** (0.5 mmol), dibenzoylmethane **7** (0.5 mmol), 70–75 °C, 24 h. ^b Determined from ¹H NMR spectra of isolated crude reaction mixtures. ^c In the dark. ^d TEMPO (10 mol %).



Scheme 1. Control reactions.

Based on the results of the control experiment presented in (Scheme 1), the proposed reaction pathway is presented in (Scheme 2). It was reported that the R-X bond of halosuccinimides as the precatalysts was activated by its reaction with the addition of Lewis base. Therefore, it seems plausible that transient halogen bonding could be responsible for the catalytic effect of NXS [33,34].

The term halogen bonding which is defined as non-covalent interaction of a halogen atom X in one molecule with a negative site in another, such as the lone pair electrons of a Lewis base [35,36]. The halogen bonding adducts are not the activated species. Rather, halonium (X^+) transfer will generate the intermediate forming succinimide anion and HOX, which further promotes the course of the reaction. HOX decomposes by disproportionation to X_2 and HXO^3 . It is well known that X_2 forms HOX and HX in reactions with water, where HOX regenerate for following catalysis. Thus, the water resulting as an only side product of the reaction might be acting as a supporter in acceleration of the reaction.

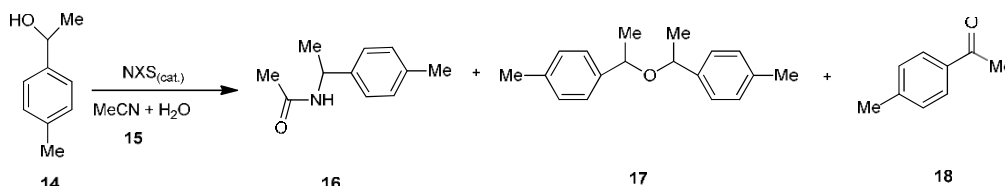


Scheme 2. Plausible reaction course.

The assumption that NXs are actually precatalysts producing HOX, X₂, and protons during the process while these species could catalyze nucleophilic substitutions seems to be reasonable [37]. The discrimination between the oxidation process observed in the case of the use of molar excess of NXs (1.3 to 2 equivalent excess) [26–28] was achieved using substoichiometric amounts of NIS and by the selection of alcohols, which could after realizing hydroxyl group form, through resonance or strong inductive effect, stabilize carbocation intermediates which readily collapse with present nucleophile sources. Alkyl alcohols or some primary benzyl alcohols were inactive under our reaction conditions. In contrast, in some cases of secondary alcohols, the formations of trace amounts of oxidation products were observed besides target products (see Tables).

Furthermore, we studied the role of *N*-halosuccinimides as mediators for direct C-N bond formation in the reaction of the alcohol with acetonitrile and water solution, and results are summarized in Table 3. The transformation of 1-(*p*-tolyl)ethan-1-ol **14**, with acetonitrile **15** in the absence of any of the NXs, no reaction occurred (entry 1, Table 3), while, in the presence of *N*-halosuccinimides as the mediator the corresponding *N*-acyl benzyl product **16** (entries 2–4), in moderate to high yield was observed, accompanied with the formation of a small amount of dimeric ether **17** (entry 4) and oxidized alcohol **18** (entries 2–4, Table 3). We found that NCS and NIS were slightly more convenient mediators for this transformation than NBS. Under the typical reaction conditions in the dark or the presence of radical scavenger, the high conversion of starting material **14** into the corresponding product **16** was established, accompanied by the formation of a small amount of dimeric ether **17** and oxidized alcohol **18** [38] (entries 5–6, Table 3).

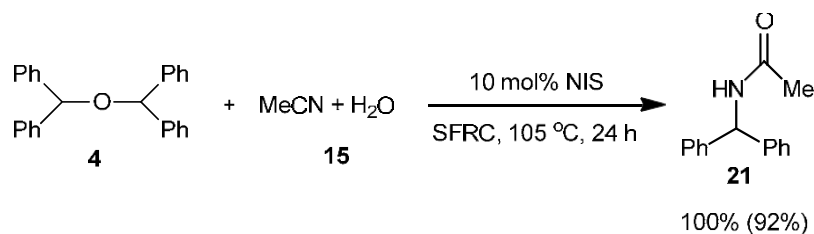
In order to support the assumption that the dimeric ether **4** might be the intermediate of this dehydrative coupling [32], few control reactions were performed (Scheme 3). Under the typical conditions when diphenylmethanol **1** was efficiently and selectively converted into dimeric ether **4** In order to support the assumption that the dimeric ether **4** might be the intermediate of the reaction course, few control reactions were performed (Scheme 3). Under the typical conditions when diphenylmethanol **1** was efficiently and selectively converted into dimeric ether **4** (Scheme 1), which was used as a starting material instead of **1** in the reaction with MeCN/H₂O **15** providing the corresponding *N*-acyl benzyl product **21** in nearly quantitative yield (Scheme 3).

Table 3. The effect of NXS as the mediator on conversion of 1-(*p*-tolyl)ethan-1-ol **14** in acetonitrile solution ^a.


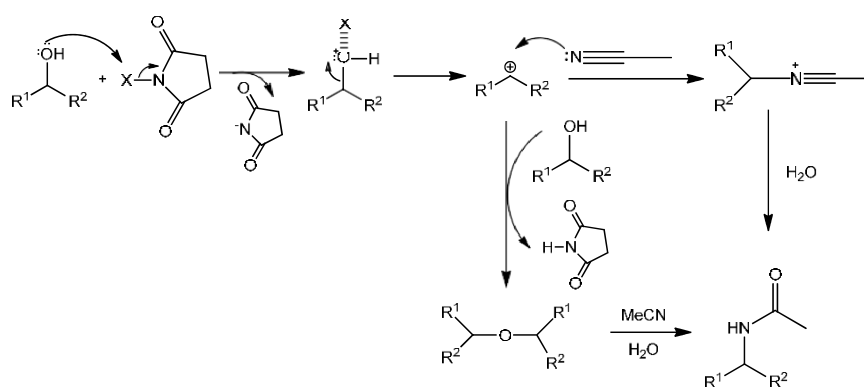
Entry	NXS	Mol %	Conversion ^b (%) of 14	Relative Distribution ^b (%)		
				16	17	18
1	-	-	-	-	-	-
2	NBS	10	78	67	-	11
3	NCS	10	82	72	-	10
4	NIS	10	84	80	2	2
5 ^c	NIS	10	84	80	2	2
6 ^d	NIS	10	84	80	2	2

^a Reaction conditions: 1-(*p*-tolyl)ethan-1-ol **14** (0.5 mmol), MeCN (1/2 mL), H₂O (2 mmol), 70–75 °C, 24 h.

^b Determined from ¹H NMR spectra of isolated crude reaction mixtures. ^c In the dark. ^d TEMPO (10 mol %).

**Scheme 3.** Control experiment.

According to these results, the dimeric ether **4** can generate the benzylic carbocation which can be trapped by MeCN to generate a nitrilium ion followed by the presence of water providing the corresponding product **21**. However, another probable pathway could be a direct reaction between carbocation and MeCN [39,40] (Scheme 4).

**Scheme 4.** Plausible reaction pathway.

The catalytic effect of NIS was applied for tertiary alkyl alcohol, in the reaction of etherification of α,α -dimethylbenzenepropanol **5** with MeOH under HCRC (entry 1, Table 4). The efficient and selective transformation was observed in the reaction with tertiary benzyl alcohol. The 2-phenylpropan-2-ol **33** was readily catalyzed by NIS providing the corresponding product **34** in nearly quantitative yield (entry 2, Table 4).

The stereochemical course of the etherification was monitored by the reaction between (S)-(-)-1-phenylethanol **11** and MeOH **2** in the presence of NIS providing the corresponding product **12** in moderate yield (entry 3, Table 4) accompanied with a small amount of acetophenone **13**. The specific rotation of pure product **12** gave the value $[\alpha] = +15^\circ$, revealing that we are not dealing with completely S_N1 or S_N2 processes but with the combination of both. It seems that the dimerization is the S_N1 , and final etherification is the S_N2 process. The details of this analysis are given in Supplementary Materials. In the case of the reaction of primary benzyl alcohol **27**, only a trace amount of benzaldehyde was observed (entry 4, Table 1).

Furthermore, under the typical reaction conditions, we checked the reaction of primary and tertiary alcohol with dibenzoylmethane **7** in the presence of NIS as the mediator under SFRC. In contrast, no reaction was observed with benzyl alcohol **27** as the type of primary alcohol due to lower reactivity and triphenylmethanol **36** as the type of tertiary alcohol, which may be attributed to the steric hindrance (entries 6 and 7, Table 4).

Additionally, the catalytic effect of NIS for direct C–C bond formation by direct coupling of phenyl substituted alkene with secondary benzyl alcohol under SFRC was studied. NIS as the mediator was found to most efficiently and selectively promote the direct coupling of diphenylmethanol **1** with 1,1-diphenylethene **9** under SFRC, furnishing the corresponding substituted alkene **10** in nearly quantitative yield (entry 8, Table 4).

On the other hand, we checked the reactions of primary and tertiary alcohol with aqueous acetonitrile **15** in the presence of NIS as the mediator under SFRC, no reaction was observed with benzyl alcohol **27** (only a trace amount of benzaldehyde was observed) and triphenylmethanol **36**, which may be attributed to the steric hindrance (entries 9 and 10, Table 4).

Additionally, the direct coupling of aniline [41], bearing deactivated group **19**, with diphenylmethanol **1** in the presence of NIS as the mediator under SFRC, afforded the corresponding *N*-alkylated compound in high yield **20** accompanied with a small amount of oxidized alcohol (entry 10, Table 4). By increasing the temperature to 105 °C, the direct coupling of diphenylmethanol **1**, 1-phenylethanol **11** with acetonitrile and water solution **15** in the presence of NIS as the mediator afforded the corresponding *N*-acyl benzyl products **21** and **22** (entries 12 and 13, Table 4) in nearly quantitative yield.

Table 4. Nucleophilic substitution of alcohols using NIS mediator ^a.

Entry	Bond Formation	R ¹ , R ² , R ³	NuY	Product	Conversion. ^b (%) (Yield ζ %)
1	C–O	R ¹ = R ² = Me R ³ = (CH ₂) ₂ Ph 5	MeOH 2		74 (64)
2		R ¹ = Ph, R ² = R ³ = Me 33	MeOH 2		93 (90)
3		R ¹ = Ph, R ² = H, R ³ = Me 11	MeOH 2		67 ^f (61) ^g

Table 4. Cont.

$$\text{R}^1\text{C}(\text{OH})(\text{R}^2)\text{R}^3 + \text{NuY} \xrightarrow{\text{NIS}_{(\text{cat.})}} \text{R}^1\text{C}(\text{Nu})(\text{R}^2)\text{R}^3$$

Entry	Bond Formation	R ¹ , R ² , R ³	NuY	Product	Conversion. ^b (%) (Yield (%)) ^c
4		R ¹ = Ph, R ² = R ³ = H 27	MeOH 2		2 ^f -
5		R ¹ = 4-MePh, R ² = H, R ³ = Ph 30	TMSOEt 23c		100 ^f (89)
6	C-C	R ¹ = R ² = R ³ = Ph 36			-
7		R ¹ = Ph, R ² = R ³ = H 27			-
8		R ¹ = R ² = Ph, R ³ = H 1			100 ^e (92)
9	C-N	R ¹ = Ph, R ² = R ³ = H 27	MeCN/H ₂ O 15		8 ^f -
10		R ¹ = R ² = R ³ = Ph 36	MeCN/H ₂ O 15		-
11		R ¹ = R ² = Ph, R ³ = H 1			85 ^f (79)
12		R ¹ = R ² = Ph, R ³ = H 1	MeCN/H ₂ O 15		100 ^f (90)
13		R ¹ =Ph, R ² =H, R ³ =Me 11	MeCN/H ₂ O 15		100 ^f (93)
14		R ¹ = 4-MePh, R ² = H, R ³ = Ph 30	TMSNCS 23b		100 (97)

Table 4. Cont.

$$\text{R}^1\text{C}(\text{OH})(\text{R}^2)(\text{R}^3) + \text{NuY} \xrightarrow{\text{NIS}_{(\text{cat.})}} \text{R}^1\text{C}(\text{Nu})(\text{R}^2)(\text{R}^3)\text{Cl}$$

Entry	Bond Formation	R ¹ , R ² , R ³	NuY	Product	Conversion, ^b (%) (Yield ζ %)
15 ^h	C–Cl	R ¹ = 3-NO ₂ Ph, R ² = R ³ = H 24	TMSCl 23a		[42]
16		R ¹ = 3-NO ₂ Ph, R ² = R ³ = H 24	TMSCl 23a		91 ^d (76)
17 ^h		R ¹ = Ph, R ² = R ³ = H 27	TMSCl 23a		69 (65) [42]
18		R ¹ = Ph, R ² = R ³ = H 27	TMSCl 23a		100 ^d (88)
19 ^h		R ¹ = 4-MePh, R ² = H, R ³ = Me 14	TMSCl 23a		90 (86) [42]
20		R ¹ = 4-MePh, R ² = H, R ³ = Me 14	TMSCl 23a		100 (98)
21 ^h		R ¹ =Ph, R ² =R ³ =Me, 33	TMSCl 23a		100 (98) [42]

^a Reaction conditions: alcohol (0.5–1 mmol), NuY (0.5 mmol–2 mL), NIS (1–10 mol %), 50–105 °C, 6.5–48 h.^b Determined from ¹H NMR spectra of the isolated crude reaction mixture. ^c Purified products. ^d Dimeric ether 6%–8%. ^e Oxidized alcohol and dimeric ether 4%. ^f Oxidized alcohol 2%–10%. ^g Specific rotation [α] = +15°. ^h Without catalyst.

We further investigated the impact of *N*-halosuccinimide as a mediator in reactions of primary benzyl alcohol with trimethylchlorosilane (TMSCl) **23a** under SFRC. Primary benzyl alcohol bearing a strong deactivated group was not converted into the corresponding product using TMSCl [42]. Thus, we studied the impact of *N*-halosuccinimides as a mediator on the course of reaction of 3-nitrobenzyl alcohol **24** with TMSCl under SFRC. Gratifyingly, the addition of a substoichiometric amount of NIS was found to effectively and selectively promote the reaction of 3-nitrobenzyl alcohol **24** with TMSCl under SFRC (entries 15 and 16, Table 4). To increase the yield of 1-(chloromethyl)-3-nitrobenzene **25**, different concentrations of the mediator were employed, and the results are given in the Supplementary Materials (Table S1). The role of NIS as the mediator was further examined in the improvement in yields of the chlorinated products. In the case of benzyl alcohol **27**, 1-(*p*-tolyl)ethanol **14** using TMSCl under mediator-free and SFRC the good- to high-yielding formation of the corresponding chlorides **28**

and **29** were accompanied by 10% of the symmetric ethers in both cases [42]. In the case of the reaction of benzyl alcohol **27** by adding NIS as the mediator, quantitative conversion of the starting material into the corresponding chloride **29** was observed, while only a small amount of dimer was detected as the side product (entries 17 and 18, Table 4). In the case of 1-(*p*-tolyl)ethanol **14**, the improvement of 98% yield **28** without the formation of the dimeric ether was attained by adding the NIS as the mediator (entries 19 and 20, Table 4).

The efficient and selective transformation was observed in the case with 2-phenylpropan-2-ol **33** into the corresponding product **35** [42] (entry 21, Table 4).

Inspired by this results with TMSCl **23a**, we performed the reactions of phenyl(*p*-tolyl)methanol **30** as the model compound under the mentioned reaction conditions, using (trimethylsilyl)isothiocyanate (TMSNCS) **23b** or ethoxytrimethylsilane (TMSOEt) **23c** as the sources of nucleophiles where isothiocyanate and ethoxy were introduced successfully into organic molecules **31** and **32** (entry 14 and 5, Table 4).

The collected results in Table 4 are organized primarily stressing the type of bond formation mediated by NIS as the most efficient and selective catalyst among the NXSs, enhancing the green chemical profiles of these transformations.

In order to check the thermal stability of *N*-halosuccinimides under reaction conditions, the thermal gravimetric analysis (TGA) on the NCS, NBS, and NIS catalysts were performed. It was detected that the degradation had occurred at none of the catalysts at the temperature range 25–200 °C.

3. Materials and Methods

All chemicals used for synthetic procedures were purchased from commercial sources ((Merck, Darmstadt, Germany; Sigma Aldrich, St. Louis, MO, USA) Reactions were monitored by thin-layer chromatography (TLC) (with silica gel/TLC cards, DC-Alufohlen-Kieselgel, Sigma-Aldrich, St. Louis, MO, USA). For the detection of compounds on chromatographic plates, a UV (Camag, Muttenz, Switzerland) lamp (254 nm) was used. Column chromatography (CC) was performed with silica gel Kieselgel 60 (particle size: 0.063–0.200 mm, Fluka, Sigma Aldrich). Nuclear magnetic resonance (Varian INOVA 300 NMR instrument, ¹H: at 303.0 MHz, ¹³C: at 76.2 MHz) using CDCl₃ as the solvent with SiMe₄ (TMS) as an internal reference and melting points (open capillary tube methodology; uncorrected, by Buchi 535 equipment) were used for identification and structure elucidation. General procedure for etherification of alcohols mediated by *N*-halosuccinimide on half mmol scale:

A mixture of benzyl alcohol (0.5–1 mmol) and *N*-halosuccinimide as a precatalyst (3–10 mol %), which had been powdered in a mortar in the case of solid-state reactants, was transferred to a 4 mL screw-capped vial, finally added eventual liquid component alkyl alcohol (1 mmol–1 mL) and heated at 70–75 °C for 6–24 h.

The reaction was observed by TLC. After reaction completion, the reaction mixture was cooled to room temperature; dissolved in EtOAc (3 × 5 mL); washed with saturated Na₂S₂O₃ (2 × 3 mL), saturated NaHCO₃ (2 × 3 mL), and water (2 × 5 mL); and the collected organic layers were dried over anhydrous Na₂SO₄ and the solvent was removed by evaporation under reduced pressure providing the corresponding product.

General procedure for new carbon-carbon bond formation through β-dicarbonyl compound in organic molecule mediated by NIS on half mmol scale:

A mixture of benzyl alcohol (0.5 mmol), β-dicarbonyl compound (0.5 mmol), and NIS (1 mol %) which had been powdered in a mortar, was transferred to a 4 mL screw-capped vial and heated at 70–75 °C for 24 h. The reaction was observed by TLC. After reaction completion, the reaction mixture was cooled to room temperature; dissolved in EtOAc (3 × 5 mL); washed with saturated Na₂S₂O₃ (2 × 3 mL), saturated NaHCO₃ (2 × 3 mL) and water (2 × 5 mL); and the collected organic layers were dried over anhydrous Na₂SO₄ and the solvent was removed by evaporation under reduced pressure providing the corresponding product.

General procedure for new carbon-carbon bond formation through electron rich C=C bond in organic molecules mediated by NIS on half mmol scale:

A mixture of benzyl alcohol or dimeric ether (0.5 mmol), alkene (0.5 mmol), and NIS (6 mol %), which had been powdered in a mortar in the case of solid-state reactants was transferred to a 4 mL screw-capped vial, finally, eventual liquid component was added, and it was heated at 70–75 °C for 24 h.

The reaction was observed by TLC. After reaction completion, the reaction mixture was cooled to room temperature, dissolved in EtOAc (3 × 5 mL), washed with saturated Na₂S₂O₃ (2 × 3 mL), saturated NaHCO₃ (2 × 3 mL) and water (2 × 5 mL). Through the anhydrous Na₂SO₄ the collected organic layers were dried, and the solvent was voided by evaporation under reduced pressure.

General procedure for new carbon-nitrogen bond formation in organic molecules mediated by NIS on half mmol scale:

In a 4 mL screw-capped vial, a mixture of benzyl alcohol (0.5 mmol), acetonitrile (0.5 mL), water (2 mmol) and NIS (10 mol %), or a mixture of solid reaction components previously powdered in a mortar was transferred: benzyl alcohol (0.5 mmol), aniline (0.55 mmol) and NIS (10 mol %). The vial was then heated at 70–105 °C for 24–48 h. The reaction was observed by TLC. When the reaction was completed, the reaction mixture was cooled to room temperature, dissolved in EtOAc (3 × 5 mL), washed with saturated Na₂S₂O₃ (2 × 3 mL), saturated NaHCO₃ (2 × 3 mL) and water (2 × 5 mL), and the collected organic layers were dried over anhydrous Na₂SO₄ and the solvent was removed by evaporation under reduced pressure providing the corresponding product.

General procedure for halo functionalization of organic compounds using trimethylsilyl derivatives mediated by NIS on half mmol scale:

A mixture of benzyl alcohol (0.5 mmol) and NIS (2–10 mol %) which had been powdered in a mortar in the case of solid-state reactants, was transferred to a 4 mL screw-capped vial, then trimethylsilyl derivatives (0.55 mmol) added and stirred at rt (room temperature) or heated at 70–75 °C for 6.5–25 h. The progress of the reaction mixture was monitored by TLC. When the reaction was completed, the reaction mixture was cooled to room temperature, dissolved in ethyl acetate (15 mL), washed with saturated Na₂S₂O₃ (6 mL), saturated NaHCO₃ (6 mL) and water (10 mL), and the collected organic layers were dried over anhydrous Na₂SO₄ and the solvent was removed by evaporation under reduced pressure providing the corresponding product.

4. Conclusions

In conclusion, *N*-halosuccinimides (chloro, bromo, and iodo, respectively), were introduced, tested, and applied as efficient and metal-free substoichiometric mediators for reactions of a comprehensive range of alcohols with various type of electron-rich organic molecules or reactive anionic species thus forming new carbon-carbon or carbon-heteroatom bonds in target alcohol molecules. Leading reactions enhanced green chemical profiles of these valuable transformations under solvent-free reaction conditions or high concentration reaction conditions.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4344/10/4/460/s1>, detailed experimental data, ¹H-NMR and ¹³C-NMR spectra of isolated final products and experimental data related to thermal analysis.

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Article

N-Halosuccinimides as Precatalysts for C-, N-, O-, and X-Nucleophilic Substitution Reactions of Alcohols under Mild Reaction Conditions

Njomza Ajvazi ^{1,*}  and Stojan Stavber ^{1,2} ¹ Jožef Stefan International Postgraduate School, Jamova 39, 1000 Ljubljana, Slovenia; stojan.stavber@ijs.si² Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

* Correspondence: njomza.ajvazi@rezonanca-rks.com; Tel.: +383 44 258 553

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Abstract: *N*-halosuccinimides (chloro, bromo, and iodo, respectively) were introduced, tested, and applied as efficient and non-metal precatalysts for C-, N-, O-, and X-nucleophilic substitution reactions of alcohols under solvent-free reaction conditions (SFRC) or under high substrate concentration reaction conditions (HCRC) efficiently and selectively, into the corresponding products.

Keywords: alcohols; *N*-halosuccinimides; C–C or C–heteroatom bond formation; solvent-free; green chemistry

1. Introduction

The development of protocols for the transformation of organic compounds following the principles of green chemistry [1] is currently one of the main trends in organic synthesis. In accordance with the principle of atom economy, effective catalytic approaches, replacement of volatile or toxic organic compounds used as solvents with safer reaction media is one of the main challenges in organic synthesis.

Since alcohols are readily available and inexpensive alkylating agents, their nucleophilic substitution is an important and attractive process used in the synthesis of organic compounds, which offers a potential impact on the environment, resulting in water as the only side product of the reaction.

In order to apply the direct transformation of a hydroxyl group often an additional activation is inescapable [2].

Many related transformations, including the use of Brønsted and Lewis acids, metal ions, or other supporters in a substoichiometric amount, have been reported by several reviews [3–13] and recent related advanced reports [14–18]. However, the requirement of toxic or expensive reagents, environmentally undesirable solvents, a high concentration of the mediator, prolonged reaction time, or high temperature make such a method less attractive from the green chemical aspect. Accordingly, it was essential to design environmentally friendly synthetic protocols for the C–C and C–heteroatom bond formation. A group of organic compounds bearing an active *N*-halogen bond, *N*-halosuccinimides (NXSs) (chloro, bromo, and iodo), are an inexpensive, commercially available, easy-to-handle, and metal-free compounds, employed for oxidation, hydroxyhalogenation or halogenation reactions [19,20]. NXSs have attracted significant interest as mediators for comprehensive organic transformations [21–28]. However, the use of NXSs as the mediator for the direct transformation of alcohols forming C–C or C–heteroatom bonds has not been discovered so far.

In our continuous research on developing greener synthetic routes [29–31], we wish to report herein the introduction of NXSs as a non-metal substoichiometric mediator for the comprehensive transformations of alcohols bearing newly C–C or C–heteroatom bonds, under SFRC or under HCRC.

2. Results and Discussion

Initially, diphenylmethanol **1** has been chosen as the model substrate to investigate the efficiency of *N*-halosuccinimides as a mediator of the process and to find the best reaction conditions for alcohol transformation (Table 1). As can be seen from Table 1 the transformation of diphenylmethanol **1** in the presence of methyl alcohol **2** in the absence of any of the NXs, no reaction has occurred (entry 1, Table 1). The transformation of diphenylmethanol **1** mediated by *N*-halosuccinimides in the presence of MeOH **2** under HCRC gave ether **3** (entries 2–4), while in the absence of MeOH under SFRC, dimerization has been observed, affording the dimeric ether **4**, (entry 5, Table 1). Under the typical reaction conditions in the dark or the presence of radical scavenger, the quantitative conversion of starting material **1** into the corresponding product **3** was established (entries 6–7, Table 1).

Table 1. Optimal reaction conditions for the highest conversion of diphenylmethanol **1** into the (methoxymethylene)dibenzene **3** in the presence of NXs as mediators under HCRC ^a.

Entry	NXS	Mol %	Reaction Conditions	Conversion ^b (%) of 1	Relative Distribution ^b (%)	
					3	4
1	-	-	MeOH (1mmol), 70–75 °C, 6 h	-	-	-
2	NIS	3	MeOH (1mmol), 70–75 °C, 6 h	100	100	-
3	NCS	3	MeOH (1mmol), 70–75 °C, 6 h	100	92	5 ^c
4	NBS	3	MeOH (1mmol), 70–75 °C, 6 h	100	92	5 ^c
5	NIS	2	70–75 °C, 3.5 h	100	-	100
6	NIS	2	In the dark, MeOH (1 mmol), 70–75 °C, 6 h	100	100	-
7	NIS	2	TEMPO (10 mol %), MeOH (1 mmol), 70–75 °C, 6 h	100	100	-

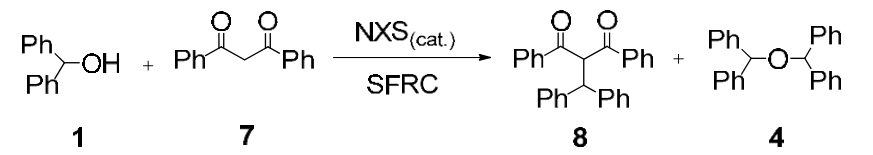
^a Reaction conditions: diphenylmethanol **1** (0.5 mmol). ^b Determined from ¹H NMR spectra of isolated crude reaction mixtures. ^c Benzophenone 3%.

Furthermore, we investigated the role of *N*-halosuccinimides as the mediators for the direct C–C bond formation by direct coupling reactions of secondary benzyl alcohol with various types of electron-rich compounds, including 1,3-dicarbonyl compounds or electron-rich alkene under SFRC. We have examined the reaction of diphenylmethanol **1** with dibenzoylmethane **7** under SFRC, and results are summarized in Table 2. The transformation of diphenylmethanol **1** in the presence of dibenzoylmethane **7** under SFRC in the absence of any of the NXs, no reaction has occurred (entry 1, Table 2). In the presence of *N*-chlorosuccinimide (NCS) as the mediator, we did not observe any conversion of the diphenylmethanol **1** (entry 2), while in the presence of *N*-bromosuccinimide (NBS) a low conversion of the diphenylmethanol **1** into the dimeric ether **4** was observed (entry 3). Moreover, in the presence of *N*-iodosuccinimide (NIS) as the mediator, the coupling of benzyl moiety with **7** took place, providing efficiently and selectively the corresponding product **8** with the new C–C bond formed between the benzyl carbon atom and C-2 carbon of dicarbonyl target **7** (entries 4 and 5). Under the typical reaction conditions in the dark or the presence of radical scavenger, the efficient and selective transformation of diphenylmethanol **1** into the corresponding product **8** was observed (entries 6–7, Table 2).

In order to support the assumption that the dimeric ether **4** might be the intermediate of this dehydrative coupling [32], few control reactions were performed (Scheme 1). Under the typical conditions when diphenylmethanol **1** was efficiently and selectively converted into dimeric ether **4**, it was applied as a starting material in the reaction with 1,1-diphenylethene **9** providing the

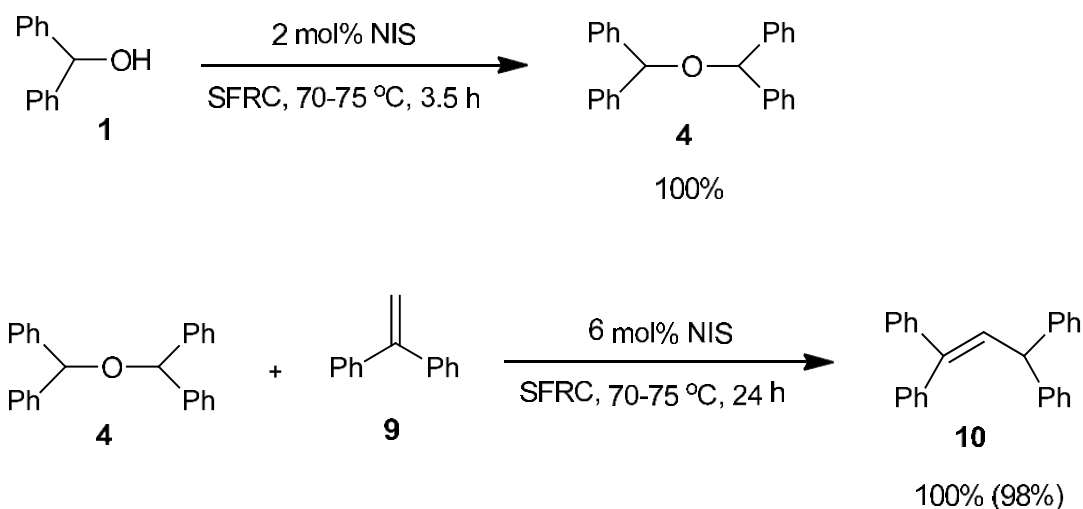
corresponding substituted alkene **10** in quantitative yield. With **9** providing the corresponding substituted alkene **10** in quantitative yield.

Table 2. The effect of substoichiometric amounts of NXS on conversion of diphenylmethanol **1** with dibenzoylmethane **7** under SFRC ^a.



Entry	NXS	Mol %	Conversion ^b (%) of 1	Relative Distribution ^b (%)	
				8	4
1	-	-	0	-	-
2	NCS	3	-	-	-
3	NBS	3	4	-	4
4	NIS	3	100	100	-
5	NIS	1	100	100	-
6 ^c	NIS	1	100	100	-
7 ^d	NIS	1	100	100	-

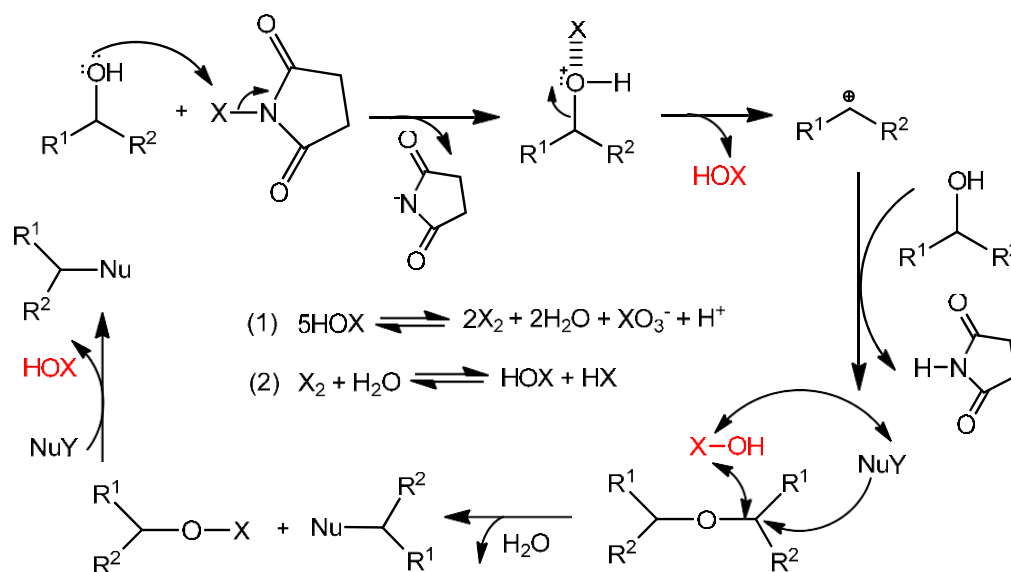
^a Reaction conditions: diphenylmethanol **1** (0.5 mmol), dibenzoylmethane **7** (0.5 mmol), 70–75 °C, 24 h. ^b Determined from ¹H NMR spectra of isolated crude reaction mixtures. ^c In the dark. ^d TEMPO (10 mol %).



Scheme 1. Control reactions.

Based on the results of the control experiment presented in (Scheme 1), the proposed reaction pathway is presented in (Scheme 2). It was reported that the R-X bond of halosuccinimides as the precatalysts was activated by its reaction with the addition of Lewis base. Therefore, it seems plausible that transient halogen bonding could be responsible for the catalytic effect of NXS [33,34].

The term halogen bonding which is defined as non-covalent interaction of a halogen atom X in one molecule with a negative site in another, such as the lone pair electrons of a Lewis base [35,36]. The halogen bonding adducts are not the activated species. Rather, halonium (X^+) transfer will generate the intermediate forming succinimide anion and HOX, which further promotes the course of the reaction. HOX decomposes by disproportionation to X_2 and HXO^3 . It is well known that X_2 forms HOX and HX in reactions with water, where HOX regenerate for following catalysis. Thus, the water resulting as an only side product of the reaction might be acting as a supporter in acceleration of the reaction.

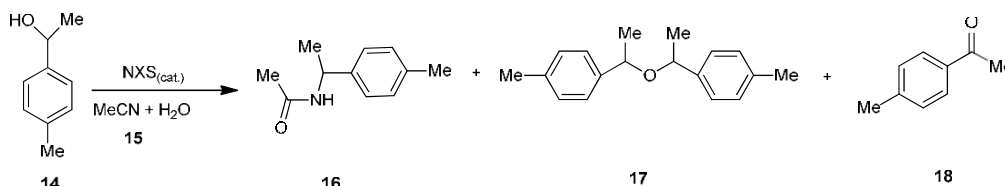


Scheme 2. Plausible reaction course.

The assumption that NXs are actually precatalysts producing HOX, X₂, and protons during the process while these species could catalyze nucleophilic substitutions seems to be reasonable [37]. The discrimination between the oxidation process observed in the case of the use of molar excess of NXs (1.3 to 2 equivalent excess) [26–28] was achieved using substoichiometric amounts of NIS and by the selection of alcohols, which could after realizing hydroxyl group form, through resonance or strong inductive effect, stabilize carbocation intermediates which readily collapse with present nucleophile sources. Alkyl alcohols or some primary benzyl alcohols were inactive under our reaction conditions. In contrast, in some cases of secondary alcohols, the formations of trace amounts of oxidation products were observed besides target products (see Tables).

Furthermore, we studied the role of *N*-halosuccinimides as mediators for direct C-N bond formation in the reaction of the alcohol with acetonitrile and water solution, and results are summarized in Table 3. The transformation of 1-(*p*-tolyl)ethan-1-ol **14**, with acetonitrile **15** in the absence of any of the NXs, no reaction occurred (entry 1, Table 3), while, in the presence of *N*-halosuccinimides as the mediator the corresponding *N*-acyl benzyl product **16** (entries 2–4), in moderate to high yield was observed, accompanied with the formation of a small amount of dimeric ether **17** (entry 4) and oxidized alcohol **18** (entries 2–4, Table 3). We found that NCS and NIS were slightly more convenient mediators for this transformation than NBS. Under the typical reaction conditions in the dark or the presence of radical scavenger, the high conversion of starting material **14** into the corresponding product **16** was established, accompanied by the formation of a small amount of dimeric ether **17** and oxidized alcohol **18** [38] (entries 5–6, Table 3).

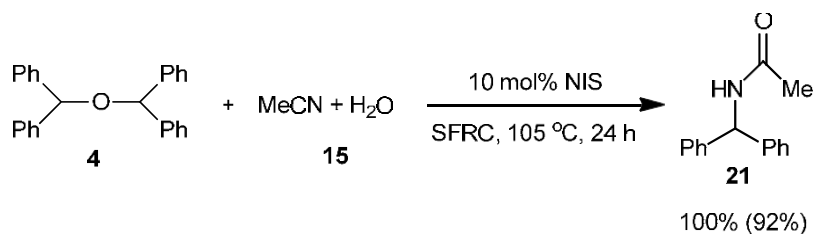
In order to support the assumption that the dimeric ether **4** might be the intermediate of this dehydrative coupling [32], few control reactions were performed (Scheme 3). Under the typical conditions when diphenylmethanol **1** was efficiently and selectively converted into dimeric ether **4** In order to support the assumption that the dimeric ether **4** might be the intermediate of the reaction course, few control reactions were performed (Scheme 3). Under the typical conditions when diphenylmethanol **1** was efficiently and selectively converted into dimeric ether **4** (Scheme 1), which was used as a starting material instead of **1** in the reaction with MeCN/H₂O **15** providing the corresponding *N*-acyl benzyl product **21** in nearly quantitative yield (Scheme 3).

Table 3. The effect of NXS as the mediator on conversion of 1-(*p*-tolyl)ethan-1-ol **14** in acetonitrile solution ^a.


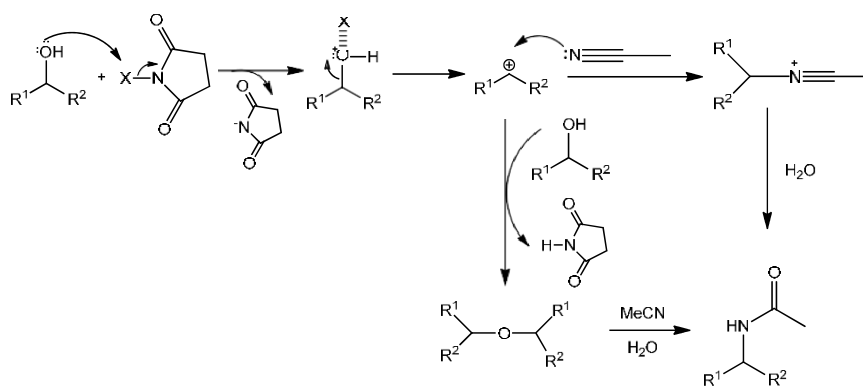
Entry	NXS	Mol %	Conversion ^b (%) of 14	Relative Distribution ^b (%)		
				16	17	18
1	-	-	-	-	-	-
2	NBS	10	78	67	-	11
3	NCS	10	82	72	-	10
4	NIS	10	84	80	2	2
5 ^c	NIS	10	84	80	2	2
6 ^d	NIS	10	84	80	2	2

^a Reaction conditions: 1-(*p*-tolyl)ethan-1-ol **14** (0.5 mmol), MeCN (1/2 mL), H₂O (2 mmol), 70–75 °C, 24 h.

^b Determined from ¹H NMR spectra of isolated crude reaction mixtures. ^c In the dark. ^d TEMPO (10 mol %).

**Scheme 3.** Control experiment.

According to these results, the dimeric ether **4** can generate the benzylic carbocation which can be trapped by MeCN to generate a nitrilium ion followed by the presence of water providing the corresponding product **21**. However, another probable pathway could be a direct reaction between carbocation and MeCN [39,40] (Scheme 4).

**Scheme 4.** Plausible reaction pathway.

The catalytic effect of NIS was applied for tertiary alkyl alcohol, in the reaction of etherification of α,α -dimethylbenzenepropanol **5** with MeOH under HCRC (entry 1, Table 4). The efficient and selective transformation was observed in the reaction with tertiary benzyl alcohol. The 2-phenylpropan-2-ol **33** was readily catalyzed by NIS providing the corresponding product **34** in nearly quantitative yield (entry 2, Table 4).

The stereochemical course of the etherification was monitored by the reaction between (S)-(-)-1-phenylethanol **11** and MeOH **2** in the presence of NIS providing the corresponding product **12** in moderate yield (entry 3, Table 4) accompanied with a small amount of acetophenone **13**. The specific rotation of pure product **12** gave the value $[\alpha] = +15^\circ$, revealing that we are not dealing with completely S_N1 or S_N2 processes but with the combination of both. It seems that the dimerization is the S_N1 , and final etherification is the S_N2 process. The details of this analysis are given in Supplementary Materials. In the case of the reaction of primary benzyl alcohol **27**, only a trace amount of benzaldehyde was observed (entry 4, Table 1).

Furthermore, under the typical reaction conditions, we checked the reaction of primary and tertiary alcohol with dibenzoylmethane **7** in the presence of NIS as the mediator under SFRC. In contrast, no reaction was observed with benzyl alcohol **27** as the type of primary alcohol due to lower reactivity and triphenylmethanol **36** as the type of tertiary alcohol, which may be attributed to the steric hindrance (entries 6 and 7, Table 4).

Additionally, the catalytic effect of NIS for direct C–C bond formation by direct coupling of phenyl substituted alkene with secondary benzyl alcohol under SFRC was studied. NIS as the mediator was found to most efficiently and selectively promote the direct coupling of diphenylmethanol **1** with 1,1-diphenylethene **9** under SFRC, furnishing the corresponding substituted alkene **10** in nearly quantitative yield (entry 8, Table 4).

On the other hand, we checked the reactions of primary and tertiary alcohol with aqueous acetonitrile **15** in the presence of NIS as the mediator under SFRC, no reaction was observed with benzyl alcohol **27** (only a trace amount of benzaldehyde was observed) and triphenylmethanol **36**, which may be attributed to the steric hindrance (entries 9 and 10, Table 4).

Additionally, the direct coupling of aniline [41], bearing deactivated group **19**, with diphenylmethanol **1** in the presence of NIS as the mediator under SFRC, afforded the corresponding *N*-alkylated compound in high yield **20** accompanied with a small amount of oxidized alcohol (entry 10, Table 4). By increasing the temperature to 105 °C, the direct coupling of diphenylmethanol **1**, 1-phenylethanol **11** with acetonitrile and water solution **15** in the presence of NIS as the mediator afforded the corresponding *N*-acyl benzyl products **21** and **22** (entries 12 and 13, Table 4) in nearly quantitative yield.

Table 4. Nucleophilic substitution of alcohols using NIS mediator ^a.

Entry	Bond Formation	R ¹ , R ² , R ³	NuY	Product	Conversion. ^b (%) (Yield ζ %)
1	C–O	R ¹ = R ² = Me R ³ = (CH ₂) ₂ Ph 5	MeOH 2		74 (64)
2		R ¹ = Ph, R ² = R ³ = Me 33	MeOH 2		93 (90)
3		R ¹ = Ph, R ² = H, R ³ = Me 11	MeOH 2		67 ^f (61) ^g

Table 4. Cont.

$$\text{R}^1\text{C}(\text{OH})(\text{R}^2)\text{R}^3 + \text{NuY} \xrightarrow{\text{NIS}_{(\text{cat.})}} \text{R}^1\text{C}(\text{Nu})(\text{R}^2)\text{R}^3$$

Entry	Bond Formation	R ¹ , R ² , R ³	NuY	Product	Conversion. ^b (%) (Yield (%)) ^c
4		R ¹ = Ph, R ² = R ³ = H 27	MeOH 2		2 ^f -
5		R ¹ = 4-MePh, R ² = H, R ³ = Ph 30	TMSOEt 23c		100 ^f (89)
6	C-C	R ¹ = R ² = R ³ = Ph 36			-
7		R ¹ = Ph, R ² = R ³ = H 27			-
8		R ¹ = R ² = Ph, R ³ = H 1			100 ^e (92)
9	C-N	R ¹ = Ph, R ² = R ³ = H 27	MeCN/H ₂ O 15		8 ^f -
10		R ¹ = R ² = R ³ = Ph 36	MeCN/H ₂ O 15		-
11		R ¹ = R ² = Ph, R ³ = H 1			85 ^f (79)
12		R ¹ = R ² = Ph, R ³ = H 1	MeCN/H ₂ O 15		100 ^f (90)
13		R ¹ =Ph, R ² =H, R ³ =Me 11	MeCN/H ₂ O 15		100 ^f (93)
14		R ¹ = 4-MePh, R ² = H, R ³ = Ph 30	TMSNCS 23b		100 (97)

Table 4. Cont.

$$\text{R}^1\text{C}(\text{OH})(\text{R}^2)\text{R}^3 + \text{NuY} \xrightarrow{\text{NIS}_{(\text{cat.})}} \text{R}^1\text{C}(\text{Nu})(\text{R}^2)\text{R}^3$$

Entry	Bond Formation	R ¹ , R ² , R ³	NuY	Product	Conversion. ^b (%) (Yield ζ %)
15 ^h	C–Cl	R ¹ = 3-NO ₂ Ph, R ² = R ³ = H 24	TMSCl 23a		[42]
16		R ¹ = 3-NO ₂ Ph, R ² = R ³ = H 24	TMSCl 23a		91 ^d (76)
17 ^h		R ¹ = Ph, R ² = R ³ = H 27	TMSCl 23a		69 (65) [42]
18		R ¹ = Ph, R ² = R ³ = H 27	TMSCl 23a		100 ^d (88)
19 ^h		R ¹ = 4-MePh, R ² = H, R ³ = Me 14	TMSCl 23a		90 (86) [42]
20		R ¹ = 4-MePh, R ² = H, R ³ = Me 14	TMSCl 23a		100 (98)
21 ^h		R ¹ =Ph, R ² =R ³ =Me, 33	TMSCl 23a		100 (98) [42]

^a Reaction conditions: alcohol (0.5–1 mmol), NuY (0.5 mmol–2 mL), NIS (1–10 mol %), 50–105 °C, 6.5–48 h.^b Determined from ¹H NMR spectra of the isolated crude reaction mixture. ^c Purified products. ^d Dimeric ether 6%–8%. ^e Oxidized alcohol and dimeric ether 4%. ^f Oxidized alcohol 2%–10%. ^g Specific rotation [α] = +15°. ^h Without catalyst.

We further investigated the impact of *N*-halosuccinimide as a mediator in reactions of primary benzyl alcohol with trimethylchlorosilane (TMSCl) **23a** under SFRC. Primary benzyl alcohol bearing a strong deactivated group was not converted into the corresponding product using TMSCl [42]. Thus, we studied the impact of *N*-halosuccinimides as a mediator on the course of reaction of 3-nitrobenzyl alcohol **24** with TMSCl under SFRC. Gratifyingly, the addition of a substoichiometric amount of NIS was found to effectively and selectively promote the reaction of 3-nitrobenzyl alcohol **24** with TMSCl under SFRC (entries 15 and 16, Table 4). To increase the yield of 1-(chloromethyl)-3-nitrobenzene **25**, different concentrations of the mediator were employed, and the results are given in the Supplementary Materials (Table S1). The role of NIS as the mediator was further examined in the improvement in yields of the chlorinated products. In the case of benzyl alcohol **27**, 1-(*p*-tolyl)ethanol **14** using TMSCl under mediator-free and SFRC the good- to high-yielding formation of the corresponding chlorides **28**

and **29** were accompanied by 10% of the symmetric ethers in both cases [42]. In the case of the reaction of benzyl alcohol **27** by adding NIS as the mediator, quantitative conversion of the starting material into the corresponding chloride **29** was observed, while only a small amount of dimer was detected as the side product (entries 17 and 18, Table 4). In the case of 1-(*p*-tolyl)ethanol **14**, the improvement of 98% yield **28** without the formation of the dimeric ether was attained by adding the NIS as the mediator (entries 19 and 20, Table 4).

The efficient and selective transformation was observed in the case with 2-phenylpropan-2-ol **33** into the corresponding product **35** [42] (entry 21, Table 4).

Inspired by this results with TMSCl **23a**, we performed the reactions of phenyl(*p*-tolyl)methanol **30** as the model compound under the mentioned reaction conditions, using (trimethylsilyl)isothiocyanate (TMSNCS) **23b** or ethoxytrimethylsilane (TMSOEt) **23c** as the sources of nucleophiles where isothiocyanate and ethoxy were introduced successfully into organic molecules **31** and **32** (entry 14 and 5, Table 4).

The collected results in Table 4 are organized primarily stressing the type of bond formation mediated by NIS as the most efficient and selective catalyst among the NXSs, enhancing the green chemical profiles of these transformations.

In order to check the thermal stability of *N*-halosuccinimides under reaction conditions, the thermal gravimetric analysis (TGA) on the NCS, NBS, and NIS catalysts were performed. It was detected that the degradation had occurred at none of the catalysts at the temperature range 25–200 °C.

3. Materials and Methods

All chemicals used for synthetic procedures were purchased from commercial sources ((Merck, Darmstadt, Germany; Sigma Aldrich, St. Louis, MO, USA) Reactions were monitored by thin-layer chromatography (TLC) (with silica gel/TLC cards, DC-Alufohlen-Kieselgel, Sigma-Aldrich, St. Louis, MO, USA). For the detection of compounds on chromatographic plates, a UV (Camag, Muttenz, Switzerland) lamp (254 nm) was used. Column chromatography (CC) was performed with silica gel Kieselgel 60 (particle size: 0.063–0.200 mm, Fluka, Sigma Aldrich). Nuclear magnetic resonance (Varian INOVA 300 NMR instrument, ¹H: at 303.0 MHz, ¹³C: at 76.2 MHz) using CDCl₃ as the solvent with SiMe₄ (TMS) as an internal reference and melting points (open capillary tube methodology; uncorrected, by Buchi 535 equipment) were used for identification and structure elucidation. General procedure for etherification of alcohols mediated by *N*-halosuccinimide on half mmol scale:

A mixture of benzyl alcohol (0.5–1 mmol) and *N*-halosuccinimide as a precatalyst (3–10 mol %), which had been powdered in a mortar in the case of solid-state reactants, was transferred to a 4 mL screw-capped vial, finally added eventual liquid component alkyl alcohol (1 mmol–1 mL) and heated at 70–75 °C for 6–24 h.

The reaction was observed by TLC. After reaction completion, the reaction mixture was cooled to room temperature; dissolved in EtOAc (3 × 5 mL); washed with saturated Na₂S₂O₃ (2 × 3 mL), saturated NaHCO₃ (2 × 3 mL), and water (2 × 5 mL); and the collected organic layers were dried over anhydrous Na₂SO₄ and the solvent was removed by evaporation under reduced pressure providing the corresponding product.

General procedure for new carbon-carbon bond formation through β-dicarbonyl compound in organic molecule mediated by NIS on half mmol scale:

A mixture of benzyl alcohol (0.5 mmol), β-dicarbonyl compound (0.5 mmol), and NIS (1 mol %) which had been powdered in a mortar, was transferred to a 4 mL screw-capped vial and heated at 70–75 °C for 24 h. The reaction was observed by TLC. After reaction completion, the reaction mixture was cooled to room temperature; dissolved in EtOAc (3 × 5 mL); washed with saturated Na₂S₂O₃ (2 × 3 mL), saturated NaHCO₃ (2 × 3 mL) and water (2 × 5 mL); and the collected organic layers were dried over anhydrous Na₂SO₄ and the solvent was removed by evaporation under reduced pressure providing the corresponding product.

General procedure for new carbon-carbon bond formation through electron rich C=C bond in organic molecules mediated by NIS on half mmol scale:

A mixture of benzyl alcohol or dimeric ether (0.5 mmol), alkene (0.5 mmol), and NIS (6 mol %), which had been powdered in a mortar in the case of solid-state reactants was transferred to a 4 mL screw-capped vial, finally, eventual liquid component was added, and it was heated at 70–75 °C for 24 h.

The reaction was observed by TLC. After reaction completion, the reaction mixture was cooled to room temperature, dissolved in EtOAc (3 × 5 mL), washed with saturated Na₂S₂O₃ (2 × 3 mL), saturated NaHCO₃ (2 × 3 mL) and water (2 × 5 mL). Through the anhydrous Na₂SO₄ the collected organic layers were dried, and the solvent was voided by evaporation under reduced pressure.

General procedure for new carbon-nitrogen bond formation in organic molecules mediated by NIS on half mmol scale:

In a 4 mL screw-capped vial, a mixture of benzyl alcohol (0.5 mmol), acetonitrile (0.5 mL), water (2 mmol) and NIS (10 mol %), or a mixture of solid reaction components previously powdered in a mortar was transferred: benzyl alcohol (0.5 mmol), aniline (0.55 mmol) and NIS (10 mol %). The vial was then heated at 70–105 °C for 24–48 h. The reaction was observed by TLC. When the reaction was completed, the reaction mixture was cooled to room temperature, dissolved in EtOAc (3 × 5 mL), washed with saturated Na₂S₂O₃ (2 × 3 mL), saturated NaHCO₃ (2 × 3 mL) and water (2 × 5 mL), and the collected organic layers were dried over anhydrous Na₂SO₄ and the solvent was removed by evaporation under reduced pressure providing the corresponding product.

General procedure for halo functionalization of organic compounds using trimethylsilyl derivatives mediated by NIS on half mmol scale:

A mixture of benzyl alcohol (0.5 mmol) and NIS (2–10 mol %) which had been powdered in a mortar in the case of solid-state reactants, was transferred to a 4 mL screw-capped vial, then trimethylsilyl derivatives (0.55 mmol) added and stirred at rt (room temperature) or heated at 70–75 °C for 6.5–25 h. The progress of the reaction mixture was monitored by TLC. When the reaction was completed, the reaction mixture was cooled to room temperature, dissolved in ethyl acetate (15 mL), washed with saturated Na₂S₂O₃ (6 mL), saturated NaHCO₃ (6 mL) and water (10 mL), and the collected organic layers were dried over anhydrous Na₂SO₄ and the solvent was removed by evaporation under reduced pressure providing the corresponding product.

4. Conclusions

In conclusion, *N*-halosuccinimides (chloro, bromo, and iodo, respectively), were introduced, tested, and applied as efficient and metal-free substoichiometric mediators for reactions of a comprehensive range of alcohols with various type of electron-rich organic molecules or reactive anionic species thus forming new carbon-carbon or carbon-heteroatom bonds in target alcohol molecules. Leading reactions enhanced green chemical profiles of these valuable transformations under solvent-free reaction conditions or high concentration reaction conditions.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4344/10/4/460/s1>, detailed experimental data, ¹H-NMR and ¹³C-NMR spectra of isolated final products and experimental data related to thermal analysis.

Author Contributions: Conceptualization, S.S.; formal analysis, N.A. and S.S.; investigation, N.A. and S.S.; methodology, N.A. and S.S.; writing—original draft, N.A. and S.S.; writing—review and editing, N.A. and S.S. All authors have read and agreed to the published version of the manuscript.

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CASE REPORT

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¹Department of Dental Emergency, University Dental Clinical Centre, Pristina, Kosovo

²Department of Periodontology and Oral Pathology, University Dental Clinical Centre, Faculty of Dentistry, "Ss. Cyril and Methodius University", Skopje, Republic of Macedonia

³"Denta Med"- Desmoret e Golakut n.n., Pristina, Kosovo

⁴Department of Prosthodontics, Dental School, Faculty of Medicine, University of Prishtina, Republic of Kosovo

⁵Department of Prosthodontics Alma Mater Europaea, Campus College Rezonanca, Prishtina, Republic of Kosovo

Corresponding author: Professor Mirjana Popovska, PhD. Department of Periodontology and Oral Pathology, University Dental Clinical Centre, Faculty of Dentistry, "Ss. Cyril and Methodius University", Skopje, Republic of Macedonia. E-mail: popovskam2002@yahoo.com. ORCID ID: <http://www.orcid.org/0000-0000-0000-0000>.

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Application of 4MATRIX and PRF in the Treatment of Infrabone Defects in Chronic Periodontopathy

Bylbyl Reçica¹, Mirjana Popovska², Urim Tefiku³, Lindita Zendeli Bedhxeti², Venera Bimbashi^{4,5}

ABSTRACT

Background: Periodontal disease also called gum disease is a serious gum infection that damages the soft tissue and, without treatment, can destroy the bone and result in different types of alveolar bone defects. That means that periodontal pockets are present and registered only in case of periodontal disease. **Objective:** The aim of this study is to observe the effect of 4MATRIX and PRF application in the treatment of infrabone defect over case report. **Material and Methods:** By using regenerative surgical periodontal therapy 4MATRIX bone substituent with 4MATRIX and PRF for elimination of deep pockets or periodontal disease was performed among patient at selected sites. **Case report:** The patient's periodontal condition has followed 3, 6- and 9-month period. The patient's oral health-related quality of life showed a marked improvement after periodontal surgery therapy. **Conclusion:** Additional application of PRF and 4MATRIX in conventional surgical treatment of infrabone defects showed decreased values of PPD and CAL registered in the segment treated after 9 months of treatment.

Keywords: Chronic periodontitis, periodontal regeneration. 4 MATRIX, PRF.

1. BACKGROUND

Periodontal disease also called gum disease is a serious gum infection that damages the soft tissue and, without treatment, can destroy the bone and result in different types of alveolar bone defects. The Periodontal disease as a Pathology by which are sick a lot of peoples and especially adults is not new, and the evidence of alveolar bone loss affecting human remains dating from around 700,000 years ago in a lot of case reports (1). Basic pathognomonic signs of periodontal disease and the most important clinical sign of it is the presence of periodontal pockets. As pathology periodontal disease is present in the general population (2, 3).

A lot of different species of bacteria are present in the dental plaque, and they are capable of inducing an inflammatory response of the periodontal tissues (4). In the patients who are more sensitive and their general condition is down, this chronic inflammation will induce a breakdown of the periodontal ligament and the surrounding alveolar bone resulting in the formation of periodontal pockets around the roots thus causing periodontitis (5).

The first step of this treatment is to mechanically clean periodontal pockets from bacteria (debridement). When there are present the big and deep periodontal pockets and there is impossible to get access to the deepest portions of the pockets and properly clean them just mechanically and to reduce the depth of pockets, than can be indicated surgery as a way of treatment (6). We have found in the literature many preparations that are used as bone substitutes, but also as stimulators of osteoblasts to produce bone matrix (7-9).

One of the most important interventions recommended in this domain is guided tissue regeneration (GTR/GBR). To achieve more satisfactory effects in periodontal surgery, a variety of treatment procedures are applied involving the use of bone substitutes of different origins (auto transplants, allografts, and alloplastic materials) (10, 11).

2. OBJECTIVE

The aim of this study is to observe the effect of 4MATRIX and PRF application in the treatment of infrabone defect over case report.

	December 2019	March 2020	July 2020	November 2020
Intervention				
Initial periodontal therapy				
Plaque control	+			
Quadrant Scaling and root planning	+			
Oral hygiene instruction	+			
Professional tooth cleaning	+			
First Reevaluation				
Plaque control		+		
Oral hygiene instruction		+		
Professional tooth cleaning		+		
Surgical periodontal Therapy				
Open flap debridement (#15,16)		+		
Regenerative therapy with 4MATRIX (#15,16)		+		
Regenerative therapy with 4MATRIX+PRF membrane		+		
Second Reevaluation				
Supportive periodontal Therapy			+	
Oral hygiene instruction			+	
Professional tooth cleaning			+	
Third Reevaluation				
Supportive periodontal Therapy				+
Oral hygiene instruction				+
Professional tooth cleaning				+

Table 1. Monitoring of the undertaken procedures during the first examination, after 3, 6 and 9 months

3. CASE REPORT

1. Baseline examination

Forty one years old patient has visited the Clinical Dentistry University Center of Pristina with the complaint of gingival swelling, periodontitis, and depth pockets in upper jaw in region of right and left premolars region.

Symptoms registered at the first examination are: redness, swelling of the gingiva and mild luxation of the teeth as objective clinical signs. The patient was monitored every 3 months. in March, July and November. Occasional deteriorations and improvements in periodontal status have been reported in the patient follow-up period.

The following procedures are undertaken for therapeutic purposes: initial periodontal therapy, periodontal regenerative therapy, treatment for recovery of oral function and treatment for recovery of oral function (Table 1).

a) Initial periodontal therapy

After the first visit, we underwent initial therapy, which included: plaque control, Quadrant Scaling and root planning, Oral hygiene instruction and Professional tooth cleaning.

Initial periodontal therapy consisted mainly of tooth brushing instruction what is mean plaque home control by patient, quadrant scaling (# 13, 14, 15, 16). An initial examination revealed that around 45 % of sites had a probing depth (PD) of >4 mm and 30 % of bleeding on probing.

Radiographic examination revealed bone resorption and infrabony defects as far as the root apex in between 14 -15, 15-16, and 24-25, 25-26, vertical bone resorption in both sites and horizontal resorption in other regions

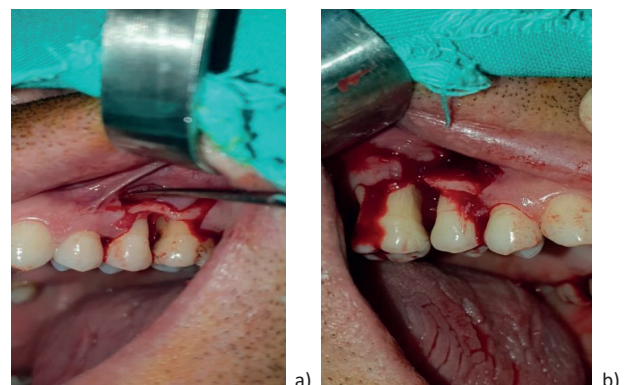


Figure 1. Flap intervention: a) open flap in between 25,26; b) open flap in between 15,16.

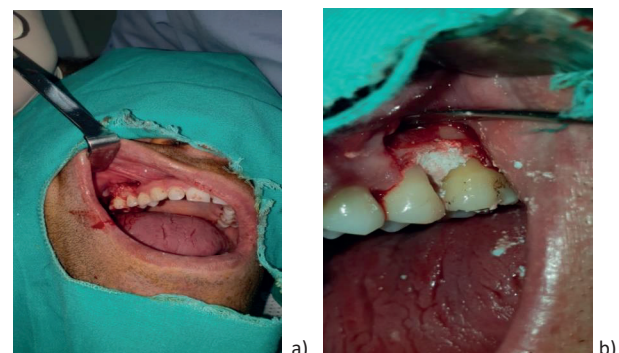


Figure 2. Flap intervention and artificial bone placed: a)14,15; b) 25-26;

in generally. Based on clinical examination and diagnosis of chronic periodontitis, the initial periodontal therapy was carried out following the periodontal surgery procedures and regenerative therapy.

2. Diagnosis

	First examination		After 3 months		After 6 months		After 9 months	
Region	14-15	15-16	14-15	15-16	14-15	15-16	14-15	15-16
PI	2	2	2	2	2	2	1	1
GI	2	2	2	2	2	2	1	1
BOP	2	2	2	2	2	2	1	1
PPD	4	4,5	4	4	4	4	3,5	4
CAL	4	4,5	4	4	4	4	3	3,5
Region	24-25	25-26	24-25	25-26	24-25	25-26	24-25	25-26
PI	2	2	2	2	2	2	1	1
GI	2	2	2	2	2	2	1	1
BOP	2	2	2	2	2	2	1	1
PPD	4	4	4	4	4	4	3,5	3,5
CAL	4	4,5	4	4	3,5	3,5	3	3

Table 2. Periodontal status at first examination, after 3, 6 and 9 months of treatment. Legend: PI - dental plaque deposits; GI- gingival inflammation; BOP - gingival bleeding; PPD - periodontal pocket CAL- clinical attachment loss

PD of > 4 mm during the first re-evaluation in addition to the conducted one plaque control, Quadrant Scaling and root planning, Oral hygiene instruction and Professional tooth cleaning. Oral hygiene instruction and Professional tooth cleaning, flap intervention was performed bilaterally in the upper jaw left and right (Figure 1). Bone substitute 4MATRIX is applied on the right, and 4MATRIX and PRF membrane are placed on the left of the intervened region (Figure 2).

3. Periodontal regenerative therapy

The first assessment of periodontal status was made by monitoring the dental plaque index (DP), gingival bleeding index (BOP), periodontal pocket depth (DP) and clinical attachment loss (CAL). In the right upper region 14-15 and 15-16 the bone substituent 4MATRIX was applied, and in the left upper quadrant (24-25, 25-26) the defect was filled with 4MATRIX and PRF membrane. Regenerative therapy with 4MATRIX bone substituent and PRF membrane by own blood of patient was then performed on tooth (24-25, 25-26).

4. Treatment for recovery of oral function

At 3 months after postoperatively there was no significant difference in bone formation in periodontal pockets where was performed 4MATRIX and PRF membrane. Six months later the finding was identical in the two treated regions of the upper jaw. After 9 months of the intervention, minimal but present bone gain is evident.

5. Supportive periodontal therapy

On re-monitoring, significantly reduced plaque accumulation, reduced gingival inflammation, but without correction of periodontal pocket depth and clinical attachment loss is evident. After 6 months the condition of the gingiva followed by gingival inflammation (GI) and gingival bleeding (BOP) was stable. After 9 months, a stable gingival periodontal finding was recorded. In this phase of re-evaluation, a PD finding was registered



Figure 3. Periodontal pockets

on the side treated with 4MATRIX and PRF membrane for CAL = 3 mm in the region 25-26 (Figure 3).

Following the findings obtained in the intervened regions with 4MATRIX bone substitute without PRF application (right) and left with 4MATRIX and PRF it is evident that during the control examinations after 3, 6 and 9 months PD, GI and BOP are unchanged. Certain changes are evident in different treated regions with 4MATRIX and 4MATRIX and PRF for DP and CAL values. The only improvement was observed in the values for DP and CAL after 9 months of the therapy in the region 14-15 (DP = 3.5, CAL = 3); 15-16 (DP = 4, CAL = 3, 5); where 4MATRIX bone substitute is applied. In the region 24-25 (DP = 3.5, CAL = 3.5); 25-26 (DP = 3, CAL = 3); where 4MATRIX is applied in combination with PRF. The results show modest clinically better therapeutic effects in the 4MATRIX and PRF-treated segment.

4. DISCUSSION

In the last twenty years, periodontal therapy has progressed significantly, from formerly used resective methods, to principles of preservation and regeneration of periodontal tissues which are used today Bembi et al., found no significant difference in the results of treatment with two materials, NovaBone Putty- CME, and Frios Algipore, followed by clinical parameters of probe depth and clinical attachment level (12).

Therefore the results of the study of Gojkov-Vukelic et al. by using Maxresorb (Bottis dental) which is composed of 60% slowly resorbing hydroxyapatite (HA) and 40% fast resorbing beta-tricalcium phosphate (β -TCP) as regenerative grafts in periodontal pockets showed significant reduction in probing depth for both groups of patients (treated by open flap surgery and treated by open flap surgery in addition with Maxresorb), one and six months after the performed surgical therapy (13). Also the results of other papers which are talking about the other biomaterials as Bio Oss, showed significant improvement for clinical parameters at sites treated with BioSS compared to control sites (14).

Experiences were also emphasized where PRF was used in periodontal regeneration BiOss, Emdogain (15-17). Though the PRF could be used as a safe and natural method for the correction of certain tissues, many researchers and clinicians in private practice tried to use PRF to regenerate the periodontal defects (18-20). Currently, many randomized clinical studies are available to compare the applied PRF (after an open curettage as a standard method or combined with other techniques) with other golden standards, such as EMD Matrix (21).

The goal of this treatment approach is healing by repair without formation of new periodontal attachment (22). The main parameters that we check after regenerative therapy are plaque index (PI), papillary bleeding index (PBI), and probing pocket depth (PPD). The ideal treatment would be to recover the periodontal tissues that have been lost (periodontal tissue regeneration). Several surgical techniques have been developed in the attempt to regenerate periodontal tissues including guided tissue regeneration (GTR), bone grafting (BG) and the use of the enamel matrix derivative (EMD). All these treatments have been shown to have the potential to regenerate at least some periodontal attachment in humans (23).

Periodontal regeneration mediated by EMD is based on a different concept. The enamel matrix is composed of a number of proteins, 90 % of which is amelogenin. One year after treatment, enamel matrix derivative (EMD) showed statistically significant improvements in probing attachment levels (PAL) (1,3mm) and probing pocket depths (PPD) reduction (1mm) in comparison with flap surgery. Henceforth, EMD seems simpler to use, may not need antibiotic coverage and does not need a second surgical intervention (if compared with non-resorptive barriers). In addition, no postoperative infections or adverse events were observed with EMD versus two cases of infection (not statistically significant) in the GTR group.

The findings from current case report presented identical results, by correction of periodontal pockets and clinical loss of attachment after 9 months of treatment with 4MATRIX substitute supplement PRF.

Platelet-rich-Plasma (PRP) is autologous plasma which has been enriched with platelets and leukocytes in addition to jellifying agents, growth factors, cytokines, bovine thrombin and anticoagulants. Comparing with PRF, PRP has some limitations because, the growth

factors are released for a very short period of time, also the bovine clotting factors may react with human clotting factors to give rise to bleeding.

For complete regeneration, the distribution of growth factors in damaged areas of the destructed bone has great significance for the potential effects of bone transplants. PRF is considered to be the second generation of concentrated platelets, consisting of concentrated platelets that emit different growth factors, which are much more demanding for the bone grains, while useful in the healing process.

Roughly, during the process of healing natural wounds, blood plays an important role in accelerating tissue regeneration by providing various cells, growth factors, cytokines, and coagulation factors. Supraphysiologic doses of platelets (Platelet Plasma) have been developed to increase platelet counts at defect sites. However, additional use of substituent is necessary to stimulate and emphasize regenerative processes, although the assessment of treatment in this study is considered not optimal (24).

An alternative method for the production of a natural autologous PDGF was first introduced using PRP, an autologous growth factor of growth derived from typical platelets after centrifugation, which are mostly used for clinical and research purposes (25, 26).

PRP was first used by many doctors in the field of oral and maxillofacial surgery and has since been widely used in the regeneration of defects, mainly in combination with bone grafts (27).

5. CONCLUSION

Additional application of PRF and 4MATRIX in conventional surgical treatment of infrabone defects showed decreased values of PPD and CAL registered in the segment treated after 9 months of treatment.

- **Patient Consent Form:** The patient was informed orally on the aim of the study and the written informed consent was obtained.
- **Author's contribution:** All authors contributed correspondingly to this work and they approved the final proofreading.
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Use of Biomaterials for Periodontal Regeneration: A Review

Bylbyl F. Reçica^{1*}, Mirjana Popovska², Amella Cana³, Lindita Zendeli Bedxeti², Urim Tefiku⁴, Spiro Spasovski⁵, Ana Spasovska-Gjorgovska⁶, Teuta Kutllovci⁷, Jehona F. Ahmed⁸

¹Department of Dental Emergency, University Dental Clinical Centre, Pristina, Kosovo; ²Department of Periodontology and Oral Pathology, University Dental Clinical Centre, Faculty of Dentistry, “Ss. Cyril and Methodius University”, Skopje, Republic of Macedonia; ³Department of Orthodontics, University Dental Clinical Centre, Skopje, Republic of Macedonia; ⁴“Denta Med” - Desmoret e Golakut n.n., Pristina, Kosovo; ⁵“Dr. Spasovski”, Skopje, Republic of Macedonia; ⁶PZU “Ilinden 96, Skopje, Republic of Macedonia; ⁷Department of Pedodontics and Preventive Dentistry, University Clinical Centre of Kosovo, Pristina, Kosovo; ⁸Department of Oral Surgery, University Dental Clinical Center Pristina, Pristina, Kosovo

Abstract

BACKGROUND: Management of bone periodontal defects, destruction, and loss of the alveolar bone is considered a challenge for modern periodontal regeneration and implant dentistry. Numerous of biomaterials are being used in periodontal regenerative treatment.

AIM: This study aims to know the characteristics of biomaterials and their efficiency in periodontal surgical treatment as regenerative therapy.

METHODS: A systematic review of the literature considering reviews, clinical studies, original papers, and articles from electronic data has been used.

RESULTS: Different biomaterials such as Straumann® *Emdogain*®, Geistlich *Bio-Oss*®, *MIS 4MATRIX – Bone Graft*, Platelet-rich fibrin (PRF), Mis Bone-4MATRIX, and PRF are being used for periodontal regeneration treatment, hence revealing more effective outcomes when combined. PRP together with conventional grafting procedures may be a beneficial treatment approach, guided tissue regeneration with bioabsorbable membranes in combination with Bio-Oss are stable on a long-term basis.

CONCLUSION: Biomaterials being used in periodontal surgical treatment have the different regenerative ability. The combined use of biomaterials might result in a better clinical outcome. There are also a number of other biomaterials used to treat periodontal regeneration, but generally all have the same ability and the same molecular structure as highlighted in this literature review.

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***Correspondence:** Bylbyl F. Reçica, Department of Dental Emergency, University Dental Clinical Centre, Pristina, Kosovo. Tel.: +38344193579.

E-mail: bardhyli59@gmail.com

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Introduction

Periodontal disease is not new. Archeological investigations have revealed evidence of alveolar bone loss affecting human remains dating from around 700,000 years ago [1].

The main chronic infective disease of the gums that affects around 10–30% of the adult population is periodontitis.

Bacteria, present in the dental plaque, are capable of inducing an inflammatory response of the periodontal tissues. In susceptible individuals, this chronic inflammation will induce a breakdown of the periodontal ligament (PDL) and the surrounding alveolar bone resulting in the formation of periodontal pockets around the roots, thus causing periodontitis [2].

Periodontitis, the main cause of tooth loss in adults, is characterized by the bacterially induced

inflammation, which frequently results in the formation of intrabony defects. It affects the supporting structures of the teeth, including the jawbone, PDL, and cementum. Apart of functional and esthetic problems in the oral cavity, periodontitis is being associated with systemic diseases, such as diabetes, preterm birth, cardiovascular disease, stroke, and pulmonary disease as well [3]. The ultimate involves the formation of new cementum on the tooth root, along with new periodontal attachment between newly formed bone and cementum [4].

Periodontitis is a disease characterized by pathological processes that involve all structures around the tooth (periodontium, cement, alveolar bone, and gingiva). During the development of the disease, complex and irreversible mechanisms induce bone destruction and resorption, which definitely leads to tooth loss [5]. It is commonly known that periodontal disease results in different types of alveolar bone defects. One of them is periodontal pockets which

represent the most important clinical sign of periodontal disease, thus are considered basic pathognomonic features of this very frequent disease present in the population [6], [7].

History

Descriptions of conditions that we would now refer to as periodontitis can be found in a number of ancient textbooks, papyruses, and manuscripts, such as *Al-Tasrif*, the medical encyclopedia written by Albucasis (936–1013) in Moorish Spain. In addition to the clinical features of periodontitis, some of these early authors also described treatment strategies for this condition.

If we spend a moment to imagine the likely oral health status of many of the people living in the Middle Ages, in the time of Albucasis, for example, we would probably conjure up images of abundant calculus deposits, inflamed gingival tissues, gingival bleeding, and halitosis. In the United States of America, Riggs (1810–1885) regarded calculus as the cause of periodontal disease, thus treated the condition by meticulous removal of calculus from pockets, “curettage” of the soft tissues and oral hygiene instruction. Furthermore, the etiological role of plaque in the development of gingival inflammation was confirmed in experimental studies on gingivitis conducted in the 1960s: Upon cessation of oral hygiene practices over periods of 3–4 weeks, plaque accumulation resulted in gingivitis, which was reversed following plaque removal and resumption of normal oral hygiene [8].

Studies on the natural history of periodontitis performed in Sri Lankan tea plantation workers between 1970 and 1985 provided basic information on oral conditions unaffected by any dental monitoring, prophylaxis or therapy and in the absence of any professionally recommended or supervised oral hygiene practices over a period of 15 years [9]. A subsequent examination of this distinct Cohort in 1990 confirmed the ongoing progression of periodontitis and the influence of tobacco consumption or betel nut chewing on attachment loss and periodontitis-related tooth loss over 20 years [10]. While clinical data from these studies generally suggested an overall increase in both prevalence and severity of the disease with advancing age, different disease progression rates within the subjects were recognized. Consequently, three subgroups of subjects were identified with various periodontal disease progression rates: (1) Rapid progression, in approximately 8% of the subjects, (2) moderate progression, in approximately 81% of the subjects, and (3) minimal progression, in approximately 11% of the subjects. Over a period of 15 years, the annual rates of clinical attachment loss in these three subgroups were up to 1.0 mm in the first group, 0.5 mm in the second group, and 0.1 mm in the third group [11].

Already some 4000 years ago, the ancient Egyptians and Chinese described periodontal diseases as inflammatory conditions, and Hippocrates (460–335B.C.) discussed the etiology and pathogenesis of different forms, including the situation when “the gums were bleeding or rotten” [12].

The early Hebrews Jews, the Romans, and later the Arabs of the middle ages contributed in various ways to the description and treatment of these diseases. However, it is probably fair to consider the 1746 publication of Pierre Fauchard [13] and *Le Chirurgien Dentiste*, as providing the first discussion of periodontal pathology and therapy with some modicum of intellectuality and usefulness. Fauchard recommended thorough scaling of the teeth with special instruments to remove calculus. He also prescribed mouthwashes, dentifrices, and the splinting of loose teeth.

The early English contribution to the understanding and management of periodontal diseases was made by John Hunter, a physiologist and surgeon of broad intellectual and scientific interests, widely known for his 1771 work on the natural history of the human teeth [14]. In another major work from 1802, a practical treatise on the diseases of the teeth, Hunter proposed a classification of the periodontal diseases that identified inflammatory processes in the gingiva as important factors in the resorption of the alveolar bone [15].

Etiology

The term “endotoxin” was first introduced to denote toxic substances within bacterial cells that were released upon the death of the bacteria. Today, the term is used synonymously with the term “lipopolysaccharide,” which is a component of the outer cell wall of Gram-negative bacteria. It was hypothesized that this endotoxin would limit the effectiveness of periodontal therapy because even if plaque and calculus were removed from the root surface, the endotoxin still presents in the cementum would continue to irritate the tissues and thus compromise healing following treatment. This presumption led to the preeminence of the treatment concept known as “root planning,” often combined as a treatment strategy with scaling, and abbreviated as “scaling and root planning.”

Periodontal Regeneration and Biomaterials

Management of bone periodontal defects, destruction, and loss of the alveolar bone in the upper

or lower jaw is considered a challenge for modern periodontal regeneration and implant dentistry [16].

There are numerous of biomaterials that are being used in the regenerative therapy of periodontal disease such as Straumann® *Emdogain*®, Geistlich *Bio-Oss*® made from the mineral part of bovine *bone*, *MIS 4MATRIX – Bone Graft*, and Platelet-rich fibrin (PRF).

The first step in the periodontal treatment procedures is the removal of supra and subgingival concerns deposits but in parallel with that an individual approach in educating and motivating patients to maintain oral hygiene, and then processing the hard and soft wall of the periodontal pocket, removal of granulation, ulceration and proliferation from the soft wall, polishing the root surface, and, where necessary, planning, and performing periodontal surgery. After the applied therapeutic procedures, it is possible to eliminate or reduce the depth of the periodontal pockets and to create a new junction epithelium [17]. The ultimate goal of any periodontal therapy is not only to prevent the progression of periodontal disease but also to regenerate the architecture and function of the periodontal complex, which involves the formation of a new cement of the root of the tooth, as well as a new epithelial attachment [4], and motivating patients to maintain proper oral hygiene.

Number of therapeutic modalities, nonsurgical, and surgical procedures, and aim to prevent progressive attachment loss, reduce probing pocket depths (PPD) and control both systemic and local risk factors associated with periodontal disease. In this literature review, we are going to highlight some of biomaterials and procedures which involve directly in periodontal.

One of the most important interventions recommended in this domain is guided tissue regeneration (GTR/guided bone regeneration). It is a surgical procedure that is aimed at restoring periodontal tissues. In general, the goal of periodontal therapy is the elimination of deep periodontal pockets. The treatment of periodontitis is cause-related, so it should be known the cause of periodontitis to treat and fight against that. The first step is to mechanically clean periodontal pockets from bacteria (debridement). The crucial role for the success of the therapy is the patient's home plaque control since pockets can be recolonized by bacteria in a few weeks. In the presence of deep pockets, surgery may also be indicated to get access to the deepest portions of the pockets to properly clean them and to reduce the depth of the pockets. The goal of this treatment approach is healing by repair without the formation of new periodontal attachment [18]. This can be done by different types of bone grafts, membranes, growth factors, etc. Using the grafts, we try to help the regeneration of lost periodontal tissue. There are many original papers which are talking about regenerative therapy in the treatment of periodontal intrabony defects. The main parameters that we check after regenerative therapy are plaque index, papillary bleeding index, and PPD. The ideal treatment

would be to recover the periodontal tissues that have been lost (periodontal tissue regeneration). Several surgical techniques have been developed in the attempt to regenerate periodontal tissues, including GTR, bone grafting (BG), and the use of the enamel matrix derivative (EMD). All these treatments have been shown to have the potential to regenerate at least some periodontal attachment in humans [19]. It's seen that the effects of Enamel Matrix Derivative and Natural Bone Mineral with Platelet-Rich Plasma on the Healing of Intrabony Defects Treated was treated very good by Döri et al. where the use of PRP does not appear to improve the results obtained with EMD+NBM (Table 1) [20]. To compare mandibular bone regeneration by applying autologous bone, platelet-rich plasma and two biomaterials (synthetic calcium hydroxyapatite, and demineralized bone matrix), and thus establish the potential benefits of these biomaterials in the regeneration of post extraction alveolar bone the faster bone formation occurred in the groups where are used autologous bone and demineralized bone matrix, respectively (Table 1) [21].

Periodontal regeneration mediated by EMD is based on a different concept. The enamel matrix is composed of a number of proteins, 90% of which is amelogenin. The only commercially available product using EMD is called Emdogain and is produced by Biora (Malmö, Sweden). One year after treatment of periodontal resorption by EMD showed statistically significant improvements in probing attachment levels (1.3 mm) and PPD reduction (1 mm) in comparison with flap surgery. However, the actual clinical advantages might be questioned since there is not yet evidence that more teeth can be saved using EMD. No evidence of major differences between EMD and GTR could be found with the expectation slightly more PPD reduction (0.6 mm) due to increased gingival recession (REC) 0.5 mm in GTR treated sites. On the other hand, EMD seems simpler to use, may not need antibiotic coverage and does not need a second surgical intervention (if compared with non-resorptive barriers). In addition, no post-operative infections or adverse events were observed with EMD versus two cases of infection (not statistically significant) in the GTR group.

Some clinical study recommends the use of Bio-Oss deproteinized bovine bone mineral alone or in combination with Emdogain for surgical regenerative procedure in human periodontal intrabony defects. In the study by Koop *et al.* [22], the use of Bio-Oss combined with EMD-Emdogain as a biologic factor in consistence with the regenerative surgical procedure is recommended for the periodontal healing. The conclusion of this study was that in the treatment of intrabony defects, the use of EMD is superior to control treatments but as effective as resorbable membranes. The additional use of EMD with a coronally advanced flap for REC coverage will give superior results compared with control but is as effective as a connective tissue graft. The use of EMD in furcations will give more

Table 1: Studies of different biomaterials used for periodontal regeneration

Authors	Application of	Year publication	Follow-up	Main results	Reference
Koop <i>et al.</i>	Enamel Matrix Derivative	2012	12 months	Effective in reduction of horizontal furcation	[22]
Najeeb <i>et al.</i>	PRF with open flap debridement	2017	12 months	Very good outcomes in regenerative therapy	[23]
Hou <i>et al.</i>	PRP in treatment of intrabony defects	2016	6 months	PRP together with conventional grafting procedures may be a beneficial treatment approach	[24]
Stavropoulos and Karring	GTR with bioabsorbable membranes+DBB (Bio-Oss)	2005	5 years	GTR with bioabsorbable membranes in combination with Bio-Oss is basically stable on a long-term basis	[25]
Agarwal <i>et al.</i>	PRF+DFDBA	2016	12 months	PRF+DFDBA more effective than DFDB with saline	[26]
Pradeep <i>et al.</i>	Platelet Rich Fibrin with 1% Metformin	2015	9 months	PRF +1% MF group showed better results in clinical parameters and radiograph defect depth reduction compare to MF, PRF, or OFD alone	[27]
Shah <i>et al.</i>	PRF+DFDBA	2012	12 months	PRF shows comparable results to DFDBA in terms of clinical parameters	[28]
Lekovic <i>et al.</i>	PRF and BPBM	2012	6 months	Both PRF and PRF-BPBM groups showed significant pocket depth reduction at 6 months compared with baseline	[29]
Panda <i>et al.</i>	Autologous platelet concentrates (APCs)	2016	9 months	APCs in surgical treatment of intrabony defects have been increasing in recent years	[30]
Esposito <i>et al.</i>	Emdogain	2009		Very good regenerative effect on bone healing	[2]
Galgut	Biodegradable oxidized cellulose and hydroxyapatite	1990	8 weeks	Guided tissue regeneration techniques have been shown to enhance new attachment formation with minimal post-operative recession	[31]
Kökdere <i>et al.</i>	PRF and PRF-mixed particulate autogenous bone graft	2015	2 months	PRF increase new bone formation and has a positive effect on early bone healing	[32]
Albanese <i>et al.</i>	PRP from the wound healing to bone regeneration	2013	12 months	Positively influence bone regeneration	[33]
Célio-Mariano <i>et al.</i>	Autologous platelet rich plasma after impacted third molar mandible surgery	2012	6 months	Autologous PRP was found to accelerate alveolar bone regeneration, and men presented better repair after tooth extraction	[34]
Dohan <i>et al.</i>	Platelet-rich fibrin (PRF)	2006	6 months	The biologic activity of the fibrin molecule is enough in itself to account for the significant cicatrice capacity of the PRF	[35]
Arenaz-Búa <i>et al.</i>	Platelet-rich plasma, hydroxyapatite, demineralized bone matrix, and autologous bone	2009	10 days	The faster bone formation occurred in the groups where we used autologous bone and demineralized bone matrix, respectively	[21]
Kobayashi <i>et al.</i>	Comparative release of growth factors from PRP, PRF, and advanced-PRF	2016	10 days	PRP can be recommended for fast delivery of growth factors whereas A-PRF is better-suited for long-term release.	[36]
Needleman <i>et al.</i>	Guided tissue regeneration for periodontal infra-bony defects	2012	12 months	GTR has a greater effect on probing measures of periodontal treatment than open flap debridement	[37]
Sebben <i>et al.</i>	Platelet-rich plasma alone and in combination on with alpha-tricalcium phosphate cement	2012	8 weeks	The data from this study suggest that treatment with α -TCP cement combined with PRP does not show any significant difference in comparison with PRP alone	[38]
Ozdemir and Okte	Beta-tricalcium phosphate alone and in combination with platelet-rich plasma	2011	6 months	After 6 months, both treatment modalities showed statistically significant clinical and radiographically improvements	[39]
Döri <i>et al.</i>	Platele-trich plasma enamel matrix derivative and natural bone mineral	2013	5 years	(1) The clinical outcomes obtained with both treatments can be maintained up to a period of 5 years and (2) the use of PRP does not appear to improve the results obtained with EMD+NBM	[20]

reduction in horizontal furcation defect depth compared with resorbable membranes.

The results of the study of Gojkov-Vukelic *et al.* [40] using Maxresorb (Botiss dental) as regenerative grafts in periodontal pockets showed a significant reduction in probing depth for both groups of patients (treated by open flap surgery and treated by open flap surgery in addition with Maxresorb), 1 and 6 months after the performed surgical therapy. By comparing the results between the groups, they did not get significantly better results for Group 2, where Maxresorb was used as bone replacement. This research is the opposite with findings of the study by Gokhale [41], whose results showed significant improvement for clinical parameters at sites treated with Bio-Oss compared to control sites.

In the research by Shivjot Chhina comparing of treatment results using standard flap surgery and flap surgery with the addition of free gingival graft revealed better treatment outcomes in the group with free gingival graft [42]. The conclusion was that the treatment of supracrestal defects with a combination of open flap debridement (OFD) and SECTG led to significantly better clinical results compared to OFD alone.

Results from meta-analysis indicated that the treatment of periodontal bone defects with intraoral BGs in periodontal regeneration is not always predictable [43]. The literature review of GTR in a lot of case studies has shown that this procedure is more effective than OFD, with an additional gain in clinical attachment level of 1.2 mm [37].

Platelet-rich plasma (PRP) is autologous plasma which has been enriched with platelets and leukocytes in addition to jellifying agents, growth factors, cytokines, bovine thrombin, and anticoagulants [44], [45]. One of the drawbacks of PRP is the fact that it has liquid nature and therefore required its combination with other biomaterials, including bone grafts derived from human cadavers (allografts) or animal products (xenografts). Data points out to the quick "burst" release of growth factors from PRP [46].

In many reviews, we noticed that PRP has been used as a regenerative substituent to promote wound healing and tissue regeneration [47]. Comparing with PRF, PRP has some limitations because the growth factors are released for a very short period of time, also the bovine clotting factors may react with human clotting factors to give rise to bleeding. There

is a reason that PRF as a second-generation platelet derivative, called PRF has been used in regenerative medicine and dentistry [35], [48], [49]. One of the main differences between PRF and previously utilized PRP is the incorporation of leukocytes in PRF. Several studies have shown their key importance during anti-infectious pathogen resistance, as well as their implications in immune regulation [50], [51], [52]. The platelet-rich fibrin when combined with open-flap debridement produces better outcomes compared to the open flap debridement alone. The regenerative potential of platelet-rich fibrin results in better augmentation and regeneration of periodontal bone defects (Table 1) [23]. The adjunctive use of PRP together with conventional grafting procedures may be a beneficial treatment approach. However, when combined with the use of a regenerative technique, such as GTR, the beneficial effect of PRP on the treatment of intrabony defects is negligible (Table 1) [24].

By used of GTR with bio absorbable membranes in combination with Bio-oss are basically stable on a long term basis and the clinical and radiographical data from baseline and from the 1- and 5-year control wich are presented on this article treatment resulted in statistically significant clinical improvements (i.e., PPD reduction, PAL gain) 1 year after surgery, which were preserved during the following 4- year observation period. PPD had increased to a minor extend (0.4 mm) from the 1- to the 5-year control visit, but the average amount of residual PPD was not significantly different between the two observation periods (Table 1) [25].

In usage of demineralized freeze-dried bone allograft (DFDBA) alone with saline and PRF with DFDBA the 12-month results indicated that both treatment modalities resulted in significant changes in all clinical and radiographic parameters. However, the PRP/DFDBA group exhibited statistically significantly greater changes compared with the DFDBA/saline group in PD, CAL, REC, bone fill and defect resolution table (Table1) [26].

Treatment of furcation defects with RSV 1.2mg in situ gel combined with autologous PRF and porus-HA bone graft, results in significant improvements of clinical and radiographic parameters when compared with OFD alone. Combining RSV with PRF and HA, implies their synergistic effects explaining their role as a regenerative material in the treatment of furcation defects (Table 1) [27].

PRF shows comparable results to DFDBA in terms of clinical parameters (Table 1) [28].

The results of this study indicate that PRF can improve clinical parameters associated with human intrabony periodontal defects, and BPBM has the ability to augment the effects of PRF in reducing pocket depth, improving clinical attachment levels and promoting defect fill (Table 1) [29].

Based on the results obtained from the systematic review by Panda et al. can be concluded that the evidence on the beneficial additive effect of APCs in surgical

treatment of intrabony defects has been increasing in recent years (Table 1) [30].

By using Biodegradable oxidized cellulose and hydroxyapatite Guided tissue regeneration techniques have been shown to enhance new attachment formation with minimal post- operative recession (Table 1) [31].

PRF alone or PRF-mixed particulate autogenous bone increase new bone formation and has a positive (Table 1) [32]. The use of PRP in the alveolar socket after tooth extractions is certainly able to improve soft tissue healing and positively influence bone regeneration but this latter effect seems to decrease a few days after extraction (Table 1) [33].

Analysis of radiographic bone density by periods 1, 2, and 3 months after extraction of impacted mandibular third molars bilaterally where in one side was used PRP and in other side just blood clot there were favorable significant differences for the PRP group. At 7 days and at 6 months, there were no statistical differences; however, higher means of radiographic bone density were observed in the PRP group (Table 1) [34].

To compare mandibular bone regeneration by applying autologous bone, platelet-rich plasma and two biomaterials (synthetic calcium hydroxyapatite, and demineralized bone matrix), and thus establish the potential benefits of these biomaterials in the regeneration of post extraction alveolar bone the faster bone formation occurred in the groups where are used autologous bone and demineralized bone matrix, respectively (Table 1) [21].

PRP, PRF, and A-PRF were able to release growth factors over time from their respective platelet formulations. Interestingly, PRP demonstrated the ability to release significantly higher levels of growth factors at very early time points whereas PRF and A-PRF had a more gradual release of growth factors up to a 10-day period (Table 1) [36].

By compare the effect of alpha-tricalcium phosphate (α -TCP) cement combined with platelet-rich plasma (PRP) on osteogenesis, and to compare the results with use of PRP alone was seen that treatment with α -TCP cement combined with PRP does not show any significant difference in comparison with PRP alone (Table 1) [38].

Beta-tricalcium phosphate alone and in combination with platelet-rich plasma as modalities for treatman of intrabony defects showed significant clinical radiographically improvements (Table 1) [39].

Unlike PRP, PRF contains a fibrin matrix instead of jellifying agents and bovine clotting factors [53]. It is important to note that PRF contains a number of cells, including platelets, leukocytes, macrophages, granulocytes, and neutrophils. Furthermore, PRF exhibits a slow and sustained release of growth factors, such as transforming growth factor- β 1, platelet-derived growth factor, and vascular endothelial growth factor which all have been proven to promote the wound healing and tissue regeneration [54].

The PRF when combined with open-flap debridement and combined by GTR produces better outcomes and better regenerative power compared to the OFD alone. The regenerative potential of PRF results in better augmentation and regeneration of periodontal bone defects. In addition, PRF may augment the regenerative potential of bone grafts. However, more long term and well-designed clinical trials are needed to ascertain the clinical efficacy of PRF and PRF containing bone grafts.

MIS 4MATRIX bone grafts is a new synthetic bone replacement product that helps in bone dental replacement procedures. The 4MATRIX composition is pure 66.6% biphasic calcium sulfate (BCS) and 33.3% hydroxyapatite (HA) and is characterized by predetermined setting time and resorption rate. 4MATRIX is the preferred augmenting-replacement product for a wide variety of dental bone replacement procedures.

Considering the bone regeneration, resorption rate and stabilization 4MATRIX have its advantages. During the administration procedure, the 4MATRIX BCS component remains intact in the presence of blood and saliva and stimulates bone growth when placed in contact with the bone or periosteum. 4MATRIX consists of two different components. BCS has a complete resorption rate in close connection with bone formation rate (4–10 weeks), while HA acts as a long-term spatial maintainer. A component such as HA contributes to the maintenance of longer-term space and provides higher mechanical strength and stabilization of new regenerated bone grafts.

Conclusion

Biomaterials being used in periodontal surgical treatment have the different regenerative ability. The combined use of biomaterials might result in a better clinical outcome.

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Hypertensive Disease in Pregnancy - A Retrospective Study

MD. PhD. Can. Sahadete ShALA

Specialist at University Clinical Center of Kosovo, Obstetrician and Gynecological Clinic (OGC) Prishtine drsahadete@gmail.com

PhD. Lumturi MERKuri

Lecturer at Faculty of Medicine European University of Tirana
lunturie.merkuri@uet.edu.al

MD. PhD. Can. Astrit GASHi

Specialist at University Clinical Center of Kosovo, Obstetrician and Gynecological Clinic (OGC) Pristine astritgashi772@gmail.com

Msc. Can. Jehona Luta

Student at University of Prishtina, Faculty of Medicine, Master of Science in Health Care Education jehonaluta13@gmail.com

Abstract

introduction: Hypertensive disease in pregnancy is a group of high blood pressure disorders that occur during pregnancy and is classified into 4 categories: pregnancy-induced hypertension (PIH), chronic hypertension, preeclampsia-eclampsia and preeclampsia superimposed on chronic hypertension. About 10% of pregnancies globally are complicated by hypertensive diseases. Hypertensive disease in pregnancy, are one of the three major causes of death in pregnancy (16%) along with postpartum bleeding (13%) and puerperal infections (2%)

Aim: The purpose of this study was to determine the frequency of hypertensive disease in pregnancy and the perinatal outcomes of women with hypertensive disease in pregnancy.

Method: This was a retrospective study of hypertensive disease in pregnancy at the Obstetrics and Gynecology Clinic / University Clinical Centre in Kosovo. The data was obtained from the hospitalized patient's medical history. Sample size: 8754 cases. Data collection, classification, and statistical analysis were performed with SPSS 21.

results: Out of 8754 observed pregnant women, 84 or 10.4% of them had the hypertensive disease in pregnancy; of them, 59.5% (50) had pregnancy-induced hypertension (PIH), 20.2% (17) preeclampsia, 14.3% (12) chronic hypertension, and 6% (5) eclampsia. The average age of women with the hypertensive disease in pregnancy was 26.6 +- 2; where 27.5% (23) of them were women over 35 years of age, while 3.5% of them were under 18

years of age. Out of 84 women; 53.5% (45) were multiparous women, while 46.5% (39) were primiparous women. 41.7% (35) of women with hypertensive pregnancy disease are born with a Cesarean section, while 58.3% (49) are born with vaginal delivery. 79.7% of infants were born with an Apgar score of over 5, 13% below 5, while 7.3% were morsfoetus.

Conclusions: The prevalence of hypertension in pregnancy at the Obstetrics and Gynecology Clinic in Pristina was 10.4%. 27.5% of women with the hypertensive disease in pregnancy were women over 35 years of age, while 3.5% of them were under 18 years of age. Hypertensive disease in pregnancy occurs more frequently in multiparous women. Due to emergencies, almost half of women with hypertensive pregnancy disease are born with a Cesarean section, and 80% of infants are born with an Apgar score of over 5.

Key words: Hypertensive disease in pregnancy; Prevalence; Kosovo

Introduction

While motherhood is a positive and enjoyable experience, many women are experiencing somewhat of a health disturbance on their pregnancy months, either that is a sort of an acute illness or a prolong disease.⁽¹⁾

Around 15% of pregnant women are expected to develop life-threatening complications during pregnancy, at delivery or post-partum.

Hypertension is the most common medical problem encountered during pregnancy, complicating 2-3% of pregnancies. Hypertensive disorders during pregnancy are classified into 4 categories, as recommended by the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy: chronic hypertension, preeclampsia-eclampsia, preeclampsia superimposed on chronic hypertension, and gestational hypertension (transient hypertension of pregnancy or chronic hypertension identified in the latter half of pregnancy)⁽²⁾

Chronic hypertension is high blood pressure that either precedes pregnancy, is usually diagnosed within the first 20 weeks of pregnancy, or does not resolve by the 12-week postpartum checkup. Two categories of severity are recognized: mild (up to 179 mm Hg systolic and 109 mm Hg) and severe (\geq 180 systolic or 110 diastolic). Chronic hypertension complicates about 5% of all pregnancies, and prevalence rates are increasing year by year because of delayed childbearing.⁽³⁾ Medications should be reviewed when pregnancy is first diagnosed or in the time when the couple is planning to get pregnant. Methyldopa is the most studied of all antihypertensive medications and is generally the first choice in pregnancy because it has a limited effect on uteroplacental blood flow⁽⁴⁾. Sometimes an alternative must be found because of elevated liver enzymes or complaints of headache. Labetalol, a combined alphablocker and beta-blocker, is the first alternative to methyldopa and is becoming a first-line choice as experience with the drug during pregnancy increases. It is generally well tolerated and has an easier (twice-a-day) dosing schedule than methyldopa.⁽⁵⁾

Most studies have not found adverse pregnancy outcomes. Nonetheless, caution should be used in cases of impaired uteroplacental perfusion, such as preeclampsia or intrauterine growth restriction. Atenolol and other pure beta-blockers should be avoided: they have been associated with babies born small for their gestational age. Angiotensin-converting enzyme (ACE) inhibitors are contraindicated in the second and third trimester because they are associated with a myriad of congenital anomalies, including renal failure, oligohydramnios, renal dysgenesis, reduced ossification, pulmonary hypoplasia, and fetal and neonatal death. ⁽⁶⁾

Complication rates are directly related to the severity and duration of elevated blood pressures. For instance, patients with severe hypertension in the first trimester have a greater than 50% risk of developing superimposed preeclampsia. ⁽⁷⁾ All hypertensive patients should undergo increased surveillance, serial laboratory tests throughout pregnancy, serial ultrasound scans to follow growth, and antenatal testing. The baby should be delivered normally that means in vaginal ways if possible.

Gestational hypertension, formerly known as pregnancy-induced hypertension or PIH, is the new onset of hypertension after 20 weeks of gestation. ⁽¹²⁾

The diagnosis can be set as soon as the patient has:

- High blood pressure (systolic ≥ 140 or diastolic ≥ 90 mm Hg, the latter measured using the fifth Korotkoff sound)
- No protein in the urine
- Previously normal blood pressures
- No manifestations of preeclampsia.

Also known as transient hypertension, gestational hypertension is actually diagnosed retrospectively when the patient does not develop preeclampsia and if blood pressure returns to normal by the 12-week postpartum visit. Fifty percent of women diagnosed with gestational hypertension are between 24 and 35 weeks develop preeclampsia ⁽⁸⁾

Preeclampsia is a multiorgan disease process of unknown etiology ⁽⁹⁾ characterized by the development of hypertension and proteinuria after 20 weeks of gestation.

Preeclampsia is defined as elevated blood pressure after 20 weeks of gestation (≥ 140 mm Hg systolic or ≥ 90 mm Hg diastolic) plus proteinuria (> 0.3 g/24 hours). In clinical practice, we usually use the criteria of two elevated blood pressure measurements 6 hours apart and a proteinuria of 300 mg in a 24-hour urine specimen. A 24-hour determination is most accurate because urine dipsticks can be affected by variable excretion, maternal dehydration, and bacteriuria ⁽¹⁰⁾

Preeclampsia can range from mild to severe. Severe preeclampsia is defined as any of the following:

- Markedly elevated blood pressure measurements (systolic ≥ 160 mm Hg or diastolic ≥ 110 mm Hg) taken at least 6 hours apart with the patient on bed rest
- Proteinuria (≥ 5 g/24 hours or $\geq 3+$ on two random samples 4 hours apart)

- Manifestations of end-organ disease: oliguria (< 500 mL in 24 hours), cerebral or visual disturbances, pulmonary edema, cyanosis, epigastric or right-upperquadrant pain, impaired liver function, thrombocytopenia, or fetal growth restriction.

HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets) used to be classified as a separate syndrome, but current thinking categorizes it as a manifestation of preeclampsia, occurring in about 20% of severe cases. It is associated with significant maternal and perinatal morbidity. A decreasing platelet count and an increasing l-lactate dehydrogenase level (indicative of both hemolysis and liver dysfunction) reflect disease severity^{(11), (12)}

Preeclampsia places both mother and fetus at risk. It is, however, a maternal disorder. The mainstay of treatment is early detection and managed delivery to minimize both maternal and fetal risks. Magnesium sulfate is still the drug of choice for preventing and arresting eclamptic seizures. It has the additional benefit of reducing the incidence of placental abruption⁽¹³⁾. Serum magnesium levels should be monitored in women with elevated serum creatinine levels, decreased urine output, or absent deep tendon reflexes⁽¹⁴⁾. Antihypertensive medications are used solely to prevent maternal morbidity and have no effect on disease progression or preventing eclampsia.

Medications must be given with caution: if blood pressure is lowered too fast, it can have a dramatic effect on uteroplacental perfusion and can cause an already compromised fetus to rapidly decompensate and become bradycardic. Preferred medications are hydralazine (5-10 mg intravenous bolus every 10-15 minutes), labetalol, nicardipine, and sodium nitroprusside. Intravenous labetalol and hydralazine are commonly used for the acute management of preeclampsia⁽¹⁵⁾.

Although many pregnant women with high blood pressure have healthy babies without serious problems, high blood pressure can be dangerous for both the mother and the fetus therefore it should be detected and soon diagnosed so we can manage it and escape from such problems.

Aim

The purpose of this study was to determine the frequency of hypertensive disease in pregnancy and the perinatal outcomes of women with hypertensive disease in pregnancy.

Materials and methods

This was a retrospective study of hypertensive disease in pregnancy at the Obstetrics and Gynecology Clinic / University Clinical Centre in Kosova. The data was obtained from the hospitalized patient's medical history. Sample size: 8754 cases. Data collection, classification, and statistical analysis were performed with SPSS 21. This study was conducted during a year from 2017 to 2018

All pregnant women that were hospitalized in University Clinical Centre of Kosova were eligible to be included in this study.

Hypertensive disorders in pregnancy were diagnosed based on the diagnostic criteria set by the National High Blood Pressure Education Program Working Group.⁽⁸⁾

Pregnant women were in a randomized selection for the study. In this study to get better results different data was collected such as their previous pregnancies, way of delivery, their babies health condition also their age.

Results

Out of 8754 observed pregnant women, 84 or 10.4% of them had the hypertensive disease in pregnancy; of them, 59.5% (50) had pregnancy-induced hypertension (PIH), 20.2%(17) preeclampsia, 14.3% (12) chronic hypertension, and 6% (5) eclampsia.

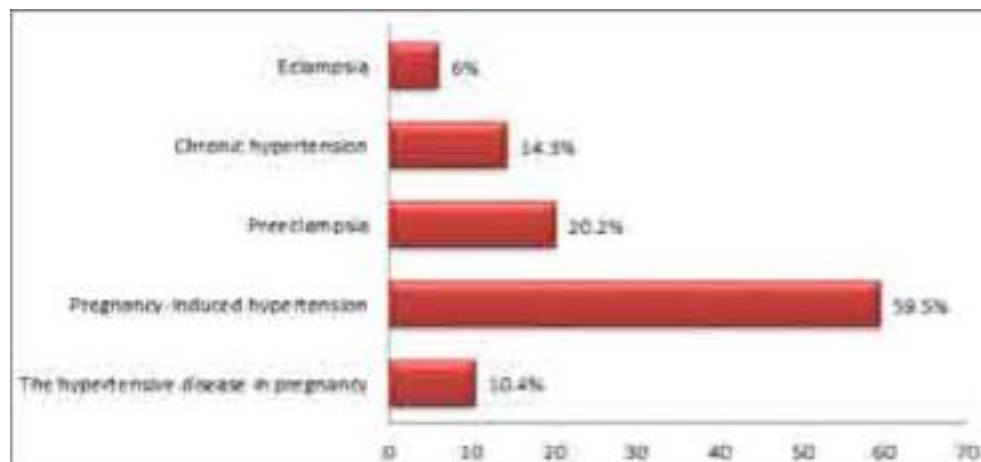
Table 1. The most common hypertensive disorder in pregnant women

	n	%
the hypertensive disease in pregnancy	84	10.4
Pregnancy-induced hypertension	50	59.5
Preeclampsia	17	20.2
chronic hypertension	12	14.3
eclampsia	5	6
total	168	100

CharT 1. The most common hypertensive disorder in pregnant women expressed in percent

The average age of women with the hypertensive disease in pregnancy was 26.6 +- 2; where 27.5% (23) of them were women over 35 years of age, while 3.5% of them were under 18 years of age.

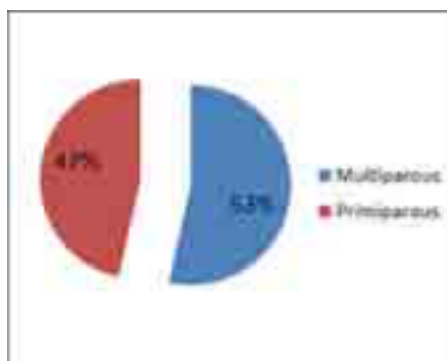
Table 2. The age groups of women that were studied



aGe	%
<18	3.5
18-34	69
>35	27.5

Out of 84 women; 53.5% (45) were multiparous women, while 46.5% (39) were primiparous women.

CharT 2.S eparated groups based on previous pregnancies of the pregnant women



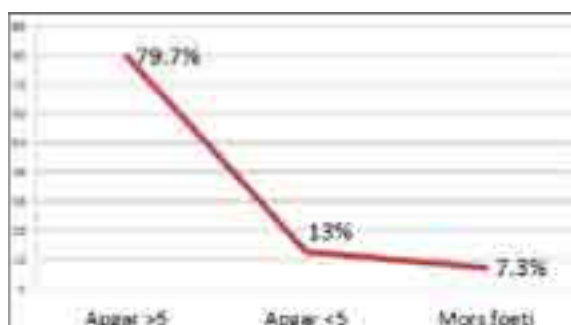
41.7% (35) of women with hypertensive pregnancy disease are born with a Cesarean section, while 58.3% (49) are born with vaginal delivery.

Table 3. The way of delivery of pregnant women with hypertensive disease in pregnancy

Way of delivery	n	%
cesarean Section	35	41.7
Vaginal delivery	49	58.3
total women with hypertensive disease in pregnancy	84	100

79.7% of infants were born with an Apgar score of over 5, 13% below 5, while 7.3% were morsfoeti Chart 3

CharT 3. Health conditions of the infants



Conclusions

We concluded that the prevalence of hypertension in pregnancy at the Obstetrics and Gynecology Clinic in Pristina was 10.4%.

About the age 27.5% of women with the hypertensive disease in pregnancy were women over 35 years of age, while 3.5% of them were under 18 years of age. Our study provides that hypertensive disease in pregnancy occurs more frequently in multiparous women such it shows in our study. Due to emergencies, almost half of women with hypertensive pregnancy disease are born with a Cesarean section, and 80% of infants are born with an Apgar score of over 5.

Discussion

This study determined adverse maternal and perinatal outcomes among women with Health Disease in Pregnancies and directly compared these adverse pregnancy outcomes between women with chronic/gestational hypertension and those with pre-eclampsia/eclampsia. The sociodemographic and reproductive characteristics did not differ significantly between the two groups except for prim paternity. Hypertensive disorders of pregnancy are the commonest medical complication of pregnancy. The incidence varies in different populations. Generally, the problem is more common in the developing countries compared to developed countries

According to the results of this meta-analysis, the pooled prevalence of hypertension disorder of pregnancy in Ethiopia was estimated to be 6.25% (95% CI: 5.23%, 7.26%). Regional variation in Health Disease In Pregnancy was observed, the highest prevalence of Health Disease In Pregnancy (18.25%) was reported in a study done in Public Health Institutions in Arba Minch town⁽¹⁶⁾

The overall pooled prevalence of hypertensive disorders of pregnancy in this study is more or less similar to the large study conducted on Health Disease In Pregnancy in China which was estimated 5.2%⁽¹⁷⁾. But, in the finding (Abalos et al., 2014) is higher than the global prevalence⁽¹⁸⁾. This difference might be due to socio-cultural, variability in maternal risk factor distribution, and the difference in antenatal care service accessibility. In addition, most of the studies included in this meta-analysis were conducted in hospitals and health centers which might increase the prevalence

Young maternal age was not associated with Health Disease In Pregnancy. Similar finding was also observed in a systematic review on pre-eclampsia. The study showed that young maternal age doesn't affect the risk of developing pre-eclampsia⁽¹⁹⁾. But, other studies showed different findings in the occurrence of Health Disease in Pregnancy among younger and older mothers.

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Risk factors for hyperemesis gravidarum requiring hospital admission during pregnancy

Abstract

Hyperemesis gravidarum (HG) is a pregnancy complication that is characterized by severe nausea, vomiting, weight loss, and possibly dehydration. The exact cause of nausea and vomiting during pregnancy is not known. However, it is believed to be caused by a rapidly rising blood level of a hormone called human chorionic gonadotropin (HCG). The symptoms of HG begin within the first six weeks of pregnancy. Nausea often doesn't go away. Risk factors include the first pregnancy, multiple pregnancy, obesity, prior or family history of HG, trophoblastic disorder, and a history of eating disorders. Diagnosis is usually made based on the observed signs and symptoms. HG has been technically defined as more than three episodes of vomiting per day such that weight loss of 5% or three kilograms has occurred and ketones are present in the urine.

Aim:

The aim of this study is to identify risk factors for hyperemesis requiring hospital admission during pregnancy.

Method:

The study included 548 pregnant women at the Clinical University Center of Kosovo from the age of 18 to 39 years that were hospitalized during the years 2012-2015. The highest participation percentage belonged to 24-26 years old pregnant women while the lowest one belonged to 36-39 years old. Data collection, classification, and statistical analysis were performed with SPSS 21.

Results:

Out of 548 pregnant women with severe nausea and vomiting 60.03% (329) were primiparous women, while 39.9% (219) were multiparous women when it comes to their blood group 28.8%

¹ Obstetrics-Gynecology Specialist, Chef of Department of post SC, Obstetric-Gynecology Clinic, University Hospitality Center Kosovo;

² Obstetrics-Gynecology Specialist, Department of Sterility, Obstetric-Gynecology Clinic, University Hospitality Center Kosovo;

³ Obstetrics-Gynecology Specialist, Department of Endocrinology, Obstetric-Gynecology Clinic, University Hospitality Center Kosovo;

⁴ PhD candidate, Medical Faculty, Nurse Section, University "Hasan Prishtina", Prishtinë

(158) of pregnant women with severe nausea and vomiting are Rh negative, while 71.2% (390) are Rh positive. The earliest pregnancy that was studied was that of 7th week of gestation and the oldest pregnancy was that of 28th week of gestation.

Conclusions:

We concluded that severe nausea and vomiting can cause major problems in a pregnancy and there are some risks factors such as dehydration and electrolyte imbalances that require hospital admission in order to avoid them and have a healthy pregnancy

Keywords:

Hyperemesis gravidarum, Kosovo, risk factors

Introduction

Physiological changes occurring during pregnancy commonly affect the gastrointestinal tract. Nausea and vomiting during pregnancy is common, affecting between 50 and 90% of gravidas (1). Of women who experience nausea and vomiting during pregnancy, 70% sustain this symptomatology between the fourth and seventh week of pregnancy. Less than 10% of patients will note this occurrence before their missed period. In approximately 90% of cases, vomiting ceases by the 16th week of gestation.[2]

Hyperemesis gravidarum-pernicious vomiting of pregnancy-is a severe form of nausea and vomiting in pregnancy often associated with weight loss, ketonemia, ketonuria, electrolyte imbalance, dehydration and possible hepatic and renal damage, which may persist throughout gestation.[3] [4]

The true incidence of hyperemesis gravidarum has not been established, yet has been reported at between 0.3 to 2%, with most authors reporting an incidence of 0.5%.[3] [5] [6]

Epidemiology

Hyperemesis gravidarum ranges in incidence between 0.3 and 2% of all deliveries. Annually in the United States more than 50,000 women are hospitalized with the diagnosis of hyperemesis gravidarum, with an average hospital stay of 4 days per patient.[7] [8]

Significant ethnic differences in the incidence of hyperemesis gravidarum have been noted among various populations. New Zealand pacific island women, United Kingdom Indian and Pakistani, and African-American women have increased rates of occurrence in comparison with ethnic European women.[9] [10] [11] Increased incidences have been noted in association with

nulliparity [odds ratio (OR), 1.6], adolescent patients, patients with increased body weight (Quetelet's index, $\text{kg/m}^2 \geq 24$, OR 1.5), multiple gestations (twins OR 1.5), gestational trophoblastic disease, fetal abnormalities including triploidy (partial mole), fetal central nervous system malformations (OR 4.0), and the occurrence of hyperemesis gravidarum in a previous pregnancy. Decreased incidences have been noted among patient with advanced maternal age (≥ 35 years of age, OR 0.5), maternal smokers (OR 0.6), and current fetal demise.[9] [12]

Etiology

The precise underlying etiology of hyperemesis gravidarum remains elusive. Numerous etiologies have been considered and include: gestation-associated hormone levels, thyrotoxicosis, serotonin, upper gastrointestinal dysmotility, psychological factors, hepatic abnormalities, autonomic nervous dysfunction, nutritional deficiencies, and *Helicobacter pylori* infection

Diagnosis

Clinical Manifestations

Hyperemesis gravidarum is a clinical diagnosis depending on typical presentation and exclusion of other causes. The disease typically occurs between the 4th and the 10th week of gestation, with resolution by the 20;pl weeks' gestation. While initially the nausea and vomiting are tolerable, gradual weight loss ($\geq 5\%$ of body weight), dehydration accompanied by abnormal serum electrolyte levels, and ketosis may occur. Hypersalivation occurs frequently, yet rarely is a major complaint.

Physical Examination

Patients with hyperemesis gravidarum presenting to the emergency department are often significantly dehydrated. These patients may manifest orthostatic changes (systolic blood pressure, mean change 8.3 ± 12.3 mmHg, and heart rate changes, mean 26.8 ± 14.5 bpm), which improve upon rehydration.[13] However, the presenting orthostatic changes lack sufficient sensitivity to be effectively applied as a quantitative screening tool for the severity of dehydration.[13] In severe cases, a dry and furry tongue (resulting from severe hypovolemia) and ketotic breath may be noted.[14] Infrequently, patients may manifest jaundice.[15] [16]

Laboratory Findings

Laboratory findings include evidence of dehydration: increased urine specific gravity and ketonuria, increase serum blood urea nitrogen and hematocrit levels, electrolyte disturbances including hyponatremia, hypokalemia and hypochloremia, which may be found in 15-25% of

patients.[17] Elevated serum amniotransferases and total bilirubin occur in between 15 to 50% of patients.[18] [19] Robertson and Miller[20] demonstrated that 24% of patients with hyperemesis gravidarum will exhibit elevated serum amylase levels with normal pancreatic amylase suggesting the source of the elevated amylase is salivary. Abnormalities in thyroid function, mainly elevated free T4 index or suppressed TSH, are found in approximately 60% of patients.[21] A number of authors have noted decreased serum vitamin B6 levels.[22] No alterations in serum zinc and copper metabolism were noted.[23]

Maternal complications.

Whereas prior to the 1940s (at which time fluid and electrolyte dynamics were not clear) maternal death complicating hyperemesis was not uncommon. In the distant past, due to the severity of potential complications of hyperemesis gravidarum, at times pregnancy termination was advocated.[14] Currently, hyperemesis gravidarum is rarely associated with death. Notwithstanding, serious life-threatening complications may occur. The most common serious reported complication involves the central nervous system.

Aim

The aim of this study is to identify risk factors for hyperemesis requiring hospital admission during pregnancy.

Materials and methods

This is a retrospective quality study that took place in the University Clinical Center of Kosovo-Prishtine. Patients that were studied mostly were in the early stages of pregnancy. They were admitted to the University Clinical Center of Kosovo because of dehydration and electrolyte imbalances. The earliest pregnancy that was studied was that of 7th week of gestation and the oldest pregnancy was of 28th +1 of gestation. The study included 548 pregnant women at the Clinical University Center of Kosovo from the age of 18 to 39 years that were hospitalized during the years 2012-2015. The highest participation percentage belonged to 24-26 years old pregnant women while the lowest one belonged to 39 years old.

Results

The earliest pregnancy that was studied was that of 7th week of gestation and the oldest pregnancy was that of 28th week of gestation. Out of 548 observed pregnant women, 61 of them were in their 7th week of gestation, most of pregnant women that were studied around 240 were

in their 8th to 13th week of pregnancy while 145 pregnant women were in their 14th to 20th week of gestation. Table 1, Chart 1

	N	%
7 th week of gestation	61	11.13
8 th -13 th week of gestation	240	43.79
14 th -20 th week of gestation	145	26.45
21 st -27 th week of gestation	82	14.96
28 th week of gestation	20	3.64
TOTAL	548	100

Table 1. The age of pregnancies studied

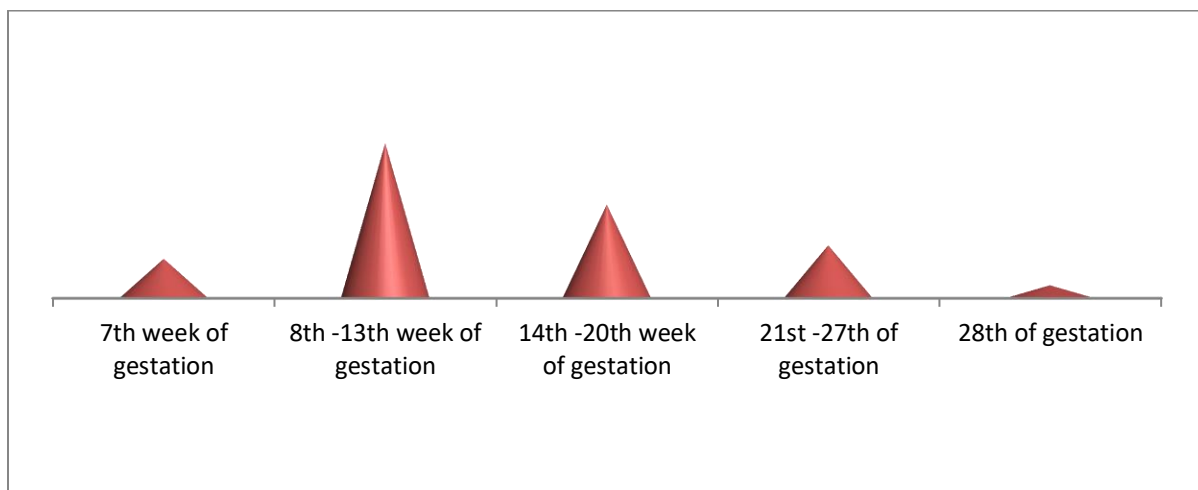


Chart 1. The age of pregnancies expressed in percent

The highest participation percentage belonged to 24 years old pregnant women with 35.8% participation while the lowest one belonged to 39 years old with 3.2% participation. Table 2.

AGE	%
18-20	6.9
21-23	11.7
24-26	35.8
27-29	21.6
30-32	14.5
33-35	6.3
36-39	3.2
TOTAL	100

Table 2. The age groups of women that were studied

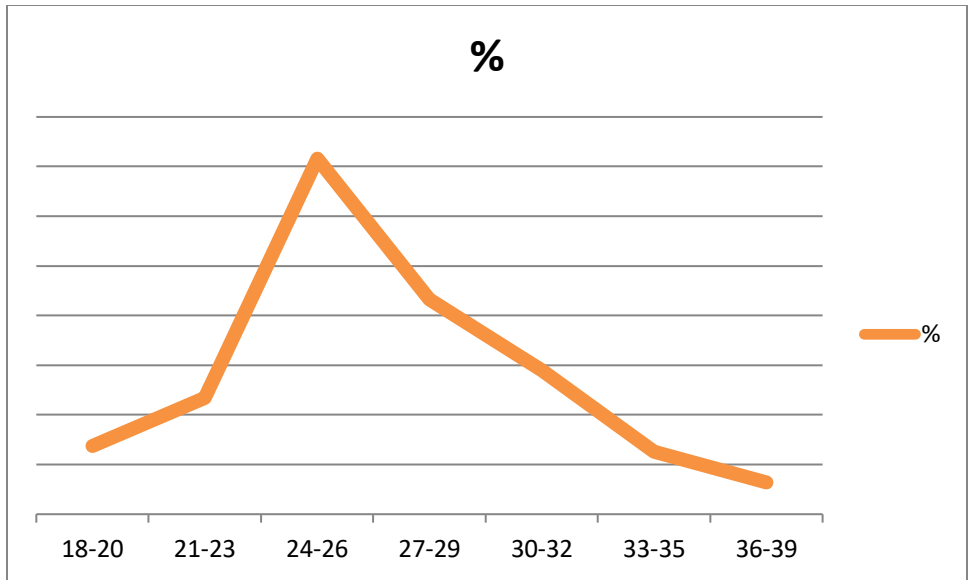


Chart 2. The age groups of women that were studied

Out of 548 women; 60.03% (329) were primiparous women, while 39.9% (219) were multiparous women. Chart 3.

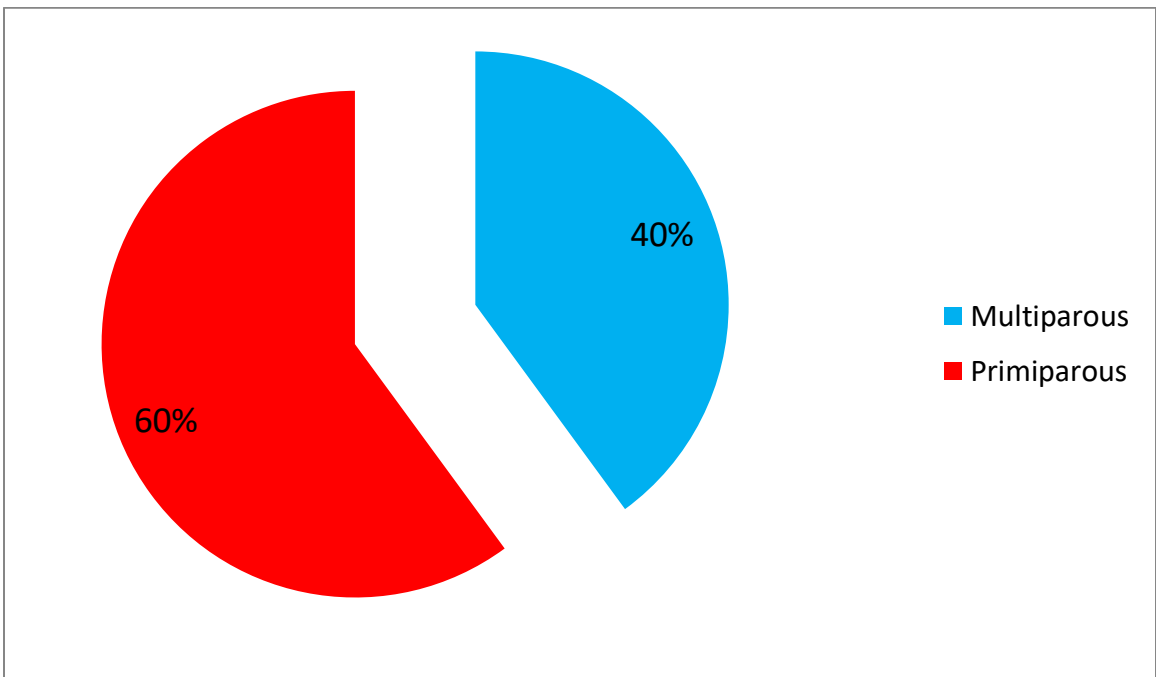


Chart 2. Separated groups based on previous pregnancies of the pregnant women

28.8% (158) of pregnant women with severe nausea and vomiting are Rh negative, while 71.2% (390) are Rh positive. Table 3.

Rh blood group	N	%
Rh negative	158	28.8
Rh positive	390	71.2
TOTAL	548	100

Table 3. Pregnant women classified in groups according to the presence of Rh factor

Conclusions

We concluded that severe nausea and vomiting can cause major problems in a pregnancy and there are some risks factors such as dehydration and electrolyte imbalances that require hospital admission in order to avoid them and have a healthy pregnancy. Out of 548 pregnant women with severe nausea and vomiting 60.03% (329) were primiparous women, while 39.9% (219) were multiparous women when it comes to their blood group 28.8% (158) of pregnant women with severe nausea and vomiting are Rh negative, while 71.2% (390) are Rh positive. The earliest pregnancy that was studied was that of 7th week of gestation and the oldest pregnancy was that of 28th week of gestation.

Discussion

Consistent with other studies, we found that hyperemesis was more common in young women [24] A number of other studies have noted an increased risk of hyperemesis among nulliparous women even after adjustment for maternal age. Nulliparous women and women pregnant for the first time have higher levels of estrogen than other women, which may predispose them to hyperemesis.[25]

It also identified several potential risk factors that merit future study. In particular, maternal preexisting diabetes, asthma, gastrointestinal disorders, and previous molar pregnancy, although not modifiable, could potentially assist in identifying women at high risk for developing hyperemesis during their pregnancies if they are established as risk factors. [5]

Hyperthyroidism has been associated with hyperemesis, although the exact mechanism is not known. Some thyroid conditions, eg, transient hyperthyroidism, are associated with higher than normal hCG levels, a characteristic of hyperemesis.[8]

Recently an association of increased seropositivity to Helicobacter Pylori among patients with hyperemesis gravidarum versus asymptomatic healthy pregnant controls.[26] Several case reports have suggested a beneficial effect of Helicobacter Pylori eradication.[27]

As treatment is often used Fluid Electrolyte and Vitamin Resuscitation, for patients experiencing continued nausea and vomiting despite institution of the above conservative treatment,

pharmacological measures may be indicated, including medications with antihistaminic, antiserotonin, or sedative/hypnotic properties or both.

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CAUSES OF INFERTILITY AMONG COUPLES IN PRISHTINA 2000-2010

Sahadete Shala^{1*}, Gjergji Theodhosi², Artan Simaku³

¹University Clinical Centre in Prishtina, Kosovo;

²Orthodox Clinic, Tirana, Albania;

³Institute of Public Health, Tirana, Albania;

*Corresponding author, Sahadete Shala, e-mail: drsahadete@gmail.com;

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ABSTRACT

The prevalence of infertility has increased significantly in recent years. The global prevalence of infertility is reported to be 10%-15% affecting approximately 10% of couples. The purpose of the study was to explore the causes of infertility among couples who visited the University Clinical Centre in Prishtina, Kosovo during the period of 2000-2010. Primary infertility was found in 229 (71.1%) couples and Secondary in 93 (28.9%) ones. Of the causes of infertility were: female factors in 127 (39.4%) of couples, male factors in 58 (18%), combined factors in 99 (30.7%) and unknown infertility in 38 (11.8%) of couples. In reviews of infertile couples, study of male and female factors is important and mostly the couples mentioned more than one reasons as the infertility factor. Finally, preconception care and counseling are recommended to all those who are planning a pregnancy to avoid failure.

Key words: Infertility, factors, primary, secondary

INTRODUCTION

Infertility is the failure to conceive (regardless of cause) after 1 year of unprotected intercourse. This condition affects approximately 10-15% of reproductive-aged couples. Reproduction requires the interaction and integrity of the female and male reproductive tracts, which involves (1) the release of a normal preovulatory oocyte, (2) the production of adequate spermatozoa, the normal transport of the gametes to the ampullary portion of the fallopian tube (where fertilization occurs), and the subsequent transport of the cleaving embryo into the endometrial cavity for implantation and development. Infertility is caused by male and/or female factors. Male and female factors each account for approximately 35% of cases. Often, there is more than one factor, with male and female factors combined causing 20% of infertility. In the remaining 10% of cases, the etiology is unknown (3). Couples with unknown etiology can be categorized as unexplained infertility or normal infertile couples (NICs), indicating that all findings from standard tests used in the infertility workup are normal. In normal infertile couples, the actual cause for infertility cannot be detected; perhaps there is dysfunctional interaction between the sperm and the oocyte, poor

quality of the embryo, or a disruption at the implantation site. In the future, identifying a mutation or the absence of a specific gene as the cause of infertility may be possible in this patient population.

Other lifestyle factors that have been associated with an increased risk of infertility include environmental and occupational factors; toxic effects related to tobacco, marijuana, or other drugs; excessive exercise; inadequate diet associated with extreme weight loss or gain; and advanced age (4).

Female Factor Infertility: Female factor infertility can be divided into several categories: cervical or uterine, ovarian, tubal, and other. Although stress and distress (anxiety or depression) have been considered factors in reducing pregnancy chances with ART, the number of studies has been limited and considerable between-study heterogeneity is noted (5). **Cervical factor infertility:** Cervical factor infertility can be caused by stenosis or abnormalities of the mucus-sperm interaction. The uterine cervix plays a pivotal role in the transport and capacitation of the sperm after intercourse. Cervical factors account for 5-10% of infertility. Cervical mucus production and characteristics change according to the estrogen concentration during the late follicular phase. At the beginning of the menstrual cycle, cervical mucus is scanty, viscous, and very cellular. The mucus forms a netlike structure that does not allow the passage of sperm. Mucus secretion increases during the mid follicular phase and reaches its maximum approximately 24-48 hours before ovulation. The water and salt concentration increases, changing the physical characteristics of the mucus. The mucus becomes thin, watery, alkaline, acellular, and elastic (spinnbarkeit) because of the increased concentration of sodium chloride, despite a fernlike pattern when the mucus is allowed to dry on a cover slide under the microscope (see the images below). At this point, the mucus organizes itself, forming multiple microchannels so the spermatozoa can travel through. During this journey, the spermatozoa simultaneously undergo activation and capacitation (6.) In addition, the mucus acts as a filter for abnormal spermatozoa and cellular debris present in the semen.

Mucus secretion may be altered by hormonal changes and medications, especially drugs like clomiphene citrate, which decrease the production. Hypoestrogenism may cause thickened cervical mucus, which impairs the passage of sperm.

Cervical stenosis can cause infertility by blocking the passage of sperm from the cervix to the intrauterine cavity. Cervical stenosis can be congenital or acquired in etiology, resulting from surgical procedures, infections, hypoestrogenism, and radiation therapy.

Uterine factor infertility: The uterus is the final destination for the embryo and the place where the fetus develops until delivery. Therefore, uterine factors may be associated with primary infertility or with pregnancy wastage and premature delivery. Uterine factors can be congenital or acquired. They may affect the endometrium or myometrium and are responsible for 2-5% of infertility cases.

Male Factor Infertility: Male factor infertility can be divided into pretesticular, testicular, and posttesticular etiologies. **Pretesticular factors:** Pretesticular causes of infertility include congenital or acquired diseases of the hypothalamus, pituitary, or peripheral organs that alter the hypothalamic-pituitary axis. Such disorders include idiopathic hypogonadotropic hypogonadism, prolactinomas, gonadotropin deficiencies, and Cushing syndrome. **Testicular factors:** Testicular factors can be genetic or nongenetic in nature. Klinefelter syndrome is the most common chromosomal cause of male infertility and results in primary testicular failure. Nongenetic etiologies include drugs, radiation, infections, trauma, and varicoceles. Aging also affects male fertility. As a man ages, testosterone levels decrease, gonadotropin levels increase, sperm concentration and semen volume change, and libido decreases. In addition, the incidence of birth defects increases. While age affects female fertility dramatically, males are not affected as much; anecdotal reports exist of men fathering children well into their 80s. **Posttesticular factors:** Posttesticular factors are those that do not allow the normal transport of sperm through the ductal system. Such factors can be congenital or acquired. Men who were exposed to DES in utero may have ductal obstruction. Congenital bilateral absence of the vas deferens is seen in men with cystic fibrosis. Additionally, infections, surgical procedures, and trauma may cause ductal blockage.

Factors Affecting Both Sexes: *Environmental and occupational factors:* Concern regarding the impact of environmental factors on fertility is increasing. Published semen analysis reports from 1985 confirm a 20% decrease of sperm concentration compared with reports published in the 1960s. Excessive radiation damages the germinal cells. Exposure to lead, other heavy metals, and pesticides has also been associated with male infertility. Many other factors, such as excessive heat exposure, microwave radiation, ultrasonography, and other health hazards are controversial as infertility-inducing factors (7).

Toxic effects related to tobacco, marijuana, and other drugs: Smoking has been associated with infertility in both males and females (8). In experimental animals, nicotine and polycyclic aromatic hydrocarbons block spermatogenesis and decrease testicular size. In women, tobacco alters the cervical mucus and the ciliary epithelium

and affects gamete transport (9). Marijuana and its metabolite, delta-9-tetrahydrocannabinol, inhibit the secretion of LH and FSH, thus inducing ovulatory disorders and luteal phase dysfunction in women (10). Marijuana use affects males by decreasing the sperm count and the quality of the sperm. Heroin, cocaine, and crack cocaine use induces similar effects but places the user at increased risk for pelvic inflammatory disease and HIV infection. Chronic alcoholism may induce ovulatory dysfunction, therefore impacting fertility. Alcohol use by males interferes with the synthesis of testosterone and has an impact on sperm concentration. Alcoholism may inhibit sexual response and cause impotence. This study was conducted to evaluate the causes of spousal infertility in the municipality of Prishtina.

MATERIAL AND METHODS

A retrospective study was conducted at University Clinical Centre in Prishtina, Kosovo. Consecutive infertile couples referred to the centre during the period of 2000-2010 were investigated thoroughly to identify the different causes of infertility. As mentioned above the WHO definition of infertility “failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse” was followed (10). The medical files of infertile couples were reviewed and the data were retrieved. Information which was extracted from the records included age, type of infertility, duration, factors identified as being responsible for infertility. The couples were assessed with a comprehensive history was taken, clinical examinations, gynecologic examination, trans-vaginal ultrasonography, hematological, hormonal profile (early follicular phase follicle stimulating hormone/luteinizing hormone or antimullerian hormone in those who are not cycling, thyroid stimulating hormone, dihydroepiandrosterone sulphate), hysterosalpingography (laparoscopy was performed if it was indicated). Male factor of infertility was assessed by two semen analyses three months apart. Then the factor(s) responsible for the infertility were identified and recorded. Male factors and sperm parameters were interpreted according the WHO reference values (11). The most common parameters of male infertility were; low sperm concentration (oligospermia), poor sperm motility (asthenospermia), and abnormal sperm morphology (teratospermia) (12). Women were diagnosed as having polycystic ovarian syndrome (PCOS) according to Rotterdam criteria (13), which was based on fulfilling two more of the following criteria: “oligomenorrhoea/anovulation, clinical or biochemical hirsutism and morphology of polycystic ovaries on ultrasonography (≥ 12 follicles in each ovary measuring 2 to 9mm in diameter)” (14). Women were diagnosed as having premature ovarian failure when there was no menarche (primary amenorrhoea) or premature depletion of ovarian follicles/ arrested folliculogenesis (secondary amenorrhoea) before the age of 40 years (15).

RESULTS

Three hundred twenty-two consecutive infertile couples were included in the study. Table 1 shows the type, causes and duration of infertility.

Table 1. Etiology and duration of infertility.

Variables	N	%	95%CI
Type of infertility			
Primary	229	71.1	65.18 – 75.99
Secondary	93	28.9	24.00 – 34.18
Causes of infertility			
Female factor	127	39.4	34.02 – 44.97
Male factor	58	18.0	13.96 – 22.64
Combined factor	99	30.7	25.70 – 36.05
Unknown	38	11.8	8.48 – 15.83

Primary infertility was found in 229 (71.1%) couples and Secondary in 93 (28.9%) ones. Of the causes of infertility were: female factors in 127 (39.4%) of couples, male factors in 58 (18%), combined factors in 99 (30.7%) and unknown infertility in 38 (11.8%) of couples.

Table 2 shows the comparison of infertility factors, age and marriage duration in primary and secondary infertility. There was no significant difference regarding the causes between primary and secondary infertility. Also, there was no significant difference between primary and secondary infertility with regard to duration of infertility ($p=0.4$). The age of females ($p<0.01$), males ($p<0.01$) and marriage duration ($p<0.01$) was significantly higher in secondary fertility.

Table 2. Comparison of infertility factors, age and marriage duration in primary and secondary infertility

Variables	Total	Infertility		P
		Primary (n=229)	Secondary (n=93)	
Causes of infertility				
Female factor	127	88 (38.4)	39 (41.9)	0.5
Male factor	58	42 (18.3)	16 (17.2)	0.8
Combined factor	99	69 (30.1)	30 (32.3)	0.7
Unknown	38	30 (13.1)	8 (8.6)	0.2
Age of females, M (SD)	32 (± 6.9)	30.8 (± 6.7)	33.9 (± 6.8)	<0.01
Age of males, M (SD)	35.9 (± 7.9)	34.2 (± 7.4)	38.7 (± 7.8)	<0.01
Duration of marriage, M (SD)	8.2 (± 5.7)	7.5 (± 5.7)	9.5 (± 5.5)	<0.01
Duration of infertility, n (%)				
1-2 yr	22 (9.7)	13 (8.3)	9 (13.0)	0.4
3-5 yr	91 (40.3)	64 (40.8)	27 (39.1)	
>5 yr	113 (50.0)	80 (51.0)	33 (47.8)	

Infertility is a prevalent disease that results in profound health and socio-economic impacts on both the person and the community (16).

Infertility is divided into primary and secondary categories based on the presence or absence of a previous pregnancy. Both female and male factors can lead to infertility. Menstrual and ovulation disorders and uterine factors are among the most common causes of female infertility. Male infertility factors are known to reduce the production of sperms with normal morphology and progressive motility. Although infertility affects human fertility health status and there is a great deal of concern about identifying its risk factors, there are no comprehensive epidemiological studies about infertility risk factors (17).

The prevalence of infertility has increased significantly in recent years. The global prevalence of infertility is reported to be 10%-15% (19). The rates of male infertility in North America, Australia, and Europe were reported to be 4%-6%, 8%, and 7.5%, respectively. A meta-analysis of the causes of infertility among the patients who referred to several infertility clinics of Iran showed that 78.4% of the couples suffered from primary and 21.6% from secondary fertility problems. Totally, 34% of them had male factor, 43.5% had female factor and 17% had both male and female factors and 8.1% had no specified cause for their infertility (18). Finally, preconception care and counseling are recommended to all those who are planning a pregnancy to avoid failure which can make the couples prefer to remain childless or consider an agreement or non-spousal sperm options (19).

The present study was conducted to investigate the type and etiology associated with infertility in the commune of Prishtina. In the present study, the female factor infertility accounted for the highest rate of infertility (39.4%), which was consistent with the results of other studies conducted in various countries (20).

According to the literature, infertility seems to be a multidimensional health issue which occurs not only due to health problems related to the fallopian tubes, the ovaries, and the endometrium, but it may also be a result of the choices imposed by the modern lifestyle, like the higher average age of people who get married, stress, non-conducive legal framework for assisted reproduction, etc. In our study the secondary infertility rate was 28.9%. It is widely accepted that during the last twenty years, the average age of having children has increased and this is a key factor for infertility. As the age of giving birth is increased, the reproductive capacity is decreased, the ovary

becomes less efficient, the frequency of sexual intercourse is decreased and the possibility of chromosomal abnormalities and miscarriage is increased.

CONCLUSION

Overall, the causes of female infertility in the present study accounted for the highest infertility rate. In reviews of infertile couples, study of male and female factors is important and mostly the couples mentioned more than one reasons as the infertility factor. Finally, preconception care and counseling are recommended to all those who are planning a pregnancy to avoid failure.

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CYP2D6 polymorphisms and the therapeutic outcome with Tamoxifen therapy in breast cancer patients from Kosovo

Selvete Shuleta-Qehaja^{1*}, Aleksandra Kapedanovska Nestorovska²,
Zorica Naumovska², Petar Stefanovski³, Aleksandar J Dimovski², Zoran Sterjev²,
Ljubica Shuturkova²

¹Kosovo Medicines Agency, University Clinical Center n.n., Pristina, Kosovo

²Ss Cyril and Methodius University, Faculty of Pharmacy, Majka Tereza 47, Skopje, Republic of Macedonia

³Clinical Hospital Dr. Trifun Panovski, Department of Oncology, ASNOM n.n., Bitola, Republic of Macedonia

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Abstract

Tamoxifen is a selective estrogen receptor modulator (SERM) used for the prevention of breast cancer and for the treatment of metastatic and early stage receptor positive breast cancer. It has been shown that tamoxifen is metabolized by the cytochrome P450 2D6 (CYP2D6) enzymes, especially with the CYP2D6 isoform. The aim of this study was to examine the prevalence of CYP2D6*4, CYP2D6*9 and CYP2D6*10 variants in patients with breast cancer in Kosovo as well as the association between CYP2D6 polymorphisms and the therapeutic outcome in tamoxifen treated patients. The study included 111 patients who were at the age of 25 to 70 years (45.75 ± 9.50). The overall variant allele frequency of CYP2D6*4 was 0.16. The genotypic frequencies of the CYP2D6*4 polymorphism in all patients were 0.02 for *4/*4, 0.28 for *1/*4 and 0.70 for the *1/*1 genotype. The overall CYP2D6*10 variant allele frequency was 0.30 and the frequency of *10/*10, *1/*10 and *1/*1 genotypes was 0.11, 0.37 and 0.52, respectively. In our study, a population of the CYP2D6*9 variant allele was not detected. In addition, we did not find any correlation between the evaluated genotypes for CYP2D6 polymorphisms and the therapeutic outcome with tamoxifen therapy. Although our study is a rather small-scale compared to large multicentre studies, we believe that it will contribute to determining the impact of CYP2D6 polymorphisms on the success of tamoxifen therapy in patients with a diagnosed breast cancer. Our results are pointing to the direction of the growing number of claims that there is still no strong evidence of any therapeutic connection between the polymorphisms examined and the outcome of the therapy.

Keywords: Tamoxifen, breast cancer, CYP2D6*4, CYP2D6*9, CYP2D6*10

Introduction

In women, breast cancer is the most common cancer diagnosed in both more and less developed regions, with

more cases registered in less developed (883,000 cases) than more developed regions (794,000 cases). Overall, there were nearly 1.7 million new cases diagnosed in 2012 (Ferlay et al., 2014). Despite the fact that there are numerous uncertainties surrounding the etiology of breast

* sshuleta@yahoo.com

cancer, intensive epidemiological, clinical, and genetic studies have identified a number of biological and social traits as risk factors associated with breast cancer. The first and foremost among them is the evidence of BRCA1 and BRCA2 susceptibility genes, familial history of breast cancer, age, higher socioeconomic status, ionizing radiation, life span length, alcohol consumption, and a variety of hormone and metabolic factors. Among the hormonal influences, a relevant etiological function has been ascribed to elevated levels of estrogens, their active metabolites, and androgens (Muti et al, 2006).

The diagnosed breast cancer cases can be classified as follows: hereditary (5–10%), i.e. correlated with a mutated phenotype of a specific gene segregation with a Mendelian inheritance pattern in families; familial (15–20%), i.e. one or more first- and/or second-degree relatives of the proband affected by breast cancer, in the absence of Mendelian inheritance and/or of an identified cancer-causing germline mutation of a specific gene; or sporadic (70–80%), namely the lack of breast cancer among a patient's first- and/or second-degree relatives (Marchetti et al., 2004).

The pharmacogenetics of cancer therapy has been of great importance. Many of the anticancer drugs are pro-drugs and are metabolized to an active form mediated by enzymes. The goal of personalized medicine for breast cancer will require the incorporation of the genetic background of an individual as well as their tumor characteristics into management decision algorithms (Trainer et al., 2004).

Tamoxifen, a selective ER modulator, is used as a first-line endocrine agent for the treatment of breast cancer. Tamoxifen is beneficial in pre- and post- menopausal women whose tumors are ER positive. The pharmacological activity of tamoxifen is dependent upon its conversion by the hepatic drug-metabolizing enzyme cytochrome P450 2D6 (CYP2D6) to its abundant metabolite, endoxifen. The CYP2D6 enzyme, coded by the corresponding gene, plays a major role in the biotransformation of tamoxifen to endoxifen. Therefore, the use of CYP2D6 genotype information to guide tamoxifen therapy represents an early example of a pharmacogenetic tool for optimizing the anticancer efficacy (Hoskins et al., 2009). CYP2D6 has a highly polymorphic expression, and more than 100 variant alleles have been described so far. This enzyme is involved in the metabolism of 20–25% of clinically used drugs and exhibits a clinically relevant gene polymorphism that modifies the pharmacokinetics of nearly 10% of drugs. Among major variant alleles in Caucasians, those caused by single-nucleotide polymorphisms (SNPs), namely CYP2D6*4 and CYP2D6*10, account for 15–20% of the alleles. In addition, variant alleles resulting from CYP2D6 rearrangements, characterized by CYP2D6 gene deletion (CYP2D6*5) or duplication/multiplication (CYP2D6*xn), are present with allele frequencies of 2–7% and 1–10%, respectively in Caucasian individuals (Ledema &

Agundez., 2005; Pinto & Dolan., 2011). There have been numerous studies and the effect of CYP2D6 polymorphism on tamoxifen clinical outcome is still under on-going analyses since there are contradictory results among them. The aim of our study was to determine whether or not testing for cytochrome P4502D6 (CYP2D6) polymorphisms in women with early hormone receptor positive breast cancer leads to the improvement of outcomes and is useful for health decision-making.

Material and methods

The total of 111 patients with breast cancer from Kosovo were subject to our study analyses. All patients had been on tamoxifen treatment (20 mg/day) (TAM patients) for at least 4 weeks as adjuvant therapy. Patients on co-prescribed CYP inhibitors were excluded from this study. The stage of disease, hormonal and HER-2/neu receptor status of the patients, menopausal status and the type of therapy are displayed in Table 1.

In order to evaluate the association between CYP2D6 polymorphisms and therapeutic outcome we divided the patients into two groups: patients with the disease and the second group consisted of patients with a stable condition of the illness. Genomic DNA was extracted from the whole blood, using a QIAGEN DNA extraction kit and the procedure recommended by the manufacturer (QIAGEN AS, Oslo, Norway). The presence of the CYP2D6 polymorphisms - CYP2D6*4 (*rs3892097*), CYP2D6*9 (*rs5030656*) and CYP2D6*10 (*rs1065852*) were analyzed with the allelic discrimination TaqMan assay (MxPro 3005P, Strategene, La Jolla, CA) according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). The study was approved by the Ethics Committee of the University Clinical Center of Pristina.

Observed and expected allele and genotype frequencies were evaluated for Hardy-Weinberg equilibrium using the Chi-squared test. The Fisher exact test was used to evaluate the association between CYP2D6 variant alleles and treatment outcome. A P-value < 0.05 was considered statistically significant.

Results

The study, as stated above, included 111 patients who were at the age of 25 to 70 years (45.75 ± 9.50). Regarding the results of our study, we did not find any connection between the patients' age and their different disease stages. The mean age of patients with a diagnosis stage IA+IB was 44.75 ± 7.97 (CL (95%) = 6.67), with stage II of the disease was 46.36 ± 10.10 (CL (95%) = 2.59) and with stage III was 44.36 ± 8.56 (CL (95%) = 3.03). Most of the patients, 73.87%, were on the therapeutic protocol Cyclophosphamide, doxorubicin, 5-

Table 1. Characteristics of the patients

Characteristics	TOTAL N=111	
Age		
Age at diagnosis ALL (mean)	45.75 [SD 9.50804; CL 95% 1.84908]	
Age at diagnosis-IA+IB (mean)	44.75 [SD 7.97765; CL 95% 6.66948]	
Age at diagnosis-II stage (mean)	46.36 SD 10.10616; CL 95% 2.58831]	
Age at diagnosis- III stage (mean)	44.36; [SD 8.56161; CL 95% 3.03581]	
Stage of disease	N	%
IA	5	4.50
IB	3	2.70
IIA	31	27.93
IIB	30	27.03
IIIA	11	9.91
IIIB	6	5.41
IIIC	16	14.41
Unknown	9	8.11
Receptor status		
ER		
neg	1	0.90
pos	109	98.20
unknown	1	0.90
PgR		
neg	4	3.60
pos (+low)	106	95.50
unknown	1	0.90
HER-2/neu		
neg	98	88.29
pos	10	9.01
unknown	3	2.70
Menopausal		
yes	62	55.86
no	42	37.84
unknown	7	6.31
Type of adjuvant chemotherapy		
cyclophosphamide, doxorubicin, docetaxel	12	10.81
cyclophosphamide, doxorubicin, 5-FU	82	73.87
cyclophosphamide, doxorubicin	1	0.90
cyclophosphamide, docetaxel	1	0.90
doxorubicin, 5-FU	8	7.21
unknown	7	6.31

Adjuvant radiation therapy			
	yes	42	37.84
	no	62	55.86
	unknown	7	6.31
Type of hormonal therapy			
	Tamoxifen	111	100.00
Disease progression			
	yes	18	16.22
	no	85	76.58
	unknown	8	7.21

Abbreviations: N-number; SD-standard deviation; CL-Confidence Level; ER -estrogen receptor, PgR-progesterone receptor, HER-2/neu -Human epidermal growth factor 2, 5-FU- Fluorouracil

FU. The mean age in this group of patients was 45.83 ± 9.67 (CL (95%) = 2.12). From the other patients, 10.81% were included on the therapeutic protocol with cyclophosphamide, doxorubicin, docetaxel, and 7.21% on the therapeutic protocol with Doxorubicin and 5-FU.

All 111 patients were genotyped for CYP2D6 polymorphisms. The distribution of *CYP2D6*4* and *CYP2D6*10* variant was in accordance with the Hardy-Weinberg expectations. The overall variant allele frequency of *CYP2D6*4* was 0.16. The genotypic frequencies of the *CYP2D6*4* polymorphism in all patients were 0.02 for *4/*4, 0.28 for *1/*4 and 0.70 for the *1/*1 genotype. The overall *CYP2D6*10* variant allele frequency was 0.30 and the frequency of *10/*10, *1/*10 and *1/*1 genotypes were 0.11, 0.37 and 0.52, respectively. In our study population, the *CYP2D6*9* variant allele was not detected.

Twenty-eight of all patients (25%) were homozygotes for the normal alleles for both polymorphisms (CC for *CYP2D6*4* and CC for *CYP2D6*10*). We did not identified patient who were homozygotes for variant alleles for both polymorphisms (CC for *CYP2D6*4* and CC for *CYP2D6*10*). The correlation analysis between CYP2D6 status and the therapeutic outcome was performed only in subgroup of patients with positive genotyping reaction and known disease status (Disease progression or Survived). The allele and genotype frequencies in the patient population stratified according to the treatment outcome (Disease progression vs. Stable) for *CYP2D6*4*, *CYP2D6*9* and *CYP2D6*10* polymorphisms are presented in Table 2. No significant differences in the allelic frequencies and genotype distribution between the two patient groups were observed (p Fisher Exact test > 0.05).

Discussion

The pharmacogenetics of cancer therapy is of significant priority. Many of the anticancer drugs are pro-

drugs and are metabolized to an active form mediated by enzymes. The catalytic efficiency of enzymes involved in the catalysis of pro-drugs is variable in individuals. Cytochrome P450 2D6 (CYP2D6) has a high relevance in drug metabolism. CYP2D6 is involved in the metabolism of 20–25% of clinically used drugs and exhibits a clinically relevant gene polymorphism that modifies the pharmacokinetics of nearly 10% of the drugs used (Pinto & Dolan, 2011).

The aim of this population-based case-control study was to define the prevalence of the *CYP2D6*4*, *CYP2D6*9* and *CYP2D6*10* variants in patients with breast cancer in the Republic of Kosovo and determine their influence on the therapeutic outcome in tamoxifen treated patients. The frequency of *CYP2D6*4* allelic variant in our study population (16%) was comparable to that found in other Caucasians in the European population (20%). For the *CYP2D6*10*, we estimate allelic frequency of 29.8% which is significantly different from its frequency in other Caucasians 1-2% (Table 3) (Ingelman-Sundberg, 2005).

The frequency of *CYP2D6*9* variant allele is <2% in the European population. In our study group, we did not find any patients with this allele. For that reason, *CYP2D6*9* polymorphisms were excluded from further analysis and not evaluated in relation to the tamoxifen treatment outcome.

Regarding the effect of CYP2D6 polymorphism on the tamoxifen clinical outcome, there have been numerous studies and they are still on going. The results among them are contradictory. The heterogeneity of results in the trials has led to confusion and controversy. The conclusions of these studies range from a possible longer disease-free survival interval to a substantially shorter recurrence-free survival time for patients carrying CYP2D6 genotypes conferring diminished tamoxifen metabolism. Tamoxifen-treated patients carrying the CYP2D6 alleles *4, *5, *10, *41 - are associated with an impaired formation of antiestrogenic metabolites and have significantly more recurrences of breast cancer, shorter

Table 2. Allele and genotype distribution in breast cancer patients

CYP2D6 variant	Genotype Allele	Disease progression			Stable			P (Fisher)
		n	frequencies	expected	n	frequencies	expected	
	*1/*1	8	0.66	0.63	51	0.71	0.72	0.455
	*1/*4	3	0.25	0.33	20	0.28	0.26	
	*4/*4	1	0.08	0.04	1	0.01	0.02	
	total	12	1.00	1.00	72	1.00	1.00	
CYP2D6*4	*1	19	0.79		122	0.85		0.267
	*4	5	0.21		22	0.15		
	total	24	1.00		144	1.00		
CYP2D6*9	*1/*1	18	1.00	1.00	72	1.00	1.00	1
	*1/*9	0	0.00	0.00	0	0.00	0.00	
	*9/*9	0	0.00	0.00	0	0.00	0.00	
	total	18	1.00	1.00	72	1.00	1.00	
	*1	36	1.00		144	1.00		1
	*9	0	0.00		0	0.00		
	total	36	1.00		144	1.00		
CYP2D6*10	*1/*1	10	0.63	0.56	40	0.49	0.48	0.270
	*1/*10	4	0.25	0.38	32	0.40	0.43	
	*10/*10	2	0.13	0.06	9	0.11	0.10	
	total	16	1.00	1.00	81	1.00	1.00	
	*1	24	0.74		112	0.69		0.507
	*10	8	0.26		50	0.31		
	total	32	1.00		162	1.00		

relapse-free periods and worse event-free survival rates compared with carriers of CYP2D6 functional alleles (Schroth et al., 2009).

In multicentre studies, including retrospectively and prospectively collected patient data, authors also confirmed the link between a CYP2D6 variation and the clinical outcome in women receiving adjuvant tamoxifen. They found that poor metabolizers (PM) for CYP2D6 enzyme had an almost 2-fold increased risk of developing breast cancer recurrence compared with patients with 2 functional CYP2D6 alleles (extensive metabolizers - EM) (Schroth et al., 2009).

Investigators from the Mayo Clinic and COBRA reported that carriers of a CYP2D6*4 variant allele had a significantly shorter time-to-recurrence and relapse-free survival compared with EM (Higgins & Stearns, 2010).

In the same year of the initial Mayo/COBRA report,

two large retrospective studies reported an inverse association between CYP2D6 genotype and breast cancer (Fox et al., 2016).

An international group of clinicians and scientists representing the Clinical Pharmacogenetics Implementation Consortium (CPIC) have reached a consensus that there is sufficient evidence to use the CYP2D6 genotype to assist with clinical recommendations for women who are being considered for tamoxifen for early stage estrogen receptor positive breast cancer (Goetz et al., 2018). The Dutch Pharmacogenetics Working Group has also made recommendations for a tamoxifen therapy based on the CYP2D6 genotypes. Their recommendation for both poor and intermediate metabolizers, is to consider using aromatase inhibitors for postmenopausal women due to an increased risk of breast cancer relapse with tamoxifen.

Table 3. Major human polymorphic variant CYP2D6 alleles and their global distribution (Source: Ingelman-Sundberg, 2005)

Major variant alleles	Mutation	Consequence	Allele frequencies (%)			
			Caucasians	Asians	Black Africans	Ethiopians and Saudi Arabians
CYP2D6*2xn	Gene duplication/multiduplication	Increased enzyme activity	1–5	0–2	2	10–16
CYP2D6*4	Defective splicing	Inactive enzyme	12–21	1	2	1–4
CYP2D6*5	Gene deletion	No enzyme	2–7	6	4	1–3
CYP2D6*10	P34S, S486T	Unstable enzyme	1–2	51	6	3–9
CYP2D6*17	T107I, R296C, S486T	Altered affinity for substrates	0	0	20–35	3–9

They also recommend that intermediate metabolizers should avoid the concomitant use of CYP2D6 inhibitors. Conversely, The National Comprehensive Cancer Network (NCCN) does not recommend CYP2D6 testing as a tool to determine the optimal adjuvant endocrine strategy (www.ncbi.nlm.nih.gov/books/NBK247013, 2018).

In our study, we did not find any correlation between the evaluated genotypes for CYP2D6 polymorphisms and the therapeutic outcome with tamoxifen therapy. At the beginning, we divided patients into two groups (Disease progression vs. Stable) and each polymorphism was evaluated in relation to the current disease status for the whole group of patients. Afterwards, the patients were divided into the following groups: patients who were homozygous for both normal alleles of the examined polymorphism, patients who were homozygous for both variant alleles and patients who were heterozygotes for both examined polymorphisms. Although our study is rather a small-case study compared to large multicentre studies, we believe that it will contribute to determining the impact of CYP2D6 polymorphisms on the success of a tamoxifen therapy in patients with diagnosed breast cancer. Our results are pointing to the direction of the growing number of claims that there is still no strong evidence of any therapeutic connection between the polymorphisms examined and the outcome of the therapy.

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Резиме

СYP2D6 полиморфизам и исход од терапија со Тамоксифен кај пациентки со канцер на дојка од Косово

Селвете Шулета Кехаја^{1*}, Александра Капедановска Несторовска², Зорица Наумовска², Петар Стефановски³, Александар Ј Димовски², Зоран Стерјев², Љубица Шутуркова²

¹Агенција за лекови Косово, Универзитетски Клинички Центар Косово б.б, Приштина, Косово

²Универзитет „Св Кирил и Методиј“, Фармацевтски факултет, Мајка Тереза 47, Скопје, Р. Македонија

³Клиничка болница Др. Трифун Пановски, Оддел за онкологија, ул. АСНОМ б.б, Битола, Р. Македонија

Клучни зборови: тамоксифен, карцином на дојка, CYP2D6*4, CYP2D6*9, CYP2D6*10

Тамоксифенот е селективен естроген рецепторен модулатор (SERM) кој се применува за превенција на рак на дојка и за третман на метастатски рак на дојка. Метаболизмот на тамоксифен се одвива преку цитохром P450 (CYP) ензимите (CYP2D6). Целта на оваа студија беше да се испита преваленцата на CYP2D6*4, CYP2D6*9 и CYP2D6*10 полиморфизмите во CYP2D6 генот кој ја кодира синтезата на истоимениот ензим кај пациенти со карцином на дојка во Косово и да се утврди можната поврзаност помеѓу овие генетски варијанти и терапевтскиот исход кај пациенти третирани со тамоксифен. Во студијата беа вклучени 111 пациенти на возраст од 25 до 70 години (45,75 ± 9,50). Утврдената алелна фреквенција за CYP2D6*4 полиморфизмот изнесуваше 0,16. Дистрибуцијата на *4/*4, *1/*4 и *1/*1 генотповите изнесуваше 0,02; 0,28 и 0,70, соодветно.

Вкупната алелна фреквенција на CYP2D6 * 10 беше 0,30, а фреквенцијата за *10/*10, *1/*10 и *1/*1 генотипови беше 0,11; 0,37 и 0,52, соодветно. Во испитуваната популација CYP2D6 * 9 алелна варијанта не беше идентификувана. Не е идентификувана корелација помеѓу евалуираните генотипови за полиморфизмите на CYP2D6 и терапевтскиот исход со терапија со тамоксифен. Иако нашата студија е помала во обем споредбено со големите мултицентристички студии, веруваме дека ќе придонесе за одредување на влијанието на CYP2D6 полиморфизмите врз успехот на терапијата со тамоксифен кај пациенти со дијагностициран рак на дојка. Нашите резултати се во насока на сè поголемиот број на тврдења дека сеуште не постојат силни докази за каква било терапевтска врска помеѓу CYP2D6 полиморфизмите и исходот од терапијата со тамоксифен.

ORIGINAL PAPER

Effects of Low Level Laser Therapy (LLLT) on Serum Values of Interleukin 6 (IL-6) in Patients with Periodontitis and Type 2 Diabetes Mellitus (T2DM)

Shefqet Mrasori¹, Mirjana Popovska², Biljana Rusevska³, Mirsad Shkreta⁴, Ardian Selani¹, Visar Bunjaku⁵

¹ALMA MATER EUROPEA Campus College "Rezonanca" Faculty of Dentistry, Pristina, Republic Kosovo

²Department of Oral Disease and Periodontology, Faculty of Dentistry, University "St. Cyril and Method", Skopje, Republic of North Macedonia

³Periodontology and Oral Disease Clinic, University Dentistry Clinical Center, Skopje, Skopje, Republic of North Macedonia

⁴UBT - Higher Education Institution - Faculty of Dentistry, Pristina, Republic Kosovo

⁵Department of Oral Disease and Periodontology, Faculty of Dentistry, UBT - Higher Education Institution, Pristina, Republic Kosovo

Corresponding author: Visar Bunjaku, MDD. UBT - Higher Education Institution, Faculty of Dentistry, Department of Oral Disease and Periodontology, Pristina, Republic Kosovo. E-mail: visar.bunjaku@ubt-uni.net. ORCID ID: <http://www.orcid.org/0000-0000-0000-0000>.

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ABSTRACT

Background: In patients with T2DM, the therapeutic effects of conservative treatment are quite limited, and there is a need for additional therapeutic procedures to achieve the desired satisfactory and solid effect. Low-level laser therapy (LLLT) has an anti-inflammatory effect, and is used to heal lesions. This mechanism is realized through inhibition of lipopolysaccharides (LPS), so it can be used in the treatment of periodontal disease in patients with diabetes. **Objective:** The aim of this study is to assess the effect of level laser therapy (LLLT) on serum IL-6 values in patients with periodontitis and T2DM. **Methods:** Patients at age between 35-60 years old, with chronic periodontitis (CH) where the clinical loss of attachment (CAL) was ≥ 4 mm therefore covering at least 50 % of affected teeth. In this study we included 80 patients, divided into two groups: 40 patients with type 2 diabetes mellitus (2TDM) treated with conservative periodontal treatment supplemented with laser therapy (LLLT), group A, and 40 patients with 2TDM, conservatively treated without LLLT. therapy i.e. group B. The laser light was applied to the gingiva in separate quadrants in 5 sessions for the next five days in a row. Blood samples were taken from all subjects at the first treatment, then in 6 weeks and 3 months after treatment, and interleukin 6 (IL-6) levels were measured. The blood samples in the test tubes remained for about 30 minutes and were then distributed in a biochemical laboratory, where they were centrifuged at 6,000 rpm for 10 minutes. The serum was separated from the test tube and transferred to the eppendorph. All serum samples were stored at -80°C until complete analysis and determination of IL-6, according to the standardized methodology. **Results:** In group A, on the first examination serum IL-6 levels varies in the interval 11.54 ± 1.11 pg / mL, after 6 weeks of therapy the values range between 11.26 ± 0.77 pg / mL, and after 3 months of therapy levels oscillate at intervals of 11.02 ± 0.67 pg / mL. In group B the findings are similar. At the first examination, the serum IL-6 values were 11.56 ± 0.81 pg / mL, after 6 weeks of therapy ranged from 11.59 ± 0.71 pg / mL, and after 3 months of therapy levels were recorded at intervals. 11.41 ± 0.78 pg /mL. The serum IL-6 value after 6 weeks of therapy in patients in group B for $Z = -2.04$ and $p < 0.05$ ($p = 0.04$) was significantly higher than in patients in group A, while after 3 months of therapy in patients in group B for $Z = -2.42$ and $p < 0.05$ ($p = 0.02$) is significantly higher than the value in patients in group A. **Conclusion:** LLLT resulted in significantly reduced serum IL-6 values in patients with periodontitis and T2DM after 6 weeks and 3 months of therapy in which conservative treatment was supplemented with LLLT.

Keywords: low power laser radiation (LLLT), interleukin 6 (IL-6), serum, periodontitis, type 2 diabetes mellitus (T2DM).

1. BACKGROUND

According to the data from the Center for control and prevention of diabetes, which treats people with diabetes in the United States, diabetes affects about 25.6 million people, or 11.3% of the country's population (1). The disease gradually

and quietly but systematically attacks the organs and tissues, causing micro and macro circulatory disorders, which affect the health status of the individuals. These changes also occur locally, soft and hard structures in the oral cavity (2-3). It has been clinically proven that type

2 diabetes mellitus (T2DM) is a risk factor for periodontitis, sometimes defined as a moderate association (4), sometimes as an increased risk of initiation and progression of periodontal disease (5) or very often the case is presence of both diseases in the same patient (6-7). In conditions of chronic disease and systemic disorder, the periodontium becomes a barrier which is easy to overcome, which allows the penetration of certain harmful pathogens first into the gingival tissue and then into the remaining periodontal structures. This condition results in a host response, activation of enzymes, and release of pro-inflammatory cytokines, including IL-6. IL-6 is responsible for regulating the immune and inflammatory tissue response and participates in the acute phase of the inflammatory response where it acts together with TNF- α (8). Through regulatory mechanisms, the production of TNF- α (9) is impaired, hence its effect on diabetes is indisputable. It has been shown to have a potential synergistic effect on fibroblasts, and is a stimulator of alveolar bone destruction by stimulating osteoclasts (10). In patients with T2DM, the therapeutic effects of conservative treatment are quite limited, and there is a need for additional therapeutic procedures to achieve the desired satisfactory and solid effect. Low-level laser therapy (LLLT) has very wide use in medicine, and is used to heal lesions. This mechanism is realized through inhibition of lipopolysaccharides (LPS), so it can be used in the treatment of periodontal disease in patients with diabetes (11).

The role and effect of lasers on fibroblasts and osteoblasts have been investigated and proven in hyperglycemic conditions (12-13). At the systemic level, the impact of LLLT on the secretion of pro-inflammatory mediators TNF- α and IL-6 from endothelial cell cultures has been investigated. According to some research, the link between these components is due to the structural placement of endothelial cells on the walls of blood vessels and the initial contact with blood rich in glucose (14).

LLLT as an addition to conservative treatment minimized the effects of 5-FU on the periodontium (15-16), the diode laser provided significant improvements in clinical parameters, confirming that lasers have a positive effect along with non-surgical periodontal therapy (17). The researchers suggest that LLLT reduces gingivitis and contributes to better therapeutic results when LLLT is used in conjunction with basic periodontal therapy, opposing to the classical conservative treatment only (18).

2. OBJECTIVE

The aim of this study was to evaluate the effect of LLLT on serum IL-6 values in patients with periodontitis and T2DM.

3. MATERIAL AND METHODS

Study design

Patients in this study were selected by the Department of Periodontology and Oral Diseases at the University of Kosovo, University Dental Clinical Center in Pristina, aged 35-60, with chronic periodontitis (CH) where the clinical attachment loss (CAL) was ≥ 4 mm covering at least 50% of affected teeth. The research was approved

by the Ethics Commission of the Faculty of Dentistry in Skopje(01/434/17).

The selected patients in this study were informed about the motive and course of the study. Only volunteers who agreed to be part of this research took part in the study, and written consent was submitted, signed by hand. The study included survey of 80 patients, who were divided into two groups:

- patients with type 2 Diabetes mellitus where conservative periodontal treatment was supplemented with laser therapy in 40 patients (group A);
- patients with type 2 Diabetes mellitus where conservative periodontal treatment has been performed without applied laser therapy, which also counted 40 patients (group B).

All patients in both groups regulated hyper-glycaemia with oral antidiabetic drugs (Glucophage XR tablets of 750 mgr. 2x daily, manufacturer Merck Sante, France).

Certain criteria were used in selection of the patients, respectively proposed by the World Health Organization as criteria for inclusion and exclusion in the study.

Criteria for exclusion from the study are: a) use of antibiotics in the previous 4 months; b) pregnancy; c) patients - smokers; d) malignant diseases; e) use of immunosuppressive drugs; f) medications that may affect periodontal status; and g) Fentoin, cyclosporine, calcium channel blockers, etc.

Criteria for inclusion in the study are: a) diagnosed with diabetes mellitus type 2; b) regulation of diabetes with oral antidiabetics; and c) diagnosed periodontitis with depth of periodontal pockets ≥ 4 mm in at least 50% of affected teeth.

After determined diagnosis in all patients who were part of the study, conservative treatment (removal of hard and soft deposits) of periodontal pockets was performed. After the initial measurements and after the determining of the clinical parameters, non-surgical treatment of the periodontal pockets was performed in all participants in the study. Periodontal pockets were irrigated with 1% chlorhexidine solution (three times for 5 minutes). Scaling and root planning was carried out in 5 sessions, in separate quadrants each session. The supra-gingival tartar was removed by ultrasound, and the treatment was performed with Grace's curette, model Hu-Friedy, Chicago, IL, USA by the same therapist.

All patients were instructed to maintain daily oral hygiene: tooth brushing, use of dental floss, Listerine solution use. In the first group of respondents, conservative treatment was supplemented with laser therapy. For this purpose, low level laser therapy (LLLT), laser light (660 nm, 10 mW, 8 min/day, in contact with the gingiva) was applied; model: (Hager & Werken LASER HF " confort " Vo23-17, Duisburg, Germany) for the next five days in a row. Blood was taken from all patients during the first treatment, and after in the 6th week an 3rd month of treatment.

Collecting serum

Samples of venous blood from the cubital vein were taken from each patient. Collected blood samples were transferred into a test tube with an anticoagulant

(pre-fabricated). The blood samples remained in the test tubes for about 30 minutes and were then distributed in a biochemical laboratory, where they were centrifuged at 6,000 rpm for 10 minutes. The serum was separated from the test tube and transferred to the eppendorph tube. All serum samples were stored at -80 °C until complete analysis and measurement of IL-6 levels.

Determining IL-6 levels in serum

In the test procedure, the reagents are prepared firstly. Namely, first, before use, all reagents should be brought to room temperature (10-25 oC). The standard reagent is prepared 15 minutes before starting with work. The concentration of the solution is 1000 pg/mL. Then 7 tubes are prepared containing 1.0 µL dilution for standard and are used to make a double dilution series according to the picture shown below. Each tube is vigorously mixed before the next transfer. 7 tubes with dissolved standard are obtained with the following concentrations: 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62,5 pg/mL, 31,25 pg/mL, 250 pg/mL, 15,625 pg/mL and 0 pg/mL.

Rinsing buffer - diluted with 30 mL of concentrated rinse buffer in 750 mL rinse buffer with deionized or distilled water.

Biotinylated Detection Ab - The exact amount needed (100µL / well) is calculated before the experiment begins. Before use, centrifuge the tube with the solution, and dilute with the concentrated Biotinylated Detection Ab to the working concentration using the Biotinylated Detection Ab Diluent (1: 100).

Concentrated HRP Conjugate - Before the start of the experiment, the exact amount needed (100µL / well) is calculated. Before use, the tube is centrifuged with the solution, and diluted with the concentrated HRP Conjugate to working concentration needed using the Concentrated HRP Conjugate Diluent (1: 100).

Test protocol

We added 100µL standard or samples to the appropriate well. Reference Standard and Sample diluent is added to standard wells. The solutions are added at the bottom of the microplate. Gently mix and cover with protective foil. Then incubate for 90 minutes at 37°. The liquid is then removed from each well. Immediately afterwards, 100 µL of the Biotinylated Detection Ab working solution is added to each well. Cover the microplate with protective foil, gently touch the tile to ensure thorough mixing. Incubate for 1 hour at 37° C.

Each well is aspirated and rinsed, repeating the process three times. Each well is rinsed with a rinsing buffer (approximately 350µL). After the last rinse, remove the remaining rinse buffer by aspiration or decantation. Then 100 ml of HRP Conjugate working solution is added to each well. Cover with foil and incubate for 30 minutes at 37 ° C. Each well is aspirated and rinsed, repeating the process five times. Each well is flushed with a wash buffer (approximately 350µL).

After the last rinse, remove the remaining rinsing buffer by aspiration or decantation. Add 90 µL of Substrate Solution to each well, cover the plate with foil and incubate in the dark for about 15-25 minutes at 37 ° C. Add 50 µL of Stop Solution to each well. The color immediately

turns yellow. The optical density (OD value) of each well is determined simultaneously, using a 450 nm microplate tile reader, and at the end we calculate the results.

Statistical processing and data analysis

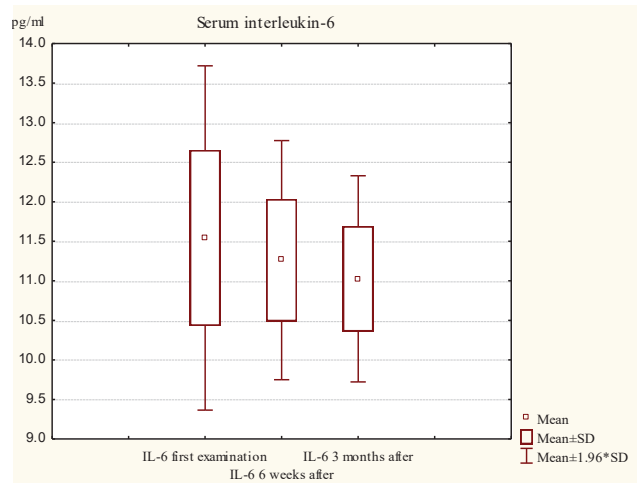
The statistical processing is performed in the statistical program Statistica 7.1 for Windows. The data is displayed as a table and graphically.

In the analysis of the data we used: a) The differences in the analyzed parameters in the first examination, after 6 weeks of therapy and after 3 months of therapy were tested using Friedman ANOVA Chi Sqr. / p; b) Differences in relations: first examination and after 6 weeks of therapy; first examination, after 3 months of therapy; after 6 weeks of therapy and after 3 months of therapy, they were tested using T-test for Dependent Samples (t / p), Wilcoxon Matched Pairs Test (Z / p) depending on the data distribution; c) The differences in the analyzed parameters between group A and group B were tested with T-test, independent, by groups (t / p) and Mann-Whitney U Test (Z / p), depending on the data distribution.

4. RESULTS

Figure 1 shows the descriptive statistics of serum IL-6 in group A. At the first examination IL-6 in serum varies in the interval 11.54 ± 1.11 pg / ml, after 6 weeks of therapy the values range between 11.26 ± 0.77 pg / mL and after 3 months of therapy they oscillate in the interval 11, 02 ± 0.67 pg / mL.

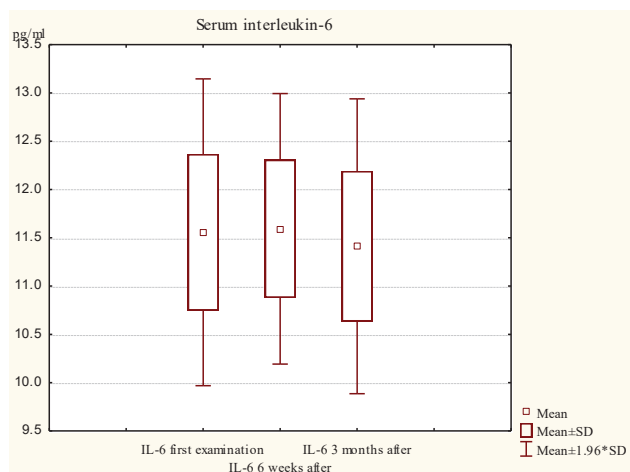
Among the IL-6 serum values in group A (first examination, six weeks, and three months) for Friedman ANOVA



Graph 1. View the values of IL-6 in serum at different time intervals in group A

IL-6 in serum	Average Rank	Sum of Ranks	Mean	Std.Dv.
Group A				
First examination	2.33	93.00	11.54	1.11
After 6 weeks	2.13	85.00	11.26	0.77
After 3 months	1.55	62.00	11.02	0.67
Group B				
First examination	1.96	78.50	11.56	0.81
After 6 weeks	2.36	94.50	11.59	0.71
After 3 months	1.68	67.00	11.41	0.78

Table 1. Differences between serum IL-6 values in groups A and B at different time intervals after therapy



Graph 2. View the values of IL-6 in serum at different time intervals in group B

IL-6 in serum	N	T	Z/ t	p-level
group A				
First examination and after 6 weeks	40	/	t=1.80	0.08
After 6 weeks and 3 months	40	141.50	3.61	0.0003
First examination and after 3 months	40	151.00	3.18	0.001
group B				
First examination and after 6 weeks	40	316.50	0.53	0.60
After 6 weeks and 3 months	40	90.00	4.30	0.000
First examination and after 3 months	40	239.50	0.99	0.32

Table 3. Differences in serum IL-6 values after 6 weeks and 3 months of treatment between groups A and B

Chi Sqr. (N = 40, df = 2) = 13.11 and p <0.01 (p = 0.001) there is a significant difference. In group B, IL-6 in serum (first examination and six weeks and three months after therapy) for Friedman ANOVA Chi Sqr. (N = 40, df = 2) = 10.11 and p <0.01 (p = 0.006) there is a significant difference (Table 1).

Figure 2 shows the descriptive statistics of serum IL-6 in group B. At the first examination, IL-6 serum values ranged from 11.56 ± 0.81 pg / mL, after 6 weeks of therapy ranged from 11.59 ± 0.71 pg / mL, and after 3 months of therapy were recorded at intervals. 11.41 ± 0.78 pg/mL.

Table 2. Intergroup differences between serum IL-6 values in groups A and B at different time intervals after therapy

In group A, the serum IL-6 value after 6 weeks of therapy for t = 1.80 and p > 0.05 (p = 0.08) was significantly lower than the value at first examination, after 3 months for Z = 3,61 and p <0.001 (p = 0.000) is significantly lower than the value after 6 weeks of therapy, and after 3 months of therapy is significantly lower than the value at the first examination, ie. Z = 3.18 and p <0.01 (p = 0.001).

In group B, after 6 weeks of therapy for Z = 0.53 and p > 0.05 (p = 0.60) the values for IL-6 were insignificantly higher than the values at first examination. Comparison made between 6 weeks and 3 months of IL-6, after 3 months of therapy for Z = 4.30 and p <0.001 (p = 0.000) is significantly lower than the value after 6 weeks, while

after 3 months of therapy for Z = 0.99 and p > 0.05 (p = 0.32) values of IL-6 are slightly lower than those at first examination (Table 2).

The serum IL-6 value after 6 weeks of therapy in patients in group B for Z = -2.04 and p <0.05 (p = 0.04) was significantly higher than in patients in group A, while again after 3 months of therapy in patients in group B for Z = -2.42 and p <0.05 (p = 0.02) is significantly higher than in patients with group A (Table 3).

5. DISCUSSION

Between chronic periodontitis and diabetes, a two-way relationship has been proven. On the one hand, destruction of the supporting apparatus of the teeth is more advanced in patients with T2DM (19, 20), while on the other hand CH may worsen glycemic control in patients with T2DM (21). This two way street is thought to be due to the presence of pro-inflammatory mediators, such as TNF-α and IL-6. It is believed that their presence is a consequence of constant microbiological stimulation or as a response of the host. In circulation, pro-inflammatory mediators come in contact with the insulin receptors, disrupting insulin function and signalization (20).

In addition to IL-10, the study of the effect of IL-6 is quite complicated and completely unclear (22, 23). The role of IL-6 is crucial because it is involved in osteoclastic activity and has a strong effect on Th-17 cells (24). In addition to this exceptional activity, it simultaneously stimulates the production of IL-1 α, which contributes to the stimulation of the anti-inflammatory process (25). There is varying information about the association between these diseases. Khosravi (26) says there is insufficient evidence to support a link between elevated IL-6 levels and alveolar destruction in periodontal disease in individuals with hyperglycaemia. While Javed et al. (27) reported that cytokines in GCF in patients with and without T2D are regulated by the intensity of periodontal infection, while the role of T2DM is quite secondary.

In group A, ie. in patients with T2DM where conservative periodontal treatment with LLLT application has been performed, the value of IL-6, TNF-α in serum after 6 weeks (11.26 ± 0.77 pg / mL, and after 3 months of therapy is significantly lower than the values of the first examination. At the first examination IL-6 in serum varies in the interval 11.54 ± 1.11 pg / mL, after 6 weeks of therapy the values range between and after 3 months of therapy they oscillate in the interval 11.02 ± 0.67 pg / mL.

Quantitative analyzes have shown that serum IL-6 values after 3 months of treatment are significantly lower than the value after 6 weeks of therapy in patients with 2TDM whose conservative therapy was supplemented with LLLT.

In group B, ie. in patients with T2DM where conservative periodontal treatment was performed without LLLT application, IL-6 values after 6 weeks (11.59 ± 0.71 pg / mL) were insignificantly higher, while after 3 months (11.41 ± 0.78 pg / mL.), they are insignificantly lower than the value at first examination (11.56 ± 0.81 pg / mL). Serum IL-6 values are significantly lower after 3 months of therapy than 6 weeks after therapy.

The results indicate that serum values of IL-6 were corrected 6 and 3 months after treatment in both groups, with a significant difference in group A $p < 0.01$ ($p = 0.001$) and in group B $p < 0.01$ ($p = 0.006$). Statistical analysis showed that in the second group, IL-6 values were significantly higher than those in group A patients after 6 weeks and 3 months of therapy. Regarding the quantification of values of IL-6 in serum after 6 weeks of therapy in patients in group B for $Z = -2.04$ and $p < 0.05$ ($p = 0.04$) is significantly higher than the value in patients with group A. After 3 months of treatment, differences between the two groups showed that the values after treatment between groups A and B for IL-6 in serum in group B were higher, ie. for $Z = -2.42$ and $p < 0.05$ ($p = 0.02$) in relation to the value in patients of group A. Intergroup differences at all time intervals showed better results in group A, confirming the effectiveness of LLLT in the treatment of periodontal disease in patients with T2DM.

The use of lasers in the treatment of periodontitis dates back not long ago, but in the beginning the recommendations for the use of LLLT in the treatment of many diseases including T2DM and periodontitis for many years were based only on vague assumptions, conclusions, reports or pilot clinical trials (28). However, experience showed that LLLT was applied to wound healing (29), against inflammation (30, 31), pain relief (32-33), swelling reduction (34-35), according to specific guidelines.

In recent years, the interest in the use of laser therapy has increased gradually, although there is still heterogeneity in research data and findings, the justification for their application has been unequivocally confirmed (36). Our findings agree with the findings of Boschi (14, 37, 38). Identical to the values obtained after applying LLLT (In GaAlP, 660 nm) in the study, the values of IL-6 and TNF- α 37 were significantly reduced.

Complexed progression of periodontal disease, with advanced inflammatory and destructive processes, as well as inadequate therapeutic effect are the basic features in patients with periodontitis in which T2DM (39-40) is diagnosed. These results are due to the presence of perio-pathogens that secrete endotoxin, which can increase the amount of many pro-inflammatory markers, including IL-6 (41-42).

In this study, in the group of patients treated conservatively with LLLT - therapy applied as adjuvant, we received 6 weeks and 3 months of reduced IL-6 values in serum. We believe that the results are due to the numerous positive properties of LLLT that are reflected on the examined pro-inflammatory mediator.

6. CONCLUSION

The low power laser radiation LLLT resulted in significantly reduced serum IL-6 values in patients with periodontitis and T2DM after 6 weeks and 3 months of therapy.

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Effect of Periodontal Treatment of Patient with Orthodontic Fix Appliance- long Term Follow-up, Case Report

Zana Sllamniku-Dalipi^{1*}, Fatmir Dragidella¹, Shefqet Mrasori², Metush Disha¹,
Kastriot Meqa¹, Visar Bunjaku¹

1. Department of Periodontology and Oral Medicine, School of Dentistry, Medical Faculty, University of Prishtina, Prishtina, Republic of Kosovo.

2. Department of Conservative Dentistry and Endodontics, Faculty of Medicine University of Prishtina, Kosovo.

Abstract

The chronic gingival inflammation caused by the accumulation of plaque around orthodontic brackets can lead to several different outcomes, such as gingival recession, attachment loss and inflammatory hyperplasia. We report a periodontal clinical management, over a period of 4 years, of a young female patient with gingival enlargement.

The orthodontic appliances often inhibits proper oral hygiene maintenance by the patient and therefore greater plaque accumulation. The development and severity of gingivitis will may depend from quantity and quality of dental plaque, immune response, morphological differences in the periodontium, etc. Gingival enlargement is one of the most tissue problems associated with fixed orthodontic appliances. Gingivectomy procedure followed up by pocket elimination and the recontouring of the diseased gingiva after 4 years, remains with no gingival sings.

The importance of appropriate periodontal treatment and plaque control interval for the patient is crucial to prevent destructive alveolar bone loss.

Case Report (J Int Dent Med Res 2020; 13(2): 351-354)

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Introduction

Gingival inflammation is usually attributed to bacterial-induced gingivitis. The modification of plaque -induced gingivitis can occur by local or systemic factors. Epidemiologic data have shown plaque-induced gingivitis to be prevalent at all ages in dentate populations¹⁻⁷.

The plaque-induced gingivitis progresses to more established forms of this disease, clinical signs and symptoms become obvious. Plaque-induced gingivitis begins at the gingival margin and may spread throughout the remaining gingival unit. The common clinical signs of plaque-induced gingivitis involve erythema, edema, bleeding, tenderness, and enlargement^{8,9}.

The severity of plaque-induced gingivitis can be influenced by factors that modify the inflammatory status of gingiva. The local factors include tooth anatomic factors, dental

restorations and appliances, root fractures and cervical root resorption¹⁰.

Systemic factors include: Sex steroid hormones: puberty, menstrual cycle, pregnancy, oral contraceptives; Hyperglycemia; Leukemia; Smoking and Malnutrition¹¹.

Gingival overgrowth is a very common condition in the orthodontic population that is characterized by gingival enlargement possibly resulting in pseudo-pocketing with or without attachment loss¹².

Placement of an orthodontic appliance in a patient's mouth is often associated with alterations in the oral hygiene habits and periodontal health. The appliances attached for the orthodontic treatment inhibits proper oral hygiene maintenance by the patient and therefore greater plaque accumulation^{13, 14, 15}. As plaque accumulates, the subgingival microflora changes from a more benign Gram-positive cocci culture to a pathogenic Gram-negative rod and spirochete culture¹⁶.

Non-surgical therapy eliminates microbial film, reduce inflammation of the periodontium and leads to clinical changes. Pseudo pockets created by the hyperplastic inflammatory changes in the gingival tissue need to be

*Corresponding author:

Fatmir Dragidella

Lagja e Spitalit street, NN, 10000 Prishtina, Republic of Kosovo

E-mail: fatmir.dragidella@uni-pr.edu

corrected with soft tissue gingivectomy.

This report presents the periodontal clinical management, over a period of 4 years, of a young female patient with gingival enlargement. The patient gave written informed consent and the case had been reviewed and approved by the University Dentistry Clinical Center of Kosovo Joint Ethics Committee.

Case Report

A case of a 15-year-old female presenting with maxillary chronic inflammatory gingival enlargement associated with prolonged orthodontic therapy. The assessment of the patient history involved chief complaint, social and family history, dental history, oral hygiene habits, smoking history and medical history and medications. Clinical examination revealed redness and swelling of the gingiva and increased tendency to bleeding. The periodontal parameters were obtained prior to intervention. In radiography of the patient was not recognized alveolar bone loss (Figure 1,2).



Figure 1. Preoperative view, gingival hyperplasia prior to periodontal surgery.



Figure 2. Postoperative view after 7 days.

Periodontal pocket management was followed with supra/subgingival debridement and oral hygiene instruction. After the re-evaluation, the pocket elimination was performed with external bevel scalpel gingivectomy combined with Low Level Laser Therapy (LLLT). Prior to surgery, patient was asked to rinse with a Chlorhexidine gluconate oral rinse for 30 seconds. Local anesthetics was administered. The bleeding points on the buccal surfaces of the upper anterior teeth marked the base of pockets. A postoperative dressing was applied.

No recurrence of gingival enlargement at the end of 4 years follow-up was observed.

The patient was in supportive periodontal care and appeared for follow-up appointments at 1 week, 1 month, 6 months, 1 year and 3 years after surgery.

Re-evaluation of the clinical response to surgical therapy showed decrease in probing pocket depth, gingival index with significantly improvement after 4 years post operatively.

Non-surgical management of gingival enlargement has shown effects in reducing gingival enlargement by eliminating its inflammatory component. Gingival overgrowth was managed by adjunctive antimicrobial oral rinses with chlorhexidine gluconate. All this procedure reduced the risk of postoperative infection and the risk of recurrence.

Due to mild gingival enlargement the gingivectomy procedure was followed up by resection of the excess tissue, pocket elimination and restoration of tissue contour and function.

There was no recurrence of the gingival enlargement in the maxillary anterior sextant (Figure 3), and the re-contouring of the diseased gingiva after 4 years, remains with no gingival sings.



Figure 3. After 4 years periodontal follow up case presentation.

Discussion

Orthodontic treatment with fixed appliances with brackets and archwires creates plaque retention sites and thus increases a patient's risk of developing tooth decay and plaque-induced gingivitis. In addition, the majority of patients undergoing orthodontic treatment are teenagers. This may also enhance the risk of poor compliance regarding plaque control and prevention¹⁷.

Gingival inflammation and gingival bleeding will increase in children at pubertal age as a result of the hormone changes that occur during puberty¹⁸.

The effects seen clinically following the insertion of orthodontic appliances into the oral cavity can contribute to chronic infection, inflammatory hyperplasia, attachment loss and gingival recession. The accumulation of microorganisms around teeth can cause gingival redness, bleeding, and edema; changes in gingival morphology; reduced tissue adaptation to the teeth; an increase in the flow of gingival crevicular fluid; and other clinical signs of inflammation¹⁹.

With gingivectomy the surgical excision of unsupported gingival tissue to the level where it is attached, creates a new gingival margin apical in position to the old. Anatomical crown becomes expose and pseudopockets are also eliminated, creating a better environment for periodontal health^{20,21}. Before receiving orthodontic treatment, the periodontal health of the patient should be the highest possible level and this has to be maintained during the treatment^{22,23}.

Therefore, determining the appropriate plaque control interval for the patient is crucial to prevent destructive alveolar bone loss. Enlarged gingival tissue harbors dentobacterial plaque, and the deeper pseudo-pocket acts as a reservoir for retention of subgingival plaque. Pseudo-pockets or gingival overgrowth or enlargement of the gingival margin and papilla, are exacerbated by poor oral hygiene. Constant periodontal care is necessary²⁴.

The location and shape of the gingival margins of the maxillary anterior teeth plays an important role in maximizing the esthetic appearance of those teeth and contributes significantly to the esthetics of a smile^{25,26,27}.

Conclusions

The successful outcomes of orthodontic treatment are influenced by the patient's periodontal status before, during, and after active orthodontic therapy. Control of gingival inflammation is essential for the primary prevention of periodontitis. Increased inflammation should be reduced to a minimum before fixed appliances are placed. Imperative for maintaining periodontal health is patient motivation and oral hygiene practices, followed up by interdisciplinary specialist cooperation.

Declaration of Interest

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Teeth and Soft Tissue Injuries as Well as Wound Healing Quality Patterns Among Primary School Students of Prishtina Region

Naim Haliti¹, Ragip Shabani², Shefqet Mrasori³, Fatmir Dragidella⁴, Hrvoje Juric⁵,
Nora Shabani Behrami⁶, Dafina Doberdoli⁷, Fehim Haliti^{7*}

1. Department of Forensic Medicine, Faculty of Medicine, University of Prishtina, Clinical Centre N.N., 10000 Prishtina, Kosovo.
2. Department of Pathology, Faculty of Medicine, University of Prishtina, Clinical Centre N.N., 10000 Prishtina, Kosovo.
3. Department of Operative Dentistry and Ednodontics, School of Dentistry, Medical Faculty, University of Prishtina, 10000 Prishtina, Kosovo.
4. Department of Periodontology and Oral Medicine, School of Dentistry, Medical Faculty, University of Prishtina, 10000 Prishtina, Kosovo.
5. School of Dental Medicine - Department of Pediatric Dentistry and Prevntion, University of Zagreb, Gundulićeva 5, 10000 Zagreb, Croatia.
6. Johannes Wesling Klinikum Minden, Institut für Pathologie, Universitatlinikum Der Ruhr – Universität Bochum, Hans-Nolte-Straße 1, 32429 Minden, Germany.
7. Department of Pediatric Dentistry, School of Dentistry, Medical Faculty, University of Prishtina, 10000 Prishtina, Kosovo.

Abstract

The aim of this study is to analyze and record the frequency and distribution of Traumatic Dental Injuries (TDI) and soft tissue injuries treated at the Emergency Department among children with various head and neck trauma. Furthermore, the quality of soft tissue healing and its impact on public oral health is described through Pediatric Health-related quality of life index (POQL Index).

This prospective interventional study was carried out at the Emergency Department of the University Clinical Center of Kosovo and University Clinical Dentistry Center between September 2017 and September of 2018 (as a part of broader clinical research) exploring the prevalence of TDI among schoolchildren at public schools (III – IX grade) in urban and suburban areas of the Prishtina Region, Kosovo. In our study 124 with various head and neck trauma are treated in the ED, and 16 children are diagnosed with fractured teeth and injured soft tissue structures. Categorized data testing was done with the X2 test or the exact Fisher test. Testing quantitative data with normal distribution with t-test or One Way ANOVA, and those without normal distribution with Mann-Whitney test. The difference is significant if $P < 0.05$.

The most fractured tooth was the left first incisor (D21) in 50 % of cases followed by the right central incisor (D 11) in 25 % of cases and second left incisor (D 22) with 12.5 %, respectfully. Male subjects had more complicated wound healing compare to female participants in our study but there was no discrepancy with significant statistical difference in the quality of healing in children with gender-based injury differentiation ($X^2 = 1.342$, $P = 0.511$). The average value of the POQL index in children included in our research was 33.9% (DS \pm 16.9%), the range 11.0-87.0%. The average value of the POQL index in injured children involved in this research was 38.8% (DS \pm 19.0%), range 14.0-69.0%. The average value of the POQL index for non-injured children included in our study was 33.1% (DS \pm 16.6%), range 11.0-87.0%.

Data obtained from this study and methodology enables the dental professionals to address the problem of inconsistent procedure protocols in ED and the immanent need for gathering contribution data which will help the institutions to make preventive measures and early interventions.

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*Corresponding author:

Ass Prof Fehim Haliti DDS, PhD.
Department of Pediatric Dentistry,
School of Dentistry, Medical Faculty,
University of Prishtina, 10000
Prishtina, Kosovo.
E-mail: feimi18@hotmail.com

Introduction

Modern living trends are very dynamic and demanding and consecutively the accident rate is much higher compare to the end of twentieth century. Environments like for example school, the home, sports facilities and the road are where traumatic events occur with the

highest frequency. Both children and adults seek care at medical or dental centers for emergencies, such as dental pain, trauma, and infection. Reports have shown that 25% of school-aged children will experience some kind of dental injury. Trauma to the oral hard and soft tissues is frequently seen in children. Epidemiologic studies have focused on the investigation of the prevalence or incidence of dental injury. The frequency of dental injuries in preschool and school children is a continuing clinical and dental public health problem. Amongst all facial injuries, dental traumas are the most common. From the total of 18% of all injuries in children up to 6 years of age are seen in the oral region.¹ One third of all children in the primary dentition stage suffer from traumatic injuries to the mouth. This is possibly related to poor motor coordination and is sometimes due to the child's inability to evaluate risks.² Resilience of the alveolar bone in young children causes dental luxations of the intrusive type to be more common than crown fractures. The predominant cause of dental injuries in younger age groups is falls, such as falling from baby carriages, falling down stairs, or falling against hard objects, and is mainly indoor injury.

Injuries to the primary dentition are common, occurring with a significantly higher annual incidence than in the permanent dentition.³ Current literature has been well documented that the prognosis for traumatized teeth depends largely upon both timely and appropriate emergency management. Furthermore, it has been reported that up to 66% of all Emergency Department (ED) visits for dental issues are for the management of traumatic dental injuries (TDI), the number of ED visits for pediatric dental care has been increasing in the last two decades.^{4,5} In addition, child abuse is highly associated with head and tooth injuries.

Facial differences and dental malocclusion affect the self-esteem of children and young adults.⁶ Severe dental injuries, can cause immediate unexpected pain and disfigurement. In addition to the economic consequences of dental injuries, trauma produces acute and chronic pain as well as a range of socioeconomic effects that include quality-of-life insults that can lead to time off school and work, lost sleep and commuting for treatment. Moreover, children may experience

anxiety produced by the unwanted attention of their peers and the inability to take part in school activities such as sports and music.^{7,8,9}

In the current studies, the majority of dental injuries involve the anterior teeth, which may lead to limitations in biting, difficulty in speaking clearly, and feeling uncomfortable to show the teeth. Consensus is reached that traumatic injuries occur more often to the maxillary than the mandibular incisors and that the central incisors are affected more than the lateral incisors.^{10,11}

The surface of the oral cavity is covered by oral mucosa, a moist lining that communicates with the external environment. The oral mucosa consists of two separate tissue components: stratified squamous epithelium (the oral epithelium) and an underlying connective tissue layer (the lamina propria). Functions of the oral mucosa include protection, sensation and secretion.¹² One of the important aspect of our study is to analyze the wound healing, as a normal biological development, which can be achieved through four distinct and highly differentiated phases: hemostasis, inflammation, proliferation, and remodeling. In order to heal effectively, all four healing stages must arise in the proper order and time related period. Many factors can impede through one or more phases of this progression, and consecutively can cause inappropriate or diminished wound healing.¹³

Wound healing can be delayed by both local and systemic or general elements. Local elements are those that exist in the wound tissue and include: the amount of exudate (which can lead to maceration if too much, or desiccation if insufficient), an abnormal bacterial balance or local infection, the presence of foreign bodies exacerbating the inflammatory response, the vascular supply to the area, pressure, friction, and shear as well as repetitive trauma to the area and wound management practices that affect wound bed temperature reducing the cellular proliferation. There is limited literature or clinical studies quantifying the impact of systemic elements on wound healing times.¹⁴

The Pediatric Health-related quality of life (POQL Index) is a valid and reliable measure of oral health-related quality of life for use in preschool, school-age and pre-teen children. Our research will analyze the quality of general and oral health after trauma for all study participants through the use of modified POQL index - at the

end of the study for all subjects.^{15, 16, 17}

The objectives of this study were to analyze and record the pattern of teeth and soft tissue injuries treated in ED and later monitored at the University Dentistry Clinical Center of Kosovo in Prishtina for the fractured teeth treatment and the quality of surrounding soft tissue wound healing.

Materials and methods

Our prospective interventional study was conceded between September 2017 and September of 2018 (as a part of broader clinical research) exploring the prevalence of TDI among schoolchildren at public schools (III – IX grade) in urban and suburban areas of the Prishtina Region, Kosovo. The study protocol and informed consent document was approved by the University of Prishtina, Medical Faculty – School of Dentistry and Kosovo Ministry of Education and Science, also it's in full concordance with the World Medical Association Declaration of Helsinki (October 2, 2011, meeting number 5, decision number 5).

A total of 124 children were referred for treatment at the emergency room due to various head and neck trauma at the University Clinical Center of Kosovo, and 16 children are diagnosed with fractured teeth and injured soft tissue structures. The research data are presented as percentage, arithmetic mean and standard deviation and range. Categorized data testing was done with the X2 test or the exact Fisher test. Testing quantitative data with normal distribution with t-test or One Way ANOVA, and those without normal distribution with Mann-Whitney test. The difference is significant if $P < 0.05$. Data processing is done using the statistical package SPSS 22.0. The sample size was calculated to give a standard error of 2.0%. The minimum sample size needed to satisfy the requirements was estimated to be 112 individuals. A correction factor equal to 1.7 was applied for design effect (deff).

Results

As far as gender distribution 59 or 47.6% were female and 65 or 52.4% male. Using the X2-test we did not obtain a difference with significant statistical implication regarding gender in our research ($X^2=0.29$, $P=0.590$).

The students involved in the research were from grades III to grade IX, so all grades of junior high school and grades III, IV and V of primary school. The smallest number 6 or 4.8% were third grade students, then fifth to ninth grade students were 94 or 24.2%.

Based on gender, with X2-test we did not obtain a difference with significant statistical consequence in the presence of injuries (F 13.6% vs. M 12.3%), ($X^2 = 0.003$, $P = 0.951$), respectively. (Table 1)

Presence of injury	Gender				Total	
	F		M			
	N	%	N	%	N	%
Yes	51	86.4	57	87.7	108	87.1
No	8	13.6	8	12.3	16	12.9
Total	59	100.0	65	100.0	124	100.0
X ² -test, P-value	X ² =0.003, P=0.951					

Table 1. Schoolchildren included in research based on the presence of injuries and gender.

Further statistical analysis didn't show any correlation between the presence of injuries and grades or age. Injuries occurred in 16.7% of children in 3rd grade, then dropped to 4.2% in 4th grade, 6.9% in fifth grade, to increase again in sixth grade 18.8%, seventh grade 23.1% (Table 2).

Grade	Presence of Injuries				Total	
	Yes		No			
	N	%	N	%	N	%
III	1	16.7	5	83.3	6	100.0
IV	1	4.2	23	95.8	24	100.0
V	2	6.9	27	93.1	29	100.0
VI	3	18.8	13	81.3	16	100.0
VII	3	23.1	10	76.9	13	100.0
VIII	3	15.0	17	85.0	20	100.0
IX	3	18.8	13	81.3	16	100.0
Total	16	12.9	108	87.1	124	100.0

Table 2. Children included in the study based on injuries and grade.

From 124 children included in the study, 9 or 7.3% had allergies, one or 0.8% had anemia, one or 0.8% rheumatic fever, one or 0.8% heart problems, one or 0.8% peripheral blood circulation deficiency and one or 0.8% was suffering from severe form of scoliosis. Without systemic disease were 110 or 88.7% of children included in this study.

The total number of children with teeth fractures and soft tissue injuries is 16, and eight of them or 50.0 % of children had the tooth 21 fractured, followed by 4 or 25.0% had dental injury of tooth 11, two or 12.5% had dental injuries of tooth 22. Tooth injuries on teeth 12; 31; 41; 61 and 62 were present in single cases or 6.3% (Table 3).

Fractured teeth	Total	
	N	%
*teeth number	16	100.0
11	4	25.0
12	1	6.3
21	8	50.0
22	2	12.5
31	1	6.3
41	1	6.3
61	1	6.3
62	1	6.3

Table 3. Fractured teeth among injured children.

In our study, the quality of wound healing was assessed independently by three evaluators' (Specialists of Pediatric Dentistry) which have scored the progression of wound healing in three categories (normal healing, healing with moderate difficulties and complicated healing), taking into consideration soft tissue laceration patterns, dehiscence, hematoma, presence of fibrin stripes above the wound, bleeding, traces of wound concussion, bone exposures and presence of foreign bodies inside the wound.

The first assessment was done 24 hours after the injury, the second assessment took place on the fourth day after the trauma and the third assessment was done on the seventh day. Photos and video recordings of wounds are well documented during the evaluation procedures for each subject included in this study. The overall score was calculated from all three evaluators and proceeded for further analysis.

In children with injuries included in the study based on the quality of wound healing, 37.5% of female had healing without difficulty compared to male subjects represented with 25.0%. While male with 37.5% had more healing with complications compared to female with only 12.5% complications during healing period. However, utilizing the X2-test we did not obtain a divergence with significant statistical difference in the quality of healing in children with gender-based injury differentiation ($X^2 = 1.342$, $P = 0.511$), (Table 4).

Wound Healing Quality	Gender				Total		
	F		M				
	N	%	N	%	N	%	
Normal Healing	3	37.5	2	25.0	5	31.3	
Healing with Moderate Difficulties	4	50.0	3	37.5	7	43.8	
Complicated Healing	1	12.5	3	37.5	4	25.0	
Total	8	100.0	8	100.0	16	100.0	
X ² -test, P-value		X ² =1.342, P=0.511					

Table 4. Gender related Quality of Wound Healing.

In children with injuries involved in the research based on quality of wound healing, healing without difficulty had 31.3%, healing with moderate difficulty was noted on 43.8% of subjects and recovery with complications in 25.0% of children.

Another statistical analysis between children in different grades demonstrated that the children of the 9th grade had more recovery without difficulty (66.7%), (Table 5).

Grade	Quality of Wound Healing						Total	
	Normal healing		Healing with moderate difficulties		Complicated Healing			
	N	%	N	%	N	%	N	%
III	-	-	-	-	1	100.0	1	100.0
IV	-	-	1	100.0	-	-	1	100.0
V	1	33.3	1	33.3	1	33.3	3	100.0
VI	-	-	1	50.0	1	50.0	2	100.0
VII	1	33.3	2	66.7	-	-	3	100.0
VIII	1	33.3	2	66.7	-	-	3	100.0
IX	2	66.7	-	-	1	33.3	3	100.0
Total	5	31.3	7	43.8	4	25.0	16	100.0

Table 5. Grade related quality of wound healing.

Children from third to sixth grade are more prone to complications during healing period (three children, 4.10 %)

Injuries in children included in this study most often in 43.8% of cases were caused by falls, in 12.5% were caused by accidents, in 6.3% of cases injuries were caused by very decayed teeth during mastication, 6.3% had fractures caused by stoning, 6.3% had intrusion of tooth 61 and in 25.0% of cases they don't remember or they don't know the injury mechanism (Table 6).

Injury Mechanism	Gender				Total	
	F		M			
	N	%	N	%	N	%
Fall	2	25.0	5	62.5	7	43.8
Accident	1	12.5	1	12.5	2	12.5
Decay/ followed by fracture	1	12.5	-	-	1	6.3
Stoning	-	-	1	12.5	1	6.3
Don't know or don't remember	3	37.5	1	12.5	4	25.0
Intrusion of D 61	1	12.5	-	-	1	6.3
Total	8	100.0	8	100.0	16	100.0

Table 6. Injury mechanism of children based on gender.

The average value of the POQL index in children included in our research was 33.9% (DS ± 16.9%), the range 11.0-87.0%. The average value of the POQL index in injured children involved in this research was 38.8% (DS ± 19.0%), range 14.0-69.0%. The average value of the POQL index for non-injured children included in our study was 33.1% (DS ± 16.6%), range 11.0-87.0%.

Utilizing the Mann-Whitney test we did not obtain a difference with significant statistical importance of POQL index values based on the presence of injuries (Table 7).

The average value of the POQL index in children with teeth fracture and soft tissue injuries included in our research was 38.8% (DS ± 19.0%), the range 14.0-69.0%. The average value of the POQL index in children involved in research with normal wound healing was 45.0% (DS ± 21.3%), range 24.0-69.0%. The average value of the POQL index in children included in research with moderate wound healing was 42.7% (DS ± 18.6%), range 17.0-67.0%. The average value of the POQL index in children involved in research with complicated healing

was 24.3% (DS ± 11.4%), range 14.0-36.0%.

With One Way ANOVA we did not obtain a difference with significant statistical difference between the POQL index values according to the quality of wound healing (F = 1.73, P = 0.578), (Table 8).

POQL Index (%)	Presence of Injury		
	Yes	No	Total
N	16	108	124
Median	38.8	33.1	33.9
DS	19.0	16.6	16.9
Min	14.0	11.0	11.0
Max	69.0	87.0	87.0
Mann-Whitney test, P-value	U'=1034.5, P=0.205		

Table 7. The results of POQL –index for all study participants.

POQL Index (%)	Wound quality of healing			Total
	Normal wound healing	Moderate wound healing	Complicated wound healing	
N	5	7	4	16
Median	45.0	42.7	24.3	38.8
DS	21.3	18.6	11.4	19.0
Min	24.0	17.0	14.0	14.0
Max	69.0	67.0	36.0	69.0
One Way ANOVA	F=1.73, P=0.578			

Table 8. Results of POQL-index based on wound quality of healing.

Based on available literature, POQL-index clustered best into four dimensions – Physical Functioning, Role Functioning, Social Functioning and Emotional Functioning. Meanwhile, all scales excluding for Role Functioning showed good consistency and strong associations with the total POQL for both groups (with and without injuries). The scales were discreetly inter-correlated, as projected, with the strongest association seen amongst the Social and Emotional scales.

Discussion

The incidence of teeth fractures and soft tissue injuries as a result of various trauma is well documented in the current available

literature. Epidemiological studies results showed that globally, the incidence/ year of dental trauma reaches 4.5%: approximately a third of children and toddlers and a quarter of adolescents and adults.¹⁸

In our study group the most fractured tooth was the left first incisor (D21) in 50 % of cases followed by the right central incisor (D 11) in 25 % of cases and second left incisor (D 22) with 12.5 %, respectfully.

Similar statistical data in comparison to our study was presented by Eigbobo et al., in their research have found that 59 (60.8%) of participants were male and 38 (39.2%) females presented with dental trauma, the commonest cause of TDI was falls (85.2%), and most (76.2%) incidents occurred in homes. The maxillary central incisors were the most (84.6%) commonly injured teeth, almost occurring equally on both sides of the jaw.¹⁹ In another study, similar results were found with 16.5% prevalence of dental trauma. Boys experienced double the number of girls' injuries. The maxillary central incisors were the teeth most affected, totaling 84.8%.²⁰

In another study conducted in Brazil, 1,650 patients were referred for treatment at the emergency room of the Oral and Maxillofacial Trauma and Surgery Service at Hospital das Clínicas. From this total, 78 (4.7%) patients presented some kind of dento-alveolar trauma, which is in vast discrepancy with our study findings 12.9% (but in much smaller sample).²¹

Involvement of soft tissue injuries as a part of multiple trauma injuries of head and neck was present in all subjects included in our study, Akuldiz et al. in their study, reported that cases of TDI including soft tissue injuries had lower percentages (22.5%).²² In another prospective study, Acton et al., analyzing the traumatic maxillofacial injuries in young bicycle riders reported that the most common oral/maxillofacial injuries were facial abrasions, cuts and lacerations (50.3%); soft tissue injuries to the mouth (30.9%); and dento-alveolar trauma (9.7%).²³

Study by Lygidakis et al., included 1271 children aged 8 months to 12 years, 680 boys and 591 girls presented as emergencies, and from this total number of children 142 (11%) subjects (90 males and 52 females) who presented following trauma had injuries to 20 primary and 221 permanent teeth, this results are

statistically similar to our findings regarding the overall patient percentage and involved teeth.²⁴ Majority of study reviews exposed the fact that the central maxillary incisors are the most frequently fractured teeth followed by maxillary second incisors.^{25, 26, 27,28,29}

Many times the private clinics receive patients with TDI and the treat them successfully, in a study done by Oulis et al., 68% of the patients sought treatment 3 days or more after the trauma had occurred (delayed treatment), while only 28% within the first days (delayed treatment). The main reasons for delayed treatment were neglect (50 %), unawareness (37%) and miscellaneous reasons (13%). The highest incidence of dental trauma was observed at the age of 10. Furthermore, important finding, which is in agreement with the literature, was that there was no difference in the probability of traumatizing teeth of one side of the oral cavity in comparison to the other.³⁰

In majority of cases documented in ED databases pediatric soft tissue injuries are commonly overlooked when discussing pediatric trauma. Yet they occur in association with facial fractures 29–56 % of the time. In our study the incidence of soft tissue injuries was 20.1 %. The modalities of management of such injuries are similar to that of adults but it has to be kept in mind that healing process starts faster so chances of formation of hypertrophic scar or keloid is more possible.³¹

The problem of very low number of children seeking medical and dental emergency treatment right after the injury was well described in a study conducted by Folakemi et al, based on the results majority of the injuries happened in the home followed by school. Only 36.4% of parents/caregivers sought dental care for their children/ward within one week after dental trauma. Parents and teachers are advised to be more safety conscious and provide protective appliances such as mouth guard for children during sporting activities.³²

Evidence regarding the importance of information technology for patient record and tracking was very well described in the paper published by Glendor et al., with the focus on promotion, prevention and curative care. An “individual risk profile”, especially for younger individuals who have received their first trauma episode, may be useful in the co-operation

between the patient, parents and their caregiver. This risk profile could be a combination of systematic information from the “experts” on the dental trauma episode, the patient and parents, and standard trauma information in dental record. A database, consisting of systematically and continuously compiled information for such risk profile, would provide better knowledge about how to avoid multiple dental trauma episodes.³³ In addition, through this described ideas by Professor Glendor it is feasible to identify children with locomotor and neurologic system deficiencies and also to proceed with their treatment in specialized healthcare institutions. In a similar detailed epidemiologic study Alan et al. are emphasizing the role that emergency medicine can play in the initial management of tooth injuries, stressing that population-based epidemiologic data on injuries must be used to reassess conventional wisdom about injuries and to target future efforts at prevention of dento-alveolar injuries.³⁴

Tumen et al., also is focused on the idea that there is a great need for more focus and health promotion policies to encourage precautionary policies dedicated to parents and teachers to inform them about the significance of traumatic dental injuries and the benefit of instant respond for dental treatment.³⁵

In our study modified POQL for both groups (with and without injuries), was shown very balanced / equal for the obtained pattern for all study participants ($U=1034.5$, $P=0.205$), which are in agreement with the results found by Gilchrist and Traebert.^{36, 37}

The use of herbal products which have accelerating wound healing properties are not uncommon, some of those studies are done on humans and animals as well. All this herbal products are rich in chemical compounds, such as saponins, tannins, alkaloids, triterpenoid, flavonoid, phenolics, steroids, and glycosides. In the second phase of our research it's our intention to use this products too, and to measure their effectiveness. Many authors are presenting their research findings in which there is a significant increase of quantitative values of Tumor Growth Factor $\beta 1$ (TGF $\beta 1$) expression but with no significant reduction of inflammatory cells, proliferation of mature collagen is deposits, faster and compact wound closure, stimulation of fibroblast cell production, etc.^{38, 39, 40}

Based on the results of our research, the course of one week wound healing was evaluated by three evaluators' which have scored the progression of wound healing in three categories (normal healing, healing with moderate difficulties and complicated healing), further analysis demonstrated that the POQL index scores for both groups didn't show any significant statistical discrepancy. The results of our research are very similar to the studies carried out by Politis, Guo and Szpaderska.^{41,42,43}

Conclusions

Based on the findings of this study dental professionals need to address the problem of inconsistent procedure protocols in ED and the immanent need for gathering contribution data which will help the institutions to make preventive measures and early interventions. These efforts would enhance the long term outcomes for young patients sustaining dental trauma and treated in ED, private dental practice or tertiary treatment institutions.

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Declaration of Interest

The authors report no conflict of interest.

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Granulomatosis with polyangiitis associated with alveolar bone loss – Case presentation

Zana Sllamniku-Dalipi¹, Fatmir Dragidella¹, Shefqet Mrasori², Metush Disha¹,
Kastriot Meqa¹, Visar Bunjaku¹

1. Department of Periodontology and Oral Medicine, School of Dentistry, Medical Faculty, University of Prishtina, Prishtina, Republic of Kosovo.

2. Department of Conservative Dentistry and Endodontics, Faculty of Medicine University of Prishtina, Kosovo.

Abstract

Granulomatosis with polyangiitis (GPA) is a long-term systemic disorder that involves both granulomatosis and polyangiitis, if untreated can be lethal. Granulomatosis with polyangiitis is also known to cause oral mucosal lesions as nonspecific erosive- ulcerative lesions and enlargement of gingivae and alveolar bone loss.

We report a case of GPA with a severe generalized phase with oral manifestations: gingival bleeding and severe gingival inflammation. The radiographic examination of the patient revealed the periodontal bone loss and tooth loss of anterior mandibular region.

Periodontal professional care is necessary to improve the oral health of patients with GPA as a part of multidisciplinary approach.

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Introduction

Granulomatosis with polyangiitis (GPA), formerly Wegener's granulomatosis, is one of a group of blood vessel disorders called vasculitis. It is a rare multisystem autoimmune disease of unknown etiology that is pathologically characterized by an inflammatory reaction pattern (necrosis, granulomatous inflammation and vasculitis) that occurs in the upper and lower respiratory tracts and kidney.¹ The affected tissues may develop areas of inflammation called granulomas, which can affect all areas of the body: the upper respiratory tract, lungs, kidneys and other organs, including the oral cavity.^{2,3}

The Chapel Hill 2012 Consensus Criteria define GPA as "necrotizing granulomatous inflammation usually involving the upper and lower respiratory tract and necrotizing vasculitis affecting predominantly small to medium vessels".⁴

Granulomatosis with polyangiitis (GPA) is a severe autoimmune disease which is highly associated with anti-neutrophil cytoplasmic antibodies (ANCA). GPA is mediated by a T-cell reaction,⁵ where the production of pro-inflammatory cytokines TNF- α , interleukin 1-beta (IL1 β) and IFN- γ , will induce the production of anti- cytoplasmic proteinase 3 antibodies (anti -PR 3 antibodies), after the expression of surface antigens on activated neutrophil granulocytes. Under the influence of cytokines and ANCA, it will lead to degranulation of neutrophil granulocytes and will contribute to the aggression of the vessel wall and be responsible for the tissue damage.⁶

Generally, the clinical manifestation of the disease varies from patient to patient. Rapid progression in combination with multiorgan failure will lead to death if untreated.⁷ Infections, environmental factors, epigenetic modifications, and a genetic predisposition may influence the risk of developing.^{8,9}

GPA typically evolves into two phases: an initial phase characterized by ear, nose and throat (ENT) manifestations, such as chronic sinusitis and otitis, ulceration of the oral cavity and pharynx, as well as pulmonary nodules

*Corresponding author:

Zana Sllamniku- Dalipi
Department of Periodontology and Oral Medicine,
School of Dentistry, Medical Faculty,
University of Prishtina,
Prishtina, Republic of Kosovo
E-mail: zanasllamniku@hotmail.com

and a severe generalized phase, defined by the occurrence of rapidly progressive glomerulonephritis, pulmonary hemorrhage, and arthritis.¹⁰

Early diagnosis and treatment of granulomatosis with polyangiitis may lead to a full recovery. Without treatment, granulomatosis with polyangiitis can be fatal.^{11,12}

10% of patients with GPA have oral manifestations, and oral lesions that present as an initial manifestation of GPA are rare. The most characteristic oral lesion is hyperplastic gingivitis presenting with a "strawberry like" appearance.^{13,14} Oral symptoms of Wegener Granulomatosis are: nonspecific erosive/ulcerative lesions of oral cavity, Ulcers in oral mucosa, gingival involvement can be seen as gingival enlargement, strawberry gingivitis, erythema, petechiae, hemorrhage and necrosis.^{15,16} In some cases ulcers, necrosis on tongue, bone alveolar loss, tooth loss and mobility, ulcers on mucosa of palate, nodular hyperplasia and desquamation of lips.¹⁷ Osteomyelitis or necrosis of the underlying bone can develop with subsequent mobility and loss of teeth.¹⁸ The diagnosis of GPA is made based on clinical symptoms and signs, the presence of the anti-neutrophil cytoplasmic antibody (ANCA), and a positive biopsy.¹⁹

We report a case of 47-year-old female patient with GPA with oral involvement gingival bleeding and severe gingival inflammation.

The patient gave written informed consent. and the case had been reviewed and approved by the University Dentistry Clinical Center of Kosovo Joint Ethics Committee.

Case Report

A 47 year old female nonsmoker patient was referred to the Department of Periodontology and Oral Medicine at the University Clinical Dental Center in Prishtina, Kosovo at May 2018 with complaining of gingival swelling since approximately 1 year, soreness and bleeding from gingiva.

Patient history. The disease started as an acute, self-limited condition characterized by the abrupt onset of symmetrical fixed red papules on the skin, later on diagnosed as Erythema exudative multiformis on May 2015. After 2 weeks, her lungs were affected and she

had shortness of breath, coughing, weight loss and diagnosed with interstitial pneumonia. While hospitalized, patient had cardiac manifestations of pericarditis and myocardial infarction. Within 1 month she developed rapidly progressive glomerulonephritis leading to renal failure. The patient was diagnosed with GPA in September 2015.

The histological proof of the diagnosis of GPA was obtained by kidney biopsy. Immunofluorescence of renal biopsy specimens presented negative staining (anti IgA, IgG, C3 and C1q) and all glomeruli were with crescent formation, with characteristics of pauci-immune rapidly progressive glomerulonephritis, positive ANCA where changes of renal function are observed, urinary sediment abnormalities.

When the patient was referred to our department the oral hygiene status was poor, gingival enlargement, gingival bleeding and severe gingival inflammation was observed. During the early phase of disease when there were only nonspecific symptoms, even the patient had previously oral complaint she didn't seek periodontal opinion, and was referred only to extract teeth's in mandibular anterior region. The patient did not receive any periodontal treatment previously.

Radiographic examination revealed generalized horizontal/ vertical bone loss on November 2016 (Figure 1) and widespread horizontal/vertical severe bone loss was more pronounced in the mandibular anterior region on January 2018 (Figure 2)). Incisors of mandibular region with terminal alveolar bone loss were extracted. Comparing the radiographic examinations, the severe periodontal destruction or early tooth loss can be observed. In this period of time the patient did not received periodontal treatment.

After including oral hygiene instructions, scaling and root planning using both hand and ultrasonic instruments, the periodontal parameters improved (Figure 3 & Figure 4).

Due to her current diagnosis with GPA, Insufficiencia renalis chr grade III, hypertension, Hypothyreosis, Arthritis, her current medications are: immunosuppressant's, oral corticosteroids, antihypertensive drugs, hypothyroidism drugs.



Figure 1. Radiography of the patient with GPA demonstrating general horizontal/vertical bone loss. (November 2016).



Figure 2. Radiography of the patient with GPA demonstrating vertical destruction of anterior maxillary region and interradicular bone loss in the maxillary first right molar (January 2018).



Figure 3. Pretreatment periodontal condition of patient with GPA.



Figure 4. Post treatment periodontal condition of patient with GPA.

Discussion

The diagnostic criteria, as defined by the American College of Rheumatology for the diagnosis of WG requires at least 2 of the following 4 criteria: (1) oral ulcers or nasal manifestation, (2) the presence of nodules, fixed infiltrate, or cavities on a chest radiograph, (3) nephritic urinary sediment (red cell cast or >5 red blood cell per high power field), and (4) granulomatous inflammation on a biopsy (20). Although oral ulceration is one of the criteria required by the ACR in the diagnosis of WG, it usually occurs late in the disease.

Proteinase 3 (PR3) is a multifunctional neutrophil-derived serine protease influencing cell cycle, differentiation, and cell death. This molecule is the main target antigen of autoantibodies in Wegener's granulomatosis (WG) known as antineutrophil cytoplasmic antibodies (PR3-ANCA).²¹ Anti-neutrophilic cytoplasmic antibodies with a cytoplasmic staining pattern (c-ANCA) have been found to have a high degree of sensitivity and specificity for Wegener's granulomatosis.²² But they are not considered diagnostic criteria according to the "The American College of Rheumatology", they have 96% specificity and a 92% sensitivity.^{23,24} If the c- ANCA are negative they do not necessarily exclude disease, but they play role once the treatment has been started.

In recent times mortality due to GPA has been significantly reduced by the use of cytotoxic therapy with cyclophosphamide and glucocorticoids.^{25,26}

Several systemic diseases exhibit characteristic oral manifestations, which dentists can identify or diagnose. Causes of gingival swelling can be a feature of chronic gingivitis and may be caused by local and systemic causes. The changes in periodontal health of patient with GPA can be improved and it requires periodontal professional care.^{27,28}

Conclusions

Health professionals should be familiar with clinical picture of GPA. The diagnosis and therapeutic treatment should be managed by a multi-disciplinary team and multidisciplinary approach involving all specialists, as well as strategies to facilitate prompt disease

recognition and to provide continued oral health care to these medically complex patients. Failure to recognize the diagnostic clinical picture of GPA is unfortunately common and this often leads to serious morbidity or even fatal outcomes.

Conflict of Interest

The authors state that there were no conflicts of interest related to this study.

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Assessment of non-surgical periodontal treatment combined with Low-Level Laser Therapy (LLLT) in chronic periodontitis patients suffering from Iron Deficiency Anemia (IDA)

Dafina Kolgeci¹, Silvana Georgieva², Shefqet Mrasori³, Blerina Kolgeci⁴, Kaltrina Kolgeci⁵

1. Department of Periodontology and Oral Disease, University Dental Clinical Center of Kosovo, Prishtina, Republic of Kosovo.
2. Faculty of Dentistry, University "St. Cyril & Methodius", Skopje, Republic of Macedonia.
3. Department of Operative Dentistry and Endodontics, University Dental Clinical Center of Kosovo, Prishtina, Republic of Kosovo.
4. Department of Pediatric and Preventive Dentistry, University Dental Clinical Center of Kosovo, Prishtina, Republic of Kosovo.
5. Faculty of Medicine, University "Hasan Prishtina".

Abstract

Hence there are many chronic diseases known as catalyzers of destructive alterations in the periodontal complex, among them Iron Deficiency Anemia, the purpose of this study is to evaluate the assessment of non-surgical periodontal treatment combined with LLLT in chronic periodontitis patients suffering from IDA.

Subjects were selected from patients treated at the University Dentistry Clinical Center of Kosovo in Prishtina, included 63 participants suffering from Iron Deficiency Anemia only, followed by patients with IDA and any concomitant chronic condition and control group consisted of subjects with Chronic Periodontitis. (from October 1st, 2016 until March 31st, 2017). Patients suffering from Chronic Periodontitis were divided into three groups, suffering from IDA (Group A, 21 subjects), IDA and Chronic Disease subjects (Group B, 21 subjects) and the control group with normal iron values suffering from chronic periodontitis (Group C, 21 subjects). In this study we had also inclusion and exclusion criteria, Criteria for classifying Debris, Loe-Silness Gingival Index. LLLT adjusted to (660 nm, 10mW, 8 min/daily, in contact with gingiva); model continuously in the next five days. Blood samples were taken at baseline and at 2-month recall visit.

As far as the gender distribution between groups, it's evident that male subjects are dominant $X(m)=39; n=63$ in all groups ($Chi=0.28; p>0.05$). There was no significant difference regarding age discrepancy among subjects ($F=11.6; p<0.01$). IDA patients groups (A and B) have more tobacco users compare to overweight/obese group 40.9% ($Chi=0.31, p>0.05$).

The differences in GI, OHI-s and CAL values were statistically significant among groups at 2 months. A p value <0.05 was considered significant. IDA patients represented in group B showed higher values for PI, GI and CAL. CPITN values are much higher in group B compare to group A and the control group C ($Chi = 17.9; p<0.05$).

Comparative data analysis of our measurements showed that root planning and scaling of periodontal pockets resulted in improved results for PI, GI, CAL after two months – compared to baseline measurement outcomes. Meanwhile, Hb and SI levels at baseline and after two months demonstrated weak correlation with periodontal parameters.

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Introduction

Periodontitis is a multifactorial inflammatory disease of periodontal tissues usually caused by extension of bacterial infection

into subgingiva, which leads to the connective tissue destruction and alveolar bone loss. The essential objective of periodontal treatment is to decrease or eliminate the responsible periopathogens, by means of removing bacterial deposits from the tooth surface. Conventional mechanical debridement (ie, scaling and root planning [SRP]) is considered to be the gold standard for inflammatory periodontal disease treatment, which can cause an interim reduction in the levels of subgingival periopathogens.^{1,2,3,4,5}

*Corresponding author:

Dafina Kolgeci
Department of Periodontology and Oral Disease,
University Dental Clinical Center of Kosovo,
Prishtina, Republic of Kosovo.
E-mail: dafina.kolgeci87@hotmail.com

Periodontal diseases are collectively the most common diseases known to mankind. Their classification is complex and takes into account the clinical presentation, age at diagnosis, rate of disease progression, and systemic and local factors that may increase risk. Periodontal diseases include gingivitis (in which the inflammation is confined to the gingiva, and is reversible with good oral hygiene) and periodontitis (in which the inflammation extends and results in tissue destruction and alveolar bone resorption). Tissue destruction in periodontitis results in breakdown of the collagen fibers of the periodontal ligament, resulting in the formation of a periodontal pocket between the gingiva and the tooth. 'Pocketing' is not evident on simple visual inspection, and assessment using a periodontal probe is essential. Periodontitis is a slowly progressing disease but the tissue destruction that occurs is largely irreversible. In the early stages, the condition is typically asymptomatic; it is not usually painful, and many patients are unaware until the condition has progressed enough to result in tooth mobility. The pockets deepen as a result of the further destruction of fibers of the periodontal ligament (referred to as attachment loss and the resorption of the alveolar bone that occurs in parallel with the progressing attachment loss. Advanced periodontitis is characterized by gingival erythema and edema, gingival bleeding, gingival recession, tooth mobility, drifting of teeth, suppuration from periodontal pockets, and tooth loss.^{6,7,8,9}

Chronic diseases are well known catalyzers of destructive alterations in the periodontal complex. Diabetes has been unequivocally confirmed as a major risk factor for periodontitis. There is emerging evidence to support the existence of a two-way relationship between diabetes and periodontitis, with diabetes increasing the risk for periodontitis, and periodontal inflammation negatively affecting glycemic control.¹⁰

Oral lesions are common in patients with Inflammatory Bowel Disease and epidemiology data vary over a wide range of 5%-50% due to contradictory studies. Non-specific lesions in the oral cavity can also be the result of malnutrition and drugs. Malnutrition, followed by anemia and mineral and vitamin deficiency, affects the oral cavity and teeth. Furthermore, all of the drug classes that are applied for the treatment of

inflammatory bowel diseases can lead to alterations in the oral cavity due to the direct toxic effects of the drugs on oral tissues, as well as indirect immunosuppressive effects with a risk of developing opportunistic infections or bone marrow suppression.^{11,12,13}

Iron Deficiency Anemia (IDA) is a major health problem worldwide. Iron deficiency of nutritional origin is the most frequent cause of microcytic hypochromic anemia, but other conditions such as bleeding, gastro-intestinal malabsorption or *Helicobacter pylori* infection can lead to iron deficiency and anemia. Anemia is a global public health problem affecting both developing and developed countries and has major consequences for human health as well as social and economic development. It affects 24.8% of the world population. The burden of anemia varies with a person's age, sex, altitude, and pregnancy. The worldwide prevalence of anemia among adolescents is 15% (27% in developing countries and 6% in developed countries). Mutually, chronic periodontitis (CP) and iron deficiency anemia (IDA) induce oxidative stress in the body and cause an imbalance between reactive oxygen species and antioxidants.¹⁴

The World Health Organization defines anemia as a level of Hb below 13.0 g/dL in male adults, below 12.0 g/dL in female adults who are not pregnant, and below 11.0 g/dL in pregnant women. Hb levels may vary across age and race, so care must be taken, particularly in the interpretation of borderline values. Furthermore, smokers and inhabitants of higher altitudes may have higher baseline Hb levels, and participation in endurance sports may alter Hb levels. The amount of circulating iron bound to transferrin is reflected by the serum iron level. The serum iron reference range is 55–160 µg/dL in men and 40–155 µg/dL in women.^{15, 16, 17, 18}

Subgingival irrigation was done using iodine. Elemental iodine or its derivatives [polyvinylpyrrolidone-iodine complex (PVP-iodine)] are probably the most broad-spectrum and potent antiseptics available. Dilute PVP-iodine may be able to kill *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and other periodontal pathogens in vitro in as little as 15s of contact and bacteria and yeasts in vivo within 5 min of contact.^{19, 20, 21, 22}

Materials and methods

Subjects were selected from patients treated at the University Dentistry Clinical Center of Kosovo in Prishtina, included 63 participants suffering from Iron Deficiency Anemia only, followed by patients with IDA and any concomitant chronic condition (Diabetes Type II, Rheumatoid Arthritis, Ulcerative Colitis or Celiac, COPD, Disease Cardiovascular Disease, etc.) and control group consisted of subjects with Chronic Periodontitis. (from October 1st, 2016 until March 31st, 2017).

All participants suffering from Iron Deficiency Anemia are selected based on the International Classification of Disease, 9th Revision [ICD – 9 – CM] (D50.-). Patients suffering from Chronic Periodontitis were divided into three groups, suffering from IDA (Group A, 21 subjects), IDA and Chronic Disease subjects (Group B, 21 subjects) and the control group with normal iron values suffering from chronic periodontitis (Group C, 21 subjects).

Inclusion criteria for participation of potential subjects in this study are:

- patients with IDA
- diagnosed chronic periodontal disease
- age 35 – 80, and
- having at least 20 remaining natural teeth were considered for the study.

Exclusion criteria are:

- no past or present history of malignant disease, particularly hematological malignancies
- patients who had hemolytic anemia or defined deficiency of vitamin B12 or folic acid
- not taking medication known to influence periodontal status, no past or present iron therapy
- not having history of any periodontal treatment in past 6 months.

In the beginning of the study data records for social status and smoking habit are noted. All patients who had history of smoking were heavy smokers (>20 cigarettes/day).

Community Periodontal Index of Treatment Needs (CPITN) as an epidemiological screening procedure for periodontal treatment needs in populations was presented and described by World Health Organization since 1978, named WHO 621 ("Trinity", with the Score 0 – 4).

Criteria for classifying Debris:

- 0 - No debris or stain present

1 - Soft debris covering not more than one third of the tooth surface, or presence of extrinsic stains without other debris regardless of surface area covered

2 - Soft debris covering more than one third, but no more than two third, of the exposed tooth surface.

3 - Soft debris covering more than two thirds of the exposed tooth surface.

Loe-Silness Gingival Index (GI) was utilized for four areas of the tooth then summed and divided by four to give the GI for the tooth, later adding the values of each tooth and dividing by the number of teeth examined, to assess the prevalence and severity of gingivitis in populations, groups and individuals.

Score 0 = Normal Gingiva

Score 1 = Mild inflammation - slight change in color, slight edema. No bleeding on probing.

Score 2 = Moderate inflammation – redness, edema, glazing. Bleeding on probing.

Score 3 = Severe inflammation – marked redness and edema, ulceration. Tendency toward spontaneous bleeding.

Clinical Attachment Level (CAL) - measured clinically from the base of the pocket – P

D to the cement-enamel junction - CEJ (in millimeters), using the formula $PD + CEJ = CAL$.

Measurements at baseline for OHI-s, GI and CAL are done using dental mirror and scaled periodontal probes. After baseline measurements, clinical parameters are noted and later all participants underwent a supragingival and subgingival full-mouth scaling and root planning (FRP), using hand instruments and ultrasonic devices under local anesthesia (SCANDONEST, Mepivacaine HCl 2%, Levonordefrin 1:20000, Septodont, USA). Pocket irrigation was done with 10% Povidon Iodine Solution, three times in 10 minutes (Viodin 10%, SQUARE Pharmaceuticals, Dhaka, Bangladesh), followed by copious irrigation with normal saline and adequate aspiration. Later, on the gingival part of the affected side LLLT was applied.

LLLT adjusted to (660 nm, 10mW, 8 min/daily, in contact with gingiva); model (Hager&Werken Laser HF "confort" V023-17, Duisburg, Germany) continuously in the next five days.

Blood samples were taken at baseline and at 2-month recall visit. Two milliliters of blood were collected using 22-gauge needle and 2 ml syringes, and immediately transferred to the

University Clinical Center of Kosovo Central Laboratory Department. The hemoglobin (Hb) and serum level of iron (SI) estimation was done by in a fully automated analyzer (Hitachi 911 Analyser, Hitachi Medical Corporation, Tokyo, Japan).

Results

As far as the gender distribution between groups, it is evident that male subjects are dominant $X(m)=39; n=63$ in all groups ($Chi=0.28; p>0.05$) (Table 1). There was no significant difference regarding age discrepancy among subjects ($F=11.6; p<0.01$). IDA patients groups (A and B) have more tobacco users compare to overweight/obese group 40.9% ($Chi=0.31, p>0.05$) (Table 2).

Variables	N=21 for each group			Test
	Group A	Group B	Group C	
Gender, n (%)				
Female	8 (38.1)	7 (33.3)	9 (42.8)	Chi=0.28, p>0.05
Male	13 (61.9)	14 (66.7)	12 (57.2)	
Age (years)				
Xb ± SD	53.3 ± 8.1	62.2 ± 9.5	64.0 ± 1.3	F=11.6, p<0.01
CV	15.20	15.27	2.03	
Smoking, n (%)				
Yes	9 (42.8)	11 (52.4)	8 (38.1)	Chi=0.31, p>0.05
No	12 (57.2)	10 (47.6)	13 (61.9)	

Xb-average

SD-standard deviation

CV-coefficient of variation applied for age and Hb/IS

Analysis of variance was used for Age and Hb/IS,

Chi square was used for Gender and Smoking

Table 1. Demographic characteristics applied for age and Hb/IS

Compared with baseline measurements, Root Planning and Scaling led to obvious reduction in the means of GI, OHI-s and CAL levels at 2 months follow up in both groups (Table 3). In view of the influence of baseline measurements, the analysis of covariance (ANOVA) was utilized to analyze the differences in periodontal indexes among all groups adjusted for the baseline covariates. The differences in GI, OHI-s and CAL values were statistically significant among groups at 2 months. A p value <0.05 was considered significant.

IDA patients represented in group B showed higher values for PI ($2.8\pm 0.4; CV = 27.8$), GI ($1.9 \pm 0.2; CV = 33.3$) and CAL ($6.1 \pm 1.3; CV = 29.3$). CPITN values are much higher in group B compare to group A and the control group C ($Chi = 17.9; p<0.05$).

Variables	N=21 for each group			Test
	Group A	Group B	Group C	
Plaque Index				
Xb ± SD	2.3 ± 0.5	2.8 ± 0.4	1.7 ± 0.5	F=11.4, p<0.01
CV	21.7	27.8	29.4	
Gingival Index				
Xb ± SD	2.2 ± 0.4	1.9 ± 0.2	1.7 ± 0.6	F=7.1, p<0.01
CV	18.2	33.3	35.3	
Clinical Attachment Level				
Xb ± SD	5.1 ± 1.1	6.1 ± 1.2	3.9 ± 1.1	F=9.0, p<0.01
CV	21.6	29.3	28.2	
CPITN, n (%)				
2 - no pocketing	2 (9.5)	3 (14.1)	2 (9.5)	Chi=19.5, p<0.05
3 - shallow pockets	7 (33.4)	5 (24.8)	11 (52.4)	
4- deep pockets	12 (57.1)	13 (61.1)	8 (38.1)	

Xb-average

SD - standard deviation

CV - coefficient of variation

Chi - square test was used for CPITN.

Table 2. Dental health in all groups.

Comparative data analysis of our measurements showed that root planning and scaling of periodontal pockets resulted in improved results for PI, GI, CAL after three months – compared to baseline measurement outcomes. Meanwhile, Hb and SI levels at baseline and after two months demonstrated weak correlation with periodontal parameters. As far as the impact of baseline measurements, covariance analysis (ANOVA) was utilized to analyze differences in periodontal indexes in both groups adapted for baseline variables. Differences of PI, GI and CAL values were statistically significant between groups after two months. Whereas, p value <0.05 is considered significant.

There is no correlation between Hb and IS levels and tooth indexes, for both baseline and follow up measurements.

There is evidence that, for baseline measurement, there is moderate correlation between Hb and IS in addition to plaque index ($r = 0.45, p < 0.01$), gingival index ($r=0.39, p<0.01$) and clinical attachment level ($r=0.49, p<0.03$).

There is weak correlation (between groups), for follow up measurement, between Hb and IS, and plaque index ($r = 0.35, p < 0.01$), gingival index ($r=0.32, p<0.05$), while the correlation is moderate between Hb and IS, and clinical attachment level ($r=0.49, p<0.01$).

Measurement time (N=63)	Plaque Index	Gingival Index	Clinical Attachment Level
Baseline			
Hemoglobine (Hb g/dL)	0.45	0.39	0.49
Iron in Serum (IS µg/dL)	8.9 ± 1.3 (49±14)	-0.01	-0.03
Follow up			
Hemoglobin (Hb g/dL)	0.35	0.32*	0.49
Iron in Serum (IS µg/dL)	9.1 ± 1.5 (54±9)	-0.005	-0.15

Table 3. Pearson Correlation between blood parameters and tooth indexes (r), p<0.01

Discussion

Some literature data are confirming that presence of periodontal disease in patients suffering from IDA can be a potential threat for health. Worsened periodontal status has the potential to make worse the primary disease.^{23, 24, 25, 26}

Chakraborty et al., in their original research paper stated that both chronic periodontitis (CP) and iron deficiency anemia (IDA) induce oxidative stress in the body and cause an imbalance between reactive oxygen species and antioxidants, such as superoxide dismutase (SOD), IDA-CP patients exhibited a higher gingival index, bleeding on probing, probing pocket depth, and percentage (%) of sites with a clinical attachment loss (CAL) of ≥6 mm (P<0.008) than CP patients. The mean salivary and serum SOD levels were significantly lower in the IDA-PH, CP, and IDA-CP patients than in the CG group (P<0.008). A significant positive correlation between salivary and serum SOD activity was observed in IDA (P<0.05); IDA patients with chronic periodontitis have more periodontal breakdowns than patients with chronic periodontitis. Serum and salivary SOD activity levels were lower in the IDA-PH, CP and IDA-CP groups than in the CG. Iron deficiency anemia influenced the serum SOD activity but did not seem to affect the salivary SOD activity in these patients.²⁷

Khan et al., found that a significant negative correlation was observed between RBC, hemoglobin, PCV, MCV, MCH, and MCHC; data analysis showed a statistically significant decrease in red blood cell parameters with increase in different grades of periodontitis.²⁸

The risk evaluation of specific biomarkers

was also studied in our institution by Sllamniku et al, her results demonstrated that periodontal disease is associated with increased circulating concentrations of TNF- α, IL-1β, IL-6 and hs-CRP.²⁹ Another study conducted by Anumolu et al., revealed a decrease in Hb and erythrocyte counts and increase in white blood corpuscles counts in chronic periodontitis when compared to healthy controls and chronic generalized gingivitis group, on the other hand there was no statistically significant difference in MCV, MCH, MCHC, and ESR among the groups.³⁰

In a 6 month follow up study Patel et al., in 50 patients had healthy periodontium and 50 patients had chronic periodontitis. Clinical parameters and red blood cell parameters of all the patients were assessed at baseline and 6 months after non-surgical periodontal therapy; from the presented study, it can be concluded that like any other chronic condition, chronic periodontitis can lead to Anemia of Chronic Disease (ACD). It also provides evidence that non-surgical periodontal therapy can improve the anemic status of patients with chronic periodontitis.³¹ On the other hand, significant increase of Matrix Metalloproteinase 9 after short term LLLT in the GCF was noted in a study conducted by Ismaili et al, and furthermore this result could be associated with remodeling of extracellular matrix and stimulation of the regenerative processes.³²

The findings from the clinical and hematological trial conducted by Kolte et al., confirmed the understanding that chronic infections such as periodontitis have systemic effects in terms of blood parameters, indicating anemia. The mean erythrocyte count for the control group was 4.32 ± 0.61 million/mm³, whereas the value for the test group was 4.09 ± 0.56 million/mm³ (P value 0.04). This decrease in the mean erythrocyte count was found to be statistically significant. Similarly, the MCHC percentage was found to be 31.64 ± 1.16 in the control group and 31.12 ± 1.35 in the test group. This decrease in the MCHC percentage was also found to be statistically significant (P value 0.03).³³

Thought-provoking results were obtained in another representative study by Gunay et al, exploring periodontal status and total antioxidant status (TAS), ferritin levels of serum and gingival cervical fluid (GCF) in patients with thalassemia major. The systemic and local ferritin level of the

thalassemia major patient groups was seen to be high compared to the control group (healthy patients), and moreover no relationship was determined between periodontal parameters, which leads to the conclusion that TM is not a risk factor associated with periodontal disease.³⁴

In another study performed by Prakash et al, showed that periodontal parameters were significantly higher ($P \leq 0.05$) in periodontitis patients. Except for the Erythrocyte Sedimentation Rate (ESR), which was significantly higher ($P = 0.03$) in the mild periodontitis group than the control group, hematological and biochemical parameters were not significantly different ($P > 0.05$) among the study groups or between the control and study groups.³⁵ Associations between periodontal status and rheumatoid arthritis using comparative cross sectional study was completed by Suhaimi et al, demonstrating that there was significantly higher number of tooth loss in Rheumatoid Arthritis (RA) patients compared to non-RA ($p = 0.011$). Tooth loss was significantly correlated with age of the subjects ($r = 0.630$; $p = 0.0001$) and duration of RA ($r = 0.457$; $p = 0.009$). No significant difference found for periodontal parameters in between two groups (control group), in conclusion the authors using limited data demonstrated that rheumatoid arthritis may indirectly influence tooth loss.³⁶

Correlation between stress-genic factors and periodontal disease was thoroughly studied in many research papers, Masulili et al investigated the relationship between academic stress with periodontal status and level of cortisol hormone, interleukin-1 β (IL-1 β) and interleukin-6 (IL-6) in gingival cervical fluid. In addition, this study was measured perceived stress and used The Dental Environment Stress (DES) and The Graduate Dental Environment Stress (GDES) questionnaire; periodontal condition using modified Russel periodontal index, and examined the levels of hormone cortisol, IL-1 β and IL-6; at the dental profession students group the relationship between academic stress level to cortisol level showed significant differences ($p = 0.025$), meanwhile IL-1 β and IL-6 showed no significance.³⁷

Smoking and tobacco consumption, to add on, further comprise a major environmental factor for periodontal disease. the mean RBC count in smokers came out to be 3.98 ± 0.18 while in nonsmokers came out to be 4.62 ± 0.27 .

Mean PCV level in smokers was 35.44 ± 1.45 and in nonsmokers was 40.54 ± 1.50 , while mean MCV level in smokers was 85.41 ± 5.34 and in nonsmokers was 85.32 ± 5.68 . MCV levels are the main determinants of some kinds of anemia.³⁸ In another comparable study related to smoking and periodontal disease Eid et al, evaluated the influence of smoking habit on the success of periodontal regeneration in treating intra-bony periodontal defects when using Platelet Rich Plasma (PRP) combined with Freeze Dried Bone Allograft (FDBA). Based on their results, clinical improvement, the amount of bone gain and the level of PDGF-BB were more in the non-smokers group, and consecutively smoking can impair the healing outcomes of periodontal regeneration.³⁹

Conclusions

Within the limitation of this study, it can be concluded that Comparative data analysis of our measurements showed that root planning and scaling of periodontal pockets resulted in improved results for PI, GI, CAL after two months – compared to baseline measurement outcomes. Meanwhile, Hb and SI levels at baseline and after two months demonstrated weak correlation with periodontal parameters.

Declaration of Interest

The authors report no conflict of interest.

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Determination of Microbiological Salivary Status in Children with Upper Respiratory Tract Disorders

Valë H Hoxha¹, Agim Begzati², Vlorë H Cakolli³

ABSTRACT

Aim: The purpose of this paper is to investigate the prevalence of dental caries and the types of streptococci mutant and *Lactobacillus* in the saliva of children with middle ear infection (MEI), enlarged tonsils (ET), and healthy children.

Materials and methods: A sample of 93 subjects (57 males and 36 females; mean age: 8.1 ± 1 years, aged between 5 years and 15 years old) were assessed: 35 patients, with MEI, and 26 with ET, formed the study group, while 32 healthy subjects formed the control group. The participants were interviewed regarding demographic data, dietary, and oral hygiene habits. Their dental caries and oral hygiene status were clinically determined, assessing diagnostic criteria for decayed, missing, and filled teeth for primary (dmft) and permanent (DMFT) dentition, oral hygiene index according to Greene-Vermillion, and the gingival index (GI) according to Silness-Löe. Stimulated salivary flow rate, and salivary mutans streptococci (MS) and lactobacilli (LB) were also determined.

Results: The groups were similar with respect to gender, using fluoride paste, visits to dentist, family income ($p > 0.05$), whereas there was a difference in age, educational level, method of feeding, use of pacifier, sweet eating habits, toothbrushing, saliva secretion, and rate of oral breathing ($p < 0.001$) between intervention and healthy group. However, higher salivary MS and LB levels were observed in MEI, and ET patients compared to controls ($p < 0.05$).

Conclusion: Our results demonstrated that patients with ET and MEI had more initial caries, gingival inflammation, dental plaque, a lower stimulated salivary secretion rate, and an increase in the level of salivary MS and LB than healthy individuals.

Clinical significance: Results from our study will help not only the pediatric dentistry specialists but also the other medical professionals like pediatricians, ENT specialists, and nurses to increase their awareness regarding the importance of the overall dental health in children with an ear infection or tonsillitis.

Keywords: Enlarged tonsils, Lactobacilli, Middle ear infection, Mutans streptococci, Oral health.

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INTRODUCTION

The association between poor oral hygiene, periodontal disease, and respiratory disease has been increasingly debated over the past several years.¹ Although the relationship between oral status and pneumonia has been well studied, the association between microorganisms in the oral cavity and upper respiratory tract infections is less clear.² Salivary enzymes associated with periodontal disease may modify the mucosal surfaces along the respiratory tract, thus facilitating colonization by pathogens.¹ Furthermore, the oral cavity is a potential reservoir for respiratory pathogens.³ Middle ear infections diseases of the ET and dental caries are highly prevalent around the globe and are the most commonly diagnosed childhood illnesses.^{4,5} Dental caries in childhood is still a major public health challenge in most industrialized and developing countries.⁶ Previous studies on dental caries demonstrated the role of salivary MS as a predictor and marker for caries risk.⁷ High level of MS in the saliva tends to contribute early colonization of these organisms in children. Children colonized by MS at an early stage developed more caries than those colonized at a later stage.⁸

The regular intake of fermentable dietary sugars, or impaired saliva flow, produces persistent conditions of low pH within the biofilm, which selects for MS and LB.⁹ Common microbiota of the oral space, MS and LB are accountable for variety of oral illnesses such as caries and periodontitis.¹⁰ Xerostomia is a common symptom and side effect of respiratory drug therapies that reduce buffering capacity and remineralization ability, leading to elevated caries risk.¹¹ Increased severity of the disease and higher numbers

^{1,2}Department of Pediatric Dentistry, School of Dentistry, University Hasan Prishtina, Prishtina, Republic of Kosovo

³Department of Periodontology and Oral Medicine, College of Medical Sciences Rezonanca, Prishtina, Republic of Kosovo

Corresponding Author: Vlorë H Cakolli, Department of Periodontology and Oral Medicine, College of Medical Sciences Rezonanca, Prishtina, Republic of Kosovo, Phone: +383 44157885, e-mail: vlora.cakolli@gmail.com

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of therapeutic medications are associated with increased caries prevalence.¹²

The aim of this research was to investigate the oral hygiene habits, gingival condition and dietary habits, salivary levels of MS and LB among MEI, ET, and healthy children.

MATERIALS AND METHODS

Subjects

The study was carried out at the Clinical Center for Pediatric and Preventive Dentistry of the University Dentistry Clinical Center of Kosovo in Pristina, ENT Clinic and the Department of Microbiology of the National Institute of Public Health of Kosovo in the period 2017/2018. The study model is prospective comparative type.

In this research a sample of 93 children with ET group ($n = 35$), middle ear infected group ($n = 26$), and a healthy children group ($n = 32$) were involved, ranging from 5 years to 15 years of age from both genders, males and females.

All of them voluntarily participated in this study and filled out the informed consent form signed by parents. The study was approved by the Research Ethics Committee of the University of Prishtina.

Inclusion and Exclusion Criteria

Children with ET and otitis media were included in the study group, but not with other health problems, chronic or acute diseases present. Children in the control group—healthy subjects were also without chronic or acute illnesses.

The study did not include patients with acute health problems of the cardiovascular system, acute and chronic pulmonary disease, gastrointestinal system disorders, metabolic disease, kidney failure, blood discrepancy, and those who did not sign the patient consent form.

All children who participated in the study were examined with dental instruments for examination using artificial light seated in dental chair.

Before dental treatment, a diagnosis of MEI and ET was based on the physical examination by an otolaryngologist (ENT specialist).

For this paper, a special study questionnaire was created, which contains:

The general (demographic) data of the patient [name and surname, gender, age, child's residence and complete medical history, education, familial economic status, nutritional background (breastfeeding vs dietary milk intake), hygiene habits (tooth brushing practices, usage of pacifier, fluoride therapy, oral breathing, and the extent of the saliva secretion)]; were acquired from their parents using a questionnaire.

Determination of dental status was done utilizing the WHO (World Health Organization) criteria¹³ by means of decay-filled teeth (dft) for primary teeth and the decay-missing-filled teeth (DMFT) index for permanent teeth. Furthermore, the DMFT score for any subject may range from 0 to 32, followed by the dft score, which may range from 0 to 20 in total numbers.¹⁴

Gingival index, according to Loe-Silness,¹⁵ has been used to evaluate the gingival status (changes in color, size, and bleeding). The evaluation is done using the exploratory probe, after drying the dental surfaces, with compressed air. All necessary instruments were sterilized before the examinations. The GI values are marked 0 to 3 where: 0—healthy sound: the gingiva has a light pink color, with grain structure, the papillae are in the interdental space, and no signs of the protrusion. The shape of the papilla depends on the shape of the tooth; 1—light inflammation: the gingival lining is slightly reddish, has slight edema, and increased gingival exudate. The gingiva does not bleed in the probe with a dental instrument; 2—average inflammation: the gingiva is reddish, it has expressed edema and increased gingival limbus, and there is bleeding after the probe, and 3—severe inflammation: the gingiva is very reddish and grown.

For the purpose of this study, oral hygiene status was assessed using the Greene and Vermillion Simplified Oral Hygiene Index (OHI-S).¹⁶ The six surfaces of the four posteriors and two anterior teeth were stained with basic fuchsin and examined later. In the posterior portion of the dentition, the first fully erupted tooth, usually the first molar (No. 16), is examined. The buccal surfaces of

the selected upper molar and the lingual surfaces of the selected lower molar were evaluated. In the anterior region of the mouth, the central incisors, specifically the labial surfaces of the upper right incisor, No. 11, and the lower-left incisor, No. 31, were scored. OHI-S values may range from 0 to 3 as described: OHI-S 0 = no debris or pigment present. OHI-S 1 = soft residue covering no more than one-third of the tooth surface or the presence of surface stains with no other debris, irrespective of the affected area covered; OHI-S 2 = soft residue overlapping more than one-third but not more than two-thirds of the exposed tooth surface. OHI-S 3 = soft residues covering more than two-thirds of the exposed tooth surface. Results of 0-1 are presented as low, and 2-3 as a high oral hygiene index (OHI-S).

Microbiological Methods for the Identification of Bacterial Species

After answering the questionnaire, clinical saliva samples were then collected from the children.

The saliva stimulated paraffin tablet was given to children who had to chew for at least 5 minutes and then collected all the saliva created in a scaled glass. After the saliva was collected, the amount of saliva was recorded as the rate of saliva flow per minute (mL/minute). Subsequently, a small amount of each saliva sample was used to count bacteria through caries risk test (CRT) (Ivoclar Vivadent Clinical, Schaan, Liechtenstein).

Bacteria Count

From the scaled glass, filled with saliva from children, a portion of the saliva sample was used to disperse into the culture medium (agar) through pipettes. A NaHCO_3 tablet was placed in the culture agar dish to promote bacterial growth and then stored in an incubator at 35–37°C for 48 hours (Fig. 1A). Later, the results obtained were evaluated (Fig. 1B). Streptococci mutant (SM) colonies were presented as small blue groups on blue agar, <1 mm in diameter (Fig. 1C), while LB colonies were presented as white colonies on translucent agar (Fig. 1D). The presence of a bacterial count higher than 10⁵ colony forming units (CFU)/mL of saliva specifies an increasing risk of emerging tooth decay. Accordingly, in this research, subjects were separated as SM and LB sums > or <10⁵ CFU/mL.¹⁷

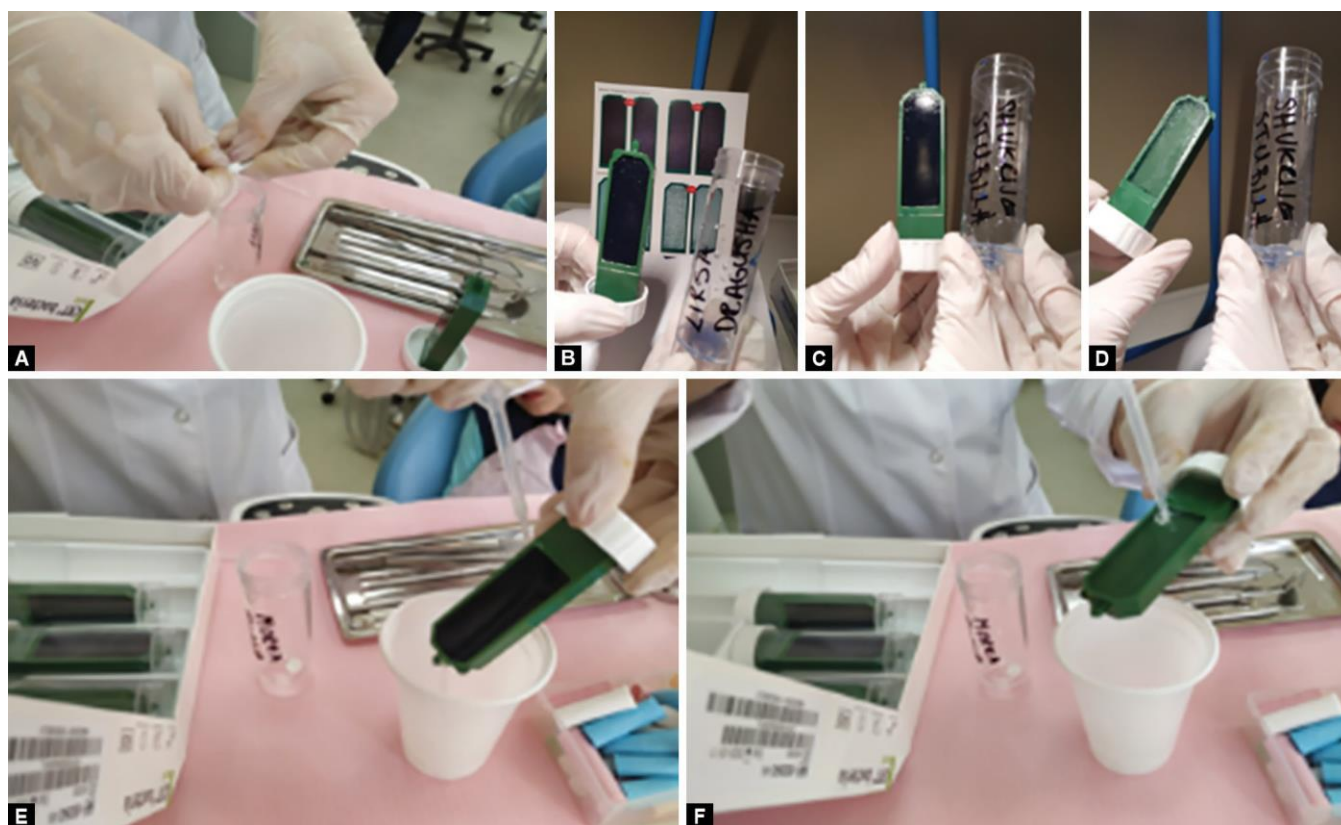
We used in total 93 both sided CRT Intro Pack-CRT agar plates. Blue agar surface: determination of MS count in saliva (Fig. 1E), bright agar surface: determination of LB count in saliva (Fig. 1F). In order to carry out this study, for the purpose of screening/evaluation, four examiners are included.

Statistical Analysis

Continuous variables were summarized as mean and standard deviation (SD), and the categorical variables were summarized as frequency (n) and percentages (%). In the statistical analysis, differences between continuous variables were tested with the one-way ANOVA and differences between categorical variables with the chi-square test (χ^2). All statistical analyses were performed using the Statistical Package for Social Science SPSS 22.0. Statistical significance was established at the $p < 0.05$ level.

RESULTS

From the total number of children involved in the study, 35 were children with ET, 26 with MEI, and 32 were healthy children as a control group. The study included 93 children, of whom 57 (61.3%) were boys and 36 (38.7%) girls. There was no significant difference



Figs 1A to F: (A) Placement of NaHCO_3 tablet in the dish of the culture agar; (B) Mutans streptococci CFU/mL saliva reading; (C) Mutans streptococci colony after incubation; (D) *Lactobacillus* colony after incubation; (E) Dispersion of the saliva sample through the culture media (agar) for mutans streptococci using pipettes; (F) Dispersion of the saliva sample through the culture media (agar) for lactobacilli using pipettes

between groups in terms of gender ratios ($p = 0.504$), but with a statistically significant difference between age in the three groups ($p < 0.001$) (Table 1). The children included in our research were between ages 5 years to 15 years. The average age of children with ET group was 7.8 years ($\text{DS} \pm 2.62$ years), with MEI was 5.7 years ($\text{DS} \pm 1.15$ years), while those of the control group was 10.6 years ($\text{DS} \pm 2.05$ years) (Table 1).

In addition, there were no significant differences between the groups in the variables, such as using fluoride paste, visits to dentist, family income, ($p > 0.05$), with the exception of family education level, method of feeding, use of pacifier, sweet eating habits, toothbrushing, saliva secretion, and rate of oral breathing ($p < 0.001$) (Table 1).

After the evaluation of the percentage of patients with SM $<$ or $> 10^5$ CFU and LB $<$ or $> 10^5$ CFU and the comparison between groups are noted that group GI has been found to have more streptococci compared to GII and the control group, whereas GII has been found to have less LB compared to the other two groups (Table 2).

The statistical analysis shows a significant difference in the OHI-S index between groups. More cases with the high oral hygiene index score were detected among children with ET (Table 3).

The statistical investigation demonstrated an expressively higher Löe-Silness GI in the study group, especially in the ET group. The GII group has been found to have more cases with 0 score of Löe-Silness GI, both for primary as well as for permanent dentition compared to other groups (Table 4).

DISCUSSION

This comparative observational study aimed to evaluate the oral health, salivary flow, and specific amount of *Streptococcus mutans* and LB in saliva between the groups with MEI, and with ET (control groups) and the healthy children group. In terms of outcomes, control groups appear to be more likely to develop a higher OHI-S and Löe-Silness GI, compared to the healthy group. Moreover, the same groups have a significantly higher risk of developing *S. mutans* and *Lactobacillus*, $> 10^5$ CFU, compared to the healthy group. Also, statistical differences were distinguished for the salivary flow rate, between healthy cases, and control groups.

There are different views in the literature on the role of socioeconomic status and family educational level in oral and microbiological health.

Our study showed a significant difference between subjects in terms of socioeconomic status and family educational level regarding oral and microbiological health.

At the same time, different authors^{4,7,14,16} reported the impact of family education, and socioeconomic status in oral health, and prevalence of dental caries while in the opposite, a study by Bakhshae et al.¹⁸ report that there were no discrepancies amongst the study groups, in terms of socioeconomic status, while there was a difference between the DMFTs. This is probably because of drug therapies for allergic rhinitis, which decreases salivary flow rate and may play a role in DMFT.^{19,20}

In our study, children with ET mostly oral breathers, have had higher plaque index compared with the other two groups, our

Table 1: Demographic, nutritional, and hygiene habits data in research groups

	<i>Intervention GI ET, n (%)</i>	<i>Intervention GII MEI, n (%)</i>	<i>Control group GIII, n (%)</i>	<i>p</i>
Age (mean \pm SD)	7.8 \pm 2.62	5.73 \pm 1.15	10.56 \pm 2.05	<0.0001
Sex				
Male	23 (65.7)	17 (65.4)	17 (53.1)	0.504
Female	12 (34.3)	9 (34.6)	15 (46.9)	
Family education level				
Primary school	10 (28.6)	10 (38.5)	2 (6.3)	0.003
High school	21 (60.0)	13 (50.0)	17 (53.1)	
Higher education level	4 (11.4)	3 (11.5)	13 (40.6)	
Family income				
<200	4 (11.4)	4 (15.4)	0 (0.0)	0.010
200-450	20 (57.1)	19 (73.1)	13 (40.6)	
>450	11 (31.4)	3 (11.5)	19 (59.4)	
Using fluoride paste				
Yes	9 (25.7)	6 (23.1)	11 (34.4)	0.683
No	20 (57.1)	14 (53.8)	13 (40.6)	
Do not know	6 (17.1)	6 (23.1)	8 (25.0)	
Teeth brush				
Once a day	15 (42.9)	16 (61.5)	14 (43.8)	0.003
2-3 per day	14 (40.0)	3 (11.5)	17 (53.1)	
Few times in the week	6 (17.1)	7 (26.9)	1 (3.1)	
Visits to dentist				
Once in 6 months	2 (5.7)	1 (3.8)	1 (3.1)	0.267
Once in the year	4 (11.4)	0 (0.0)	10 (31.3)	
Only when has pain	29 (82.9)	25 (96.2)	21 (65.6)	
Sweet eating habits				
Often	18 (51.4)	15 (57.7)	24 (75.0)	0.008
Rare	5 (14.3)	3 (11.5)	7 (21.9)	
Sometimes	12 (34.3)	8 (30.8)	1 (3.1)	
Saliva secretion				
Normal	14 (40.0)	15 (57.7)	12 (37.5)	<0.0001
High	10 (28.6)	0 (0.0)	19 (59.4)	
Low	11 (31.4)	11 (42.3)	1 (3.1)	
Method of feeding				
Brestfeeding	10 (28.6)	16 (61.5)	27 (84.4)	<0.0001
Bottle feeding	22 (62.9)	3 (11.5)	2 (6.3)	
Both	3 (8.6)	7 (26.9)	3 (9.4)	
Prolonged pacifier use				
Yes	23 (65.7)	4 (15.4)	17 (53.1)	<0.0001
No	12 (34.3)	22 (84.6)	15 (46.9)	
Mouth breath				
Yes	35 (100.0)	14 (53.8)	0 (0.0)	<0.0001
No	0 (0.0)	12 (46.2)	32 (100.0)	

ET, enlarged tonsils; MEI, middle ear infection; SD, standard deviation

results are in concordance with other authors studies too.^{17,21,22} Furthermore, it appears that the child has greater contact with airflow, during respiration and subsequently, gingival inflammation and irritation may be associated with superficial dehydration and decreased salivary flow. Also, a study by Mehta et al.²² stated that children who breathe orally appear to be predisposed to form the highest level of dental plaque.

Meanwhile, in our study, the group with tonsillar hypertrophy demonstrated a significant difference compared to healthy group as far as OHI-S index values.

In a study by Eryaman et al.⁵ conducted in Turkey amongst 80 children aged 3-12 years (38 in the intervention group and 42 in the control group), the correlation between poor oral hygiene and tonsillar hypertrophy dmft index was analyzed for

Table 2: Microbiological status of colonies of *Streptococcus mutans* and *Lactobacillus* in research groups

	Intervention GI ET, n (%)	Intervention GII MEI, n (%)	Control group GIII, n (%)	p
<i>Lactobacillus</i>				
CFU/mL <10 ⁵	2 (7.4)	20 (76.9)	4 (12.9)	<0.0001
CFU/mL >10 ⁵	25 (92.6)	6 (23.1)	27 (87.1)	
<i>Streptococcus mutans</i>				
CFU/mL <10 ⁵	13 (37.1)	22 (84.6)	17 (53.1)	0.001
CFU/mL >10 ⁵	22 (62.9)	4 (15.4)	15 (46.9)	

ET, enlarged tonsils; MEI, middle ear infection; CFU, colony-forming unit

Table 3: OHI-S status of permanent dentition in research groups

	Intervention GI ET, n (%)	Intervention GII MEI, n (%)	Control group GIII, n (%)	p
OHI-S permanent dentition				
0	7 (20.0)	22 (84.6)	3 (9.4)	<0.0001
1	9 (25.7)	1 (3.8)	23 (71.9)	
2	13 (37.1)	3 (11.5)	5 (15.6)	
3	6 (17.1)	0 (0.0)	1 (3.1)	

ET, enlarged tonsils; MEI, middle ear infection; OHI-S, oral health index-simplified; the scores of 0-1 were classified as low, and of 2-3 as high oral hygiene index (OHI-S)

Table 4: Löe-Silness gingival index status of primary and permanent dentition in research groups

	Intervention GI ET, n (%)	Intervention GII MEI, n (%)	Control group GIII, n (%)	p
Primary dentition Löe-Silness GI				
0	9 (25.7)	19 (73.1)	14 (43.8)	0.014
1	19 (54.3)	6 (23.1)	15 (46.9)	
2	6 (17.1)	1 (3.8)	3 (9.4)	
3	1 (2.9)	0 (0.0)	0 (0.0)	
Permanent dentition Löe-Silness GI				
0	7 (20.0)	22 (84.6)	4 (12.5)	<0.0001
1	15 (42.9)	1 (3.8)	23 (71.9)	
2	11 (31.4)	3 (11.5)	5 (15.6)	
3	2 (5.7)	0 (0.0)	0 (0.0)	

ET, enlarged tonsils; MEI, middle ear infection; GI, gingival index; the scores of 0-1 were classified as low, and of 2-3 as high Löe-Silness gingival index (GI)

the primary dentition, DMFT index for permanent teeth, and the OHI-S index. Results of this research have demonstrated a strong correlation between poor oral hygiene and tonsillar hypertrophy.⁵ These findings state that the dental pathogens, *S. mutans*, and *Streptococcus sobrinus*, may persuade the immunologic response progression in the tonsils, and accordingly, the question is would the chronic incentive of poor oral hygiene cause hypertrophy of the tonsils.⁵

The current research concludes that in comparison to healthy controls, children with MEI are reported to have higher OHI-S index, Löe-Silness GI, increased mean CFU of SM and LB, especially in the primary dentition.

Whether there is a correlation between oral health and dental caries in children with MEI has also been investigated by some authors.²³⁻²⁵ Kashyap et al.²³ demonstrated that the risk of early childhood caries was found to be significantly higher in children who were diagnosed with MEI. In their study, the group with middle ear infected children showed a mean CFU of 5.6013, which differed significantly (p value = 0.001) from noninfected children group. On the contrary, authors Eryaman et al.²⁴ and Nelson et al.²⁵ conclude from their clinical study that there was no statistically significant association between oral hygiene and the MEI.

They did not find any difference between the group with high OHI-S and low OHI-S in connection with present MEIs.

The results of our study are consistent with previously published

works²⁶⁻²⁹ concerning the relationship between poor oral hygiene and MS and LB colonies. Meanwhile, in a study carried out by Zhou et al.,²⁷ who found that colonization of MS in 8-32-month-old children was associated with poor oral hygiene, while Köhler et al.²⁸ stated that earlier colonization by MS in young children generally translates into increased caries rates. Also, in a study done by Köll-Klais et al.,²⁹ a positive correlation was found between the DMFS counts, and the LB counts in Estonian schoolchildren.

Limitations of the Study

A few limitations should be noted. The main weakness of this paper was the short duration of the study, along with the small number of patients, factors that might have had an effect on the outcome. Another disadvantage is that we only identified the cases we did not follow their treatment outcomes.

CONCLUSION

Our results demonstrated that patients with ET and MEI had more initial caries, gingival inflammation, dental plaque, a lower stimulated salivary secretion rate, and an increase in the level of salivary MS and LB than healthy individuals. The future study should be done in a large sampling and should be undertaken in order to obtain relevant data regarding the treatment of children with upper respiratory infections and poor oral health.

CLINICAL SIGNIFICANCE

Results from our study will help not only the pediatric dentistry specialists but also the other medical professionals like pediatricians, ENT specialists, and nurses to increase their awareness regarding the importance of the overall dental health in children with an ear infection or tonsillitis.

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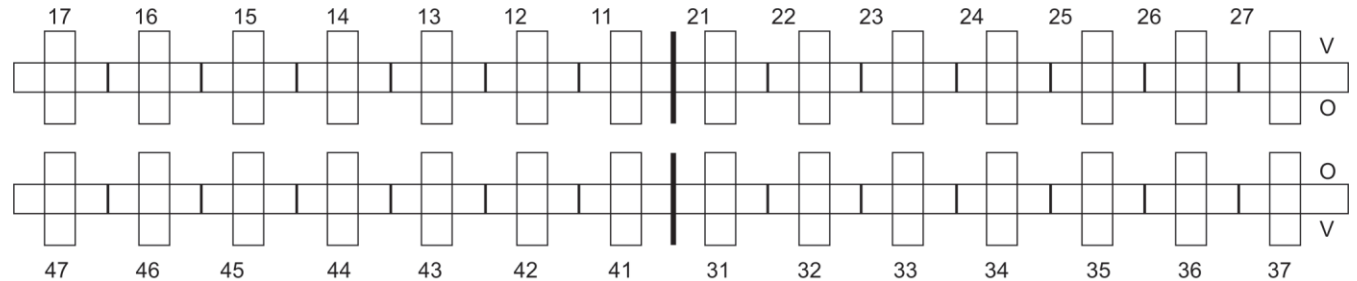
QUESTIONNAIRE FOR PARENTS AND CHILDREN DENTAL EXAMINATION

1. General data

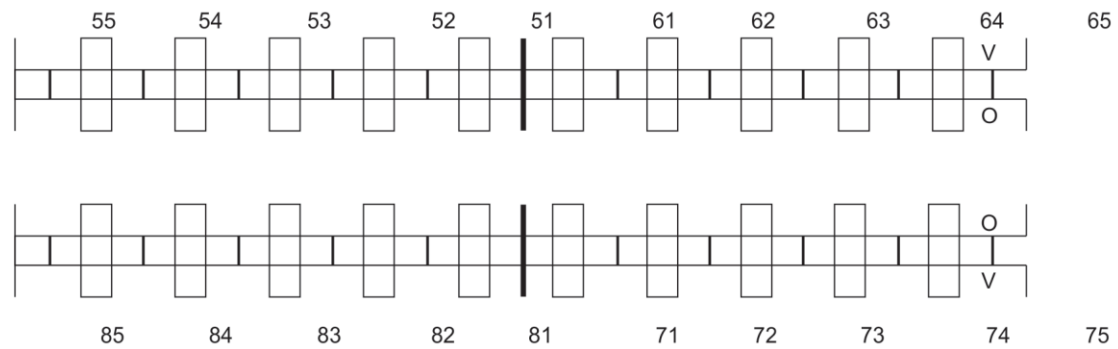
Name _____
 Surname _____
 Date of birth _____
 Age _____
 Gender (M = 1, F = 2) _____
 Code _____
 Living place (1 = Urban, 2 = Rural) _____
 Other _____

2. Dental/oral examination

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
			55	54	53	52	51	61	62	63	64				
48	47	46	85	84	83	82	81	71	72	73	74	75	36	37	38
			45	44	43	42	41	31	32	33	34	35			
dmft					d			m				f			
DMFT					D			M				F		=	



Gingival index-permanent dentition (Löe-Silness) _____

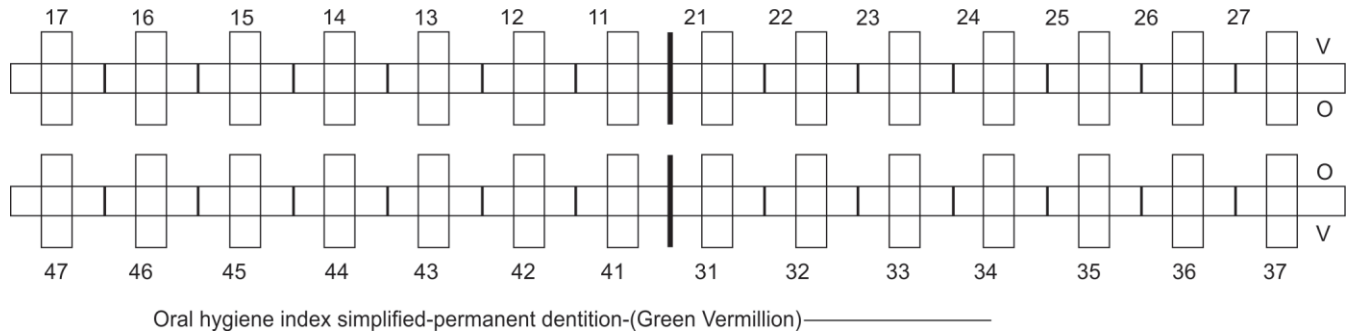


Gingival index-primary dentition (Löe-Silness) _____

Contd...

Microbiological Identification of Saliva in Children

Contd...



Sagittal direction disarrangement:

I	II	III
---	----	-----

Transversal direction disarrangement: PO JO

Vertical direction disarrangement:

Open	Deep
------	------

Streptococcus mutans 0 1 2 3

Lactobacillus 1 2 3 4

Sweet eating habits?

Often	Yes	No
Rare	Yes	No
Sometimes	Yes	No

Saliva secretion

Normal	Yes	No
High	Yes	No
Low	Yes	No

Method of feeding?

Breastfeeding	Yes	No
Bottle feeding	Yes	No
Both	Yes	No

Prolonged pacifier use?

Yes
No

Mouth breath?

Yes
No

Note:

Family education level?

Primary school	Yes	No
High school	Yes	No
Higher education level	Yes	No

Family income?

<200 Euro	Yes	No
200-450 Euro	Yes	No
>450 Euro	Yes	No

Using fluoride paste?

Yes
No
Don't know

Teeth brush

Once a day	Yes	No
2-3 per day	Yes	No
Few times in a week	Yes	No

Visit to dentist?

Once in six months	Yes	No
Once in the year	Yes	No
Only when has pain	Yes	No

Examination date:

Place:



Detection of Bacteria in the Dental Plaque in Children with Down Syndrome

Vlorë Hysenaj Cakolli¹, Sefedin Muçaj², Alberto Benedetti³, Valë Hysenaj Hoxha^{4*}

1. College of Medical Sciences "Rezonanca" - Prishtina, Republic of Kosovo.
2. National Institute of Public Health of Kosovo - Pristina, Republic of Kosovo.
3. University "St. Cyril and Methodius" - Skopje, Faculty of Dentistry, Department of Maxillofacial Surgery, Republic of Macedonia.
4. University "Hasan Prishtina" - Prishtina, Medical Faculty - School of Dentistry, Department of Pediatric Dentistry, Republic of Kosovo.

Abstract

The purpose of this paper is to investigate the prevalence of dental caries and the types of streptococci present in the dental plaque of children with Dawn Syndrome.

The study was carried out at the Clinical Center for Pediatric and Preventive Dentistry of the University Dentistry Clinical Center of Kosovo in Prishtina and at the Department of Microbiology of the National Institute of Public Health of Kosovo in the period 2016/2017. Determination of dental status was done assessing diagnostic criteria for Decayed, Missing and Filled Teeth for permanent dentition, Oral Hygiene Index according to Greene-Vermillion and the Gingival Index according to Löe-Silness. Two groups are formed consisting of 30 patients in each group, the group with Down syndrome and the group with healthy children. Smear from the dental plaque was microbiologically analyzed using automatic VITEK 2 system and the colorimetric GP stripes.

In 50% of children involved in our research *St. myth* and *St. oral* were isolated, in 21.7% *St. parasanguinis* was isolated, and in 13.3% *St. salivarius*, in 3.3% *St. sanguinis*, in 3.3% *St. Infantarius ssp coli*, in 1.7% *St. pneumoniae* and *St. constellatus ss. Pharyngitis*. In the group suffering from Down syndrome the most frequent isolated bacteria was *St. mitis*, *St. oral* (43.3%), while at the control group *St. parasanguinis* (66.7%).

Oral health status in both groups was poor. Based on groups, in the group suffering from Down Syndrome the most frequent isolated bacteria was *St. mitis*, *St. oral*, while at the control group *St. parasanguinis*.

Clinical article (J Int Dent Med Res 2019; 12(4): 1474-1480)

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Introduction

The Down Syndrome (DS), Trisomy 21 or Mongoloidism represents a chromosomal disorder as a result of genetic mutation, resulting in physical growth delay, intellectual impairment and speech disorders.^{1,2} In Kosovo there is no official statistical data regarding the persons suffering from DS. The affected community with DS in Kosovo in itself represents a specific and sensitive social category. According to the Association of Down Syndrome Kosova, the number of persons with DS in Kosovo is about

600.

Frequent oral abnormalities have been expressed in DS individuals like malformations of the small palate and maxilla, mouth breathing resulting in dry mouth, fissured tongue and lips, delayed tooth eruption, dental agenesis, low incidence of dental caries, high incidence of periodontal diseases, mucosal ulcers, candidiasis, and acute necrotizing ulcerative gingivitis, in contrast with healthy individuals.^{3,4} Patients with DS also have enlarged tongue, occlusal and soft tissues forces misbalance, open bite, impaired mastication and following difficulty in self-cleansing of teeth.^{5,6}

Dental caries represents a multifactorial diseases. A number of causal, environmental and hereditary factors influence the development of tooth decay, including nutrition habits, carbohydrate intake, oral hygiene, cariogenic microorganisms, inadequate fluoride intake,

*Corresponding author:

Valë Hysenaj-Hoxha
Department of Pediatric Dentistry,
School of Dentistry, University Clinical Center of Kosovo,
Prishtina, Republic of Kosovo
Email: vala.hoxha@hotmail.com

alveolar arch anomalies, tooth crowding and poor salivary function.⁷⁻¹¹

Low prevalence of dental caries is one of the most well-known phenomenon among DS patients, regardless of exposure to numerous risk factors, such as a caries inducing diet, impaired salivary flow, mouth breathing, deranged occlusal forces, and deprived oral hygiene. The exact mechanism of this observable fact remains unclear, regardless the reality that the etiology was verified in numerous studies. Some of the hypotheses suggested to explain the low prevalence of dental caries include the following: delayed tooth eruption in combination with an altered chronology of eruption; the high frequency of hypodontia; differences in the composition, pH, and buffering capacity of the saliva and the salivary flow and differences in the cariogenic microbiota.¹²⁻¹⁷

Variety of microorganisms consist of the number of present species (species opulence) and the number of individuals of all species (uniformity). Awareness regarding microbial variety is vital, given that a microbial population may change in terms of the number of individuals per species in response to changing conditions that favor their growth.¹⁸

Two most frequent bacterial species *Streptococcus mutans* and *Streptococcus sobrinus* are strongly related with dental caries. On the other hand, the relationship between oral streptococci and dental caries in children with DS is not well documented. Meanwhile, some studies have demonstrated that the incidence of dental caries is associated with *Streptococcus mutans* counts in children and adolescents with DS, other authors have not found such association.¹⁹⁻²²

The purpose of this paper is to investigate the prevalence of dental caries and the types of streptococci present in the dental plaque of children with DS and to compare the results with the healthy group, to evaluate the degree of oral hygiene and the gingival condition between children with DS and a group of healthy children.

Materials and methods

Subjects

The study was carried out at the Clinical Center for Pediatric and Preventive Dentistry of the University Dentistry Clinical Center of Kosovo in Pristina and the Department of Microbiology of

the National Institute of Public Health of Kosovo in the period 2016/2017. The study model is prospective, comparative type.

All children aged 7 to 15 years, who participated in the study were examined with dental instruments for examination using artificial light seated in dental chair.

Determination of dental status was done according to WHO criteria.²³ For the purpose of this paper, a special study questionnaire has been created, which contains:

- The general (demographic) data of the patient (name and surname, gender, age, child's residence and complete medical history);
- Dental Status
- Gingival Index (GI)
- Dental Plaque Index according to Green-Vermilion (0 - 3)

Microbiological status for the identification of bacterial species:

We have formed 2 groups consisting of 30 patients in each group, the group with Down syndrome and the group with healthy children.

In both groups, dental plaque samples were taken and their microbiological analysis was carried out using Vitek 2 (BioMérieux Industries, 69280 Marcy-l'Étoile - France), utilizing specific method for the identification of bacterial species.

Dental Status

Determination of dental status was done according to the WHO parameters for assessing diagnostic criteria for Decayed, Missing and Filled Teeth for permanent dentition.

Gingival index (GI) according to Löe – Silness²⁴ has been used to evaluate the gingival status (changes in color, size and bleeding). The evaluation is done using exploratory probe, after drying the dental surfaces with compressed air. All necessary instruments were sterilized before the examinations. The gingival index values are marked 0-3 where: 0 - healthy sound: the gingiva has a light pink color, with grain structure, the papillas are in the interdental space and no signs of protrusion. The shape of the papilla depends on the shape of the tooth. 1- Light inflammation. The gingival lining is slightly reddish, has slight edema and increased gingival exudate. The gingiva does not bleed in probe with dental instrument; 2- Average inflammation: the gingiva is reddish, it has expressed edema and increased gingival limbus, and there is bleeding after probe, and 3- severe inflammation: the

gingiva is very reddish and grown.

The *Oral Hygiene Index* (OHI) according to Greene-Vermillion²⁵ determines the presence of dental plaque with a value of 0-3. The evaluation is done using exploratory probe. Value 0- no dental plaque in the third gingival portion of the tooth crown, 1-dental plaque covers less than 1/3 of the tooth surface. It can not be seen with the eye but only when the probe is passed through the surface, leaving a small amount of plaque on the tip of the probe. Value 2- dental plaque has covered more than 1/3, but no more than 2/3 of the tooth surface. And finally, value 3

- a large amount of dental plaque that covers more than 2/3 of the tooth surface. The interdental space is filled with dental plaque.

The index includes only 6 representative teeth:

- the vestibular surfaces of the first maxillary molars of the right and left side (in the absence of them, the second molars or the second premolars were examined), while in the primary teeth dentition vestibular surfaces of the second maxillary molars were used for examination.

- the vestibular surfaces of the permanent right maxillary central incisor and the permanent left central mandibular incisor and the primary incisors in the case of primary dentition (in their absence, the central left maxillary incisor is examined and central right mandibular incisor),

- the lingual surfaces of the first mandibular molars or the second primary molars in the primary dentition.

Microbiological methods for the identification of bacterial species

In both groups smear from the dental plaque was removed using absorbent paper points (Patterson Brand®-1031 Mendota Heights Rd, Saint Paul, MN 55120, USA) and their microbiological analysis was done using the Vitek 2 specific method (BioMérieux Industries, 69280 Marcy-l'Étoile - France) for qualitative identification of bacterial species.

Gram positive cocci are detected using the automatic VITEK 2 system and the colorimetric GP stripes. After 6-8 hours of incubation, the reaction is read automatically. Samples were taken from the dental plaque first on the buccal surfaces of the molars of the lower jaw (primary or permanent dentition), then in the incisors of the upper jaw (primary or permanent

dentition) with sterile absorbent paper point and sent for microbiological analysis.

The samples taken from the dental plaque were first diluted with 0.9% saline and then placed on the Prepared Plates with blood agar. Plates with nourishing agar were placed in the incubator for 24^h at 37°C. After bacterial cultivation, the material from the nourishing agar plate is placed in special glass cups to create a bacterial suspension. The suspension is consisted of mixed material with 0.9% saline in the amount of 2-3 ml. The glass cup consisting the suspension is then connected through a special tube with the GP stripe. Through the tube the material from the cup is transferred to the GP stripe and then the stripe kit is placed on the Vitek 2 apparatus for the identification of bacterial strains.

Inclusion and Exclusion Criteria

In this prospective clinical research, patients with DS are included in the study group, but not with other health problems, chronic or acute diseases present. Patients in the control group - healthy subjects are also without chronic or acute illnesses.

The study did not include patients with acute health problems of the cardiovascular system, acute and chronic pulmonary disease, gastrointestinal system disorders, metabolic disease, kidney failure, blood discrepancy, and those who did not sign the patient consent form. All subjects voluntarily participated in this study and filled out the informed consent form. The study was approved by the Research Ethics Committee of the University of Prishtina.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Science SPSS 22.0. Regarding the included statistical parameters the arithmetic average values, the standard deviation (SD), and the minimum and the maximum obtained values are calculated. In the statistical analysis differences between normally distributed continuous variables were tested with the Student t-test and differences between categorical variables with the chi-square test (χ^2). For the continuous data, Fisher's exact test were applied. Level of statistical significance was set to $p < 0.05$.

Results

The study included 60 children, of whom 34 (56.7%) were boys and 26 (43.3%) girls. From the total number of children involved in the study,

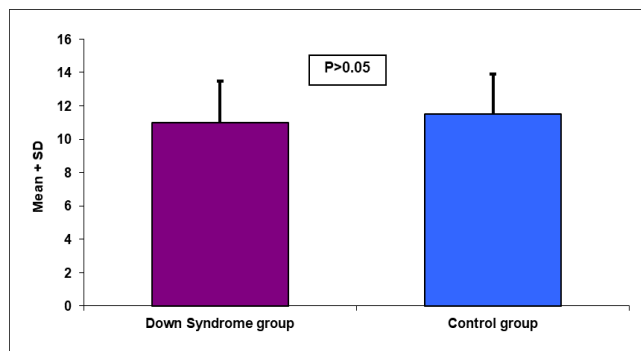
30 were children suffering from DS and 30 were healthy children as a control group. By gender, the group with DS had more boys (66.7%) compared to the control group where more than 53.3% were girls but without any important statistical significance ($P = 0.193$), (Table 1).

Gender	Down Synd. group		Control group		Total	
	N	%	N	%	N	%
Male	20	66.7	14	46.7	34	56.7
Female	10	33.3	16	53.3	26	43.3
Total	30	100.0	30	100.0	60	100.0

X^2 -test, P-value $X^2=1.69$, $P=0.193$

Table 1. Included children based on gender and groups.

The children included in our research were between ages 7 to 15 years. Those with DS were between 7 and 15 years old, while in the control group children ranged from 8 to 15 years (Table 2). The average age of children involved in the research was 11.2 years ($DS \pm 2.4$ years). The average age of children suffering from DS was 11.0 years ($DS \pm 2.5$ years), while those of the control group was 11.5 years ($DS \pm 2.4$ years). T-test did not demonstrate significant statistical significance between mean age among groups (T-test = 0.852, $P = 0.397$), (Graph 1).



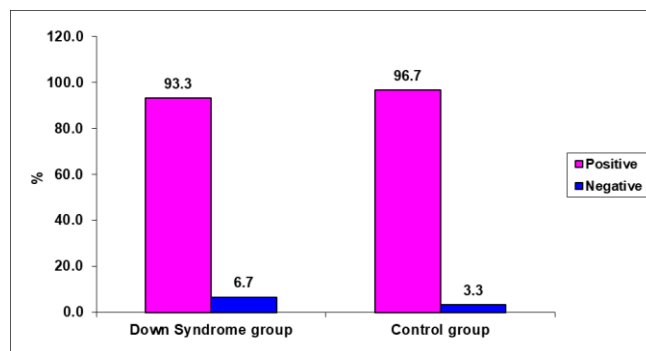
Graph 1. Average age of children included in the study based on group.

Microbiological analysis findings in 93.3% of cases was positive for the DS group, while in 96.7% of positive cases in the control group, but without significant difference ($P = 0.999$), (Table 2 and Graph 2).

Microbiologic Analysis	Down syndrome group		Control group		Total	
	N	%	N	%	N	%
Positive	28	93.3	29	96.7	57	95.0
Negative	2	6.7	1	3.3	3	5.0
Total	30	100.0	30	100.0	60	100.0

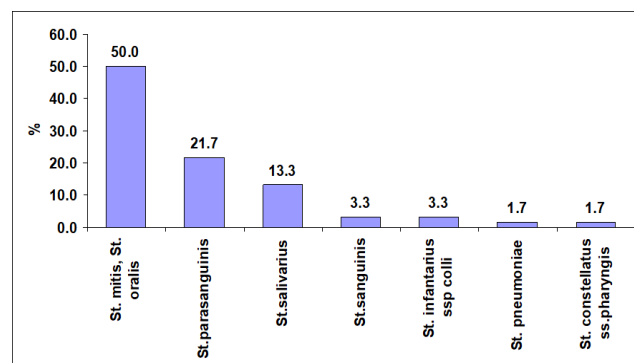
Fischer test, P-value $P=0.999$

Table 2. Microbiologic test results based on groups



Graph 2. Microbiologic analysis results of the subjects based on the group

In 50% of children involved in our research *St. myth* and *St. oral* were isolated, in 21.7% *St. parasanguinis* was isolated, and in 13.3% *St. salivarius*, in 3.3% *St. sanguinis*, in 3.3% *St. Infantarius ssp coli*, in 1.7% *St. pneumoniae* and *St. constellatus ss. Pharyngitis* (Graph 3).

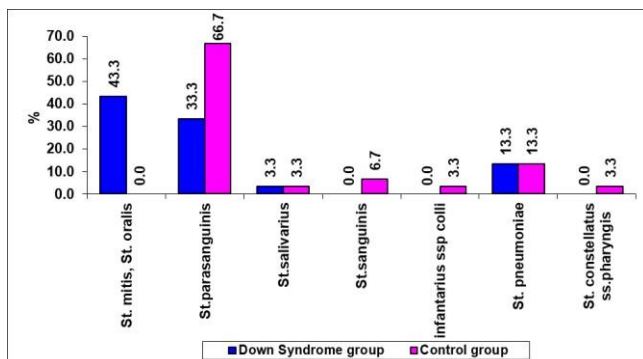


Graph 3. Microorganisms ranking isolated in all study subjects .

Based on groups, in the group suffering from DS the most frequent isolated bacteria was *St. mitis*, *St. oral* (43.3%), while at the control group *St. parasanguinis* (66.7%), (Table 3 and Graph 4).

Microbiologic analysis	Down syndrome group		Control group		Total	
	N	%	N	%	N	%
<i>St.parasanguinis</i>	13	43.3	-	-	13	21.7
<i>St. mitis</i> , <i>St. oralis</i>	10	33.3	20	66.7	30	50.0
<i>St.sanguinis</i>	1	3.3	1	3.3	2	3.3
<i>St. infantarius</i> <i>ssp colli</i>	-	-	2	6.7	2	3.3
<i>St. pneumoniae</i>	-	-	1	3.3	1	1.7
<i>St.salivarius</i>	4	13.3	4	13.3	8	13.3
<i>St. constellatus</i> <i>ss.pharyngis</i>	-	-	1	3.3	1	1.7
None	2	6.7	1	3.3	3	5.0
Total	30	100.0	30	100.0	60	100.0

Table 3. Types of isolated bacteria between children's groups.



Graph 4. Isolated Microorganisms Structure based on groups.

Discussion

Microbiological analysis findings of our cases was positive for the DS and control healthy group, but without significant difference ($P = 0.999$),

The results of our study are in contradiction with the results of the other two studies²⁶⁻²⁸ from the literature that report DS children present a lower salivary density of *S. mutans* and a lower dental caries experience than non-Down controls.

Scaloni F et al.²⁶ in their representative study aimed to assess the salivary densities of *S.*

mutans, *S. sobrinus*, and streptococci and dental caries experience, in a group of DS children and adolescents. Authors found that DS children and adolescents present a lower dental caries experience and a lower salivary density of *S.*

mutans than non-Down controls.

Meanwhile, in another systematic review and meta-analysis carried out by Deps et al.²⁷ demonstrated that based on scientific evidence, individuals with DS have fewer dental caries than individuals without DS.²⁷

In our study, we analyzed the types of streptococci in the dental plaque and find out that the most often detected bacteria was *St. Myths*, *St oralis* (43.3%) in the Down syndrome group whereas *St. parasanguinis* (66.7%) in the healthy control group.

In two independent studies Shapira J et al.⁶ and Stabholz A et al.⁹ showed that *Streptococcus mutans* counts, expressed as number of colony-forming units on mitis salivarius agar plates among the Down syndrome group, were the lowest, although not statistically significant compared with the counts of the healthy children.^{6,9}

Martinez - Martinez RE et al.²⁹ analyzed the periodontal biofilm in DS patients, with and without periodontitis, *Tannerella forsythia* was the most frequent bacteria detected in the group with and without periodontitis (95.5 and 63.3%) followed by *Treponema denticola* (88.8 and 50%) and *Porphyromonas gingivalis* (53.3 and 25% respectively). There were statistical differences between groups ($p < 0.05$).²⁹

In a five-year longitudinal study of dental caries risk related to *Streptococcus mutans* and *Streptococcus sobrinus* in subjects with intellectual disabilities Oda Y et al.³⁰ calculated the proportion of each of these strains to total bacteria, and compared dental caries incidence over 5 years, the proportion of *S. mutans* to total bacteria was moderately correlated with DMFT in year 2, Δ DMFT in years 2 and 5, and Δ SNAC in years 2 and 5 (correlation coefficient = 0.470, $P < 0.001$), while the proportion of *S. sobrinus* to total bacteria was moderately correlated with DMFT in years 2 and 5, Δ DMFT in years 1, 2, and 5, and Δ SNAC in years 2 and 5 (correlation coefficient = 0.695, $P < 0.001$). Individuals with ID who harbored both bacterial strains had a higher risk of dental caries and a significantly higher proportion of *S. sobrinus* to total bacteria.³⁰

Study carried out by Kishi et al.³¹ examining 54 mother-and-child pairs, they collected saliva samples from the mothers and the plaque samples from the children, to assess the relationships of quantitative salivary levels of *Streptococcus mutans* and *S. sobrinus* in mothers with the colonization of mutans streptococci (MS) in plaque and caries status in their 2.5-year-old children. Results from this study showed that the maternal salivary levels of *S. mutans* and *S. sobrinus* determined by real-time PCR were significantly related to MS colonization in plaque as well as dental caries in their children at 2.5 years of age.³¹

The relationship between oral streptococci and dental caries in children with DS is not well elaborated.²⁶

In the current literature, there have been many researches on oral health and prevalence of caries in children with DS, particularly in comparison with children suffering from various disabilities (mental retardation, cerebral palsy, musculoskeletal disorders, blindness, deafness, ect).

In the research, carried out by Shyama M. et al.³², in a study population comprised 832 disabled children and young adults (3-29 years; mean age 12.1 years), the mean DMFT was 5.4, and DMFS 15.2, being highest in the Down's syndrome and lowest in the blind. The proportion of caries-free subjects in permanent dentition, over 5 years of age was 24.2%. The smallest percentage of caries-free subjects was found in the hearing impaired (16.4%) and highest percentage in the blind (35.5%). The mean DMFT was 4.5 and the DMFS 8.7, being highest in the Down's syndrome and lowest in the blind.³²

In a cross-sectional study that included 33 DS individuals were included, aged 19 - 45 years, from Sarajevo and Tuzla Canton, Bosnia and Herzegovina conducted by Porović et al.³³ showed that the mean DMFT index is $15,96 \pm 8,08$; the analysis of oral hygiene of Down syndrome children by using the debris index, is found that 42,4% have very good oral hygiene, 21,2% respondents have good oral hygiene, 27,3% are with poor oral hygiene, while the very poor hygiene have 9,1% subjects, whereas CPI index value was 0,82.³³

This study had several strengths, e.g. it is a controlled design. According to subjects' characteristics at baseline, obtained results corresponded to those of similar studies.

A few limitations should be noted. First, this study utilized a small sample, did not provide power calculations and may have been underpowered. Second, we did not investigate the correlation between microbiological analyzes with other indices (oral hygiene index, gingival index).

Conclusions

Our results show that oral health condition was poor in both groups. Based on groups, in the group suffering from Down Syndrome the most frequent isolated bacteria was *St. mitis*, *St. oral*, while at the control group *St. parasanguinis*. Follow-up examinations as well as preventive approaches should be utilized for such individuals.

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Conflicts of interest

The authors declare no conflict of interest.

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