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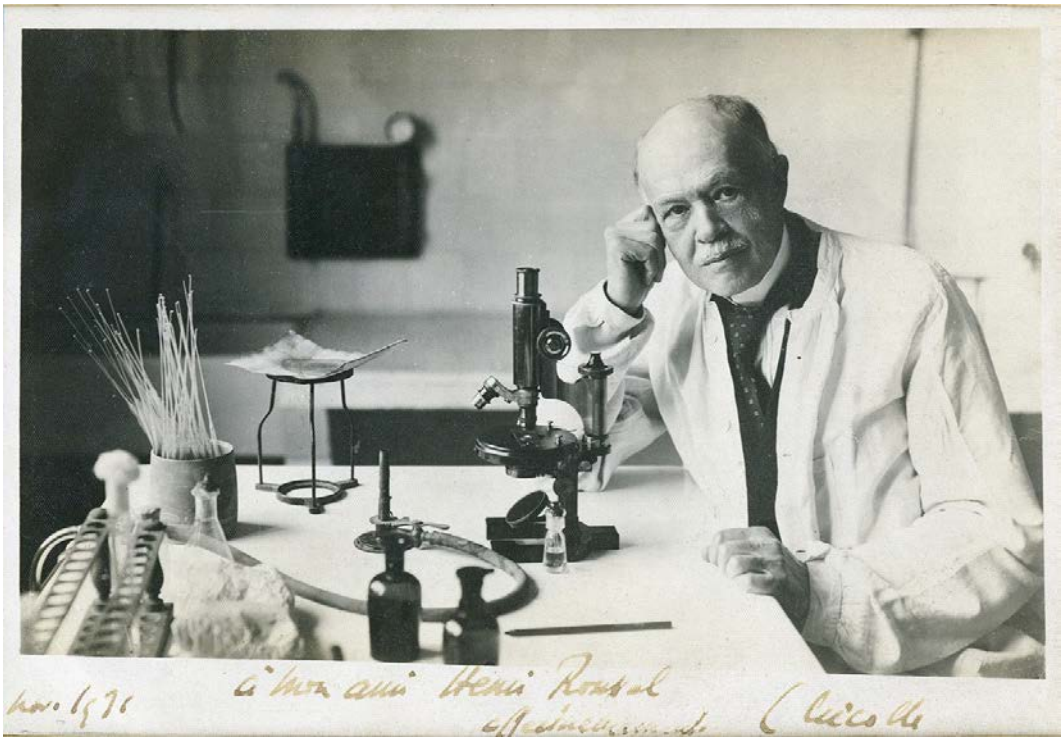

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Charles Jules Henri Nicolle (21 September, 1866–28 February, 1936) was a French bacteriologist who received the Nobel Prize in Medicine for his identification of lice as the transmitter of epidemic typhus causative agent. This year, on September 21, his 155 anniversary is marked.

Šarl Žil Anri Nikol (21. septembar 1866–28. februar 1936), francuski bakteriolog koji je 1928. godine dobio Nobelovu nagradu za medicinu za otkriće vaške kao prenosioca uzročnika epidemijskog tifusa. Ove godine, 21. septembra, obeležava se 155 godina od njegovog rođenja.

ORIGINAL ARTICLES

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Evaluation of periodontal status and treatment needs of the Serbian Armed Forces population

Procena stanja parodoncijuma i potrebe lečenja profesionalnih pripadnika Vojske Srbije

Dragana Daković*†, Margareta Lekić*†, Dubravko Bokonjić†‡, Zoran Lazić†§, Tatjana Čutović†‖, Raša Mladenović¶

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Abstract

Background/Aim. Periodontal disease is the second most common diseases after dental decay. The aim of the study was to determine frequency and seriousness of the periodontal disease, and periodontal treatment needs among the participants of the Serbian Armed Forces (SAF). **Methods.** A prospective cross-sectional study carried out on 1,411 military personnel aged 19–64 years (mean age: 35.20 ± 8.46 years). Periodontal health was recorded following the Community Periodontal Index (CPI) of Treatment Needs (CPITN). The frequency distributions were studied with regard to age groups, gender and military rank. The mean number of sextants affected per person was assessed. **Results.** Only 3% of the total SAF population had healthy periodontal tissue. The highest percentage of the studied population had the presence of calculus (42.7%), and the mean value of sextants with calculus was 1.77 per person. 35.8% of participants had the CPI score 3 (4–5 mm), and 5.4% of participants had the CPI score 4 (≥ 6 mm). Periodontal pockets (CPI score 3 and 4) were mostly present in the upper right (32%) and left (26%) sextant. Almost every subject needed better oral hygiene maintenance, but 41.2% of our participants required complex periodontal treatment. **Conclusion.** On the basis of all data, the treatment needs of SAF participants are big and vary in relation to the age, gender and military rank. Unfortunately, a dramatically small number of participants in the study had a completely healthy periodontium.

Key words:
epidemiology; military personnel; oral health; periodontium; periodontal diseases; periodontal index; serbia.

Apstrakt

Uvod/Cilj. Parodontalna bolest je, posle karijesa, druga najčešća stomatološka bolest. Cilj studije bio je da odredi učestalost i težina periodontalne bolesti kao i potrebe lečenja među pripadnicima Vojske Srbije. **Metode.** Prospektivna studija preseka izvedena je na 1 411 profesionalnih vojnika starosti 19–64 godina (prosečne godine: 35,20 ± 8,46). Periodontalno zdravlje ispitanika praćeno je indeksom stanja periodoncijuma – CPI i potrebama lečenja u zajednici (CPITN). Ispitivana je učestalost raspodele u odnosu na starosne grupe, pol i čin u vojsci. Procenjivana je srednja vrednost zahvaćenih sekstanata po osobi. **Rezultati.** Samo 3% ukupne ispitivane populacije profesionalne vojske Srbije imalo je zdrav periodoncijum. Najveći procenat ispitanika imao je prisutan zubni kamenac (42,7%), a srednja vrednost sekstanata sa zubnim kamencom bila je 1,77 po osobi. 35,8% ispitanika imalo je CPI skor 3 (4–5 mm), a 5,4% CPI skor 4 (≥ 6 mm). Dubina periodontalnih džepova (CPI skorovi 3 i 4) najčešće je bila prisutna u gornjem desnom (32%) i gornjem levom (26%) sekstantu. Skoro svaka osoba imala je potrebu za obukom u vezi sa oralnom higijenom, a 41,2% naših ispitanika imalo je potrebu za kompleksnom periodontalnom terapijom. **Zaključak.** Potrebe lečenja pripadnika profesionalne vojske Srbije velike su i raznovrsne i variraju u odnosu na godište, pol i čin u vojsci. Na žalost, zabrinjavajuće mali broj ispitanika imao je potpuno zdrav periodoncijum.

Ključne reči:
epidemiologija; vojni kolektiv; usta, zdravlje; periodoncijum; periodontalne bolesti; periodontalni indeks; srbija.

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Introduction

Periodontal disease (PD) is one of the most ubiquitous diseases of mankind, considered to be the second most common dental diseases worldwide, after dental decay [1]. Global Burden of Disease Study (GBD), 1990–2010 showed that the advanced form of periodontitis is the sixth most common condition affecting 11.2 % or 743 million people throughout the world aged between 15 and 99. Global presence of periodontal disease increased by 57.3% from 1990 until 2010 [2, 3]. Research results have demonstrated that the incidence of advanced form of periodontal disease did not change over the twenty year period. These age-standardised incidence and prevalence are similar for males and females. The prevalence of advanced periodontal disease increases with age (gradually between the third and fourth decade of life) and reach its peak at the age around 40 years [4].

Male population of the average age 38 dominate in the population of military officers participants [5, 6]. From the military service perspective, oral and periodontal health is especially important, because loss of periodontal health can be negatively reflected on the professional performance of officers and military personnel. In other words, the oral health of military personnel has a significant impact on military operations since untreated oral conditions can result in increased rates of disease and non-battle injury for deployed soldiers [7].

According to our knowledge there have been no published epidemiological studies related to gingival or periodontal health among professional soldiers in Serbia. There is only one study concerning the frequency and possible risk factors of periodontal disease among military personnel in Serbia [8]. This information is very important for establishing priorities and defining the kind and quantity of prevention and the need for medical treatment, as well as to ensure the presence of qualified personnel.

The aims of this study were to determine in detail the frequency and severity of periodontal disease, as well as the treatment needs among Serbian Army respondents, presented through the Community Periodontal Index (CPI) of treatment needs (CPITN). Also, we wanted to determine the association of age, gender and military rank with the prevalence of periodontal disease in this population, and to compare obtained results with similar studies in other countries.

Methods

A prospective cross-sectional study was designed. The sample consisted of 1,411 Serbian permanent-force military personnel, aged from 19 to 64 (mean age: 35.20 ± 8.46) years. The subjects' age, gender and military rank were recorded. The study lasted for 20 months. The sample size was calculated according to the results of the initial part of this study (25% prevalence of CPI codes 3 and 4 among Serbian permanent-force military personnel). With the test power of 0.8 (80%), alpha probability of 0.05 and allowable error of ≈ 10%, the calculated number of participants was at least 1,400. The calculation was based on formula: $N = 4PQ/d^2$; P = prevalence (initial study), Q = 100-P, d = allowable error (5%–20% of P).

The including criteria were professional military officers employed in the army in all the regions of Serbia. The excluding criteria were all system diseases that could harm in any way general or oral health of a studied participant, as well as the lack of corresponding teeth for evaluation.

The Clinical Research Study Protocol and the positive opinion were obtained from the Military Medical Academy (MMA) Ethics Committee. All participants of the study filled in a Volunteer Consent Form for participation in the study, and subsequently they were sent to the oral clinical examination. The clinical examination was conducted by three calibrated periodontists using dental mirrors and a specially designed World Health Organization (WHO) periodontal probe (CPITN probe), not employing a force greater than 20 g, following WHO criteria (WHO 1987). Six sextants were examined in every participant, and the index teeth were the following: 17, 16, 11, 26 and 27 in the upper jaw, and 37, 36, 31, 46 and 47 in the lower jaw. Only those sextants containing at least 2 functional teeth were examined; otherwise they were not included in the study.

The depth of the periodontal pockets was measured on 6 locations around each tooth. Each sextant was designed as: CPI code 0 – healthy, CPI score 1 – bleeding on probing, CPI score 2 – the presence of calculus, CPI score 3 – the depth of periodontal pockets 4–5 mm, and CPI score 4 – depth of periodontal pockets of 6 mm and more. In each sextant only the highest value for each sextant was scored and recorded [9].

Statistical analysis

Complete statistical analysis of data was done with the statistical software package SPSS Statistics 18. Most of the variables were presented as number (frequency) of certain categories, while statistical significance of differences was tested with the χ^2 test. In case of continuous data, variables were presented as mean value ± standard deviation (SD). Kolmogorov-Smirnov test was used for the evaluation of distribution of these data. Statistical significance between groups was tested by *t*-test and one-way ANOVA test. All the analyses were estimated at minimal $p < 0.05$ level of statistical significance.

Results

A total of 1,411 Serbian military officers were examined. There was a big disproportion in the number of male (89.7%) and female (10.3%) participants. Also, a statistically significant difference in the age was observed between the tested groups of men and women (35.85 ± 8.48 *vs* 29.55 ± 5.80 years, respectively). The average age of the participants was 35.2 ± 8.46 years. The majority of participants aged 25–34 years (39.1%). The ratio of officers: non-commissioned officers was 1 : 2 (Table 1). Also, non-commissioned officers were statistically significantly younger.

Table 1

Basic demographic and professional characteristics of subjects

Parameters	n (%)	Age (years) mean ± SD	Test (*p*)
Gender			
male	1,266 (89.7)	35.85 ± 8.48	*t* = 11.71
female	145 (10.3)	29.55 ± 5.80	(< 0.001)
Age category (years)			
≤ 24 .	153 (10.8)	22.39 ± 1.35	
25 – 34	552 (39.1)	29.80 ± 2.93	F = 3846.09
35 – 44	471 (33.4)	39.03 ± 2.94	(< 0.001)
45 – 64	235 (16.7)	48.56 ± 2.83	
Military rank			
officer	482 (34.2)	36.68 ± 8.77	*t* = 4.67
corporal	929 (65.8)	34.43 ± 8.19	(< 0.001)
Total	1,411 (100.0)	35.20 ± 8.46	

SD – standard deviation.

Taking into account the total examined population, only 3% of the study participants had a completely healthy periodontium (Table 2). The presence of periodontal pockets (scores 3 and 4) was detected in very high percentage (41.2%) of tested subjects. Calculus was present equally in both genders, but significantly more women had a completely healthy periodontium compared to men (26.9% *vs* 2.4%, respectively), as well as gingival inflammation (8.3% *vs* 11.5%, respectively).

In the age group from 35 to 44, shallower periodontal pockets were present in 44.8%, but the older group (45–64 years) had periodontal pockets with CPI scores 3 and 4 in 69.3% of cases. In the military rank category, there was no significant difference in comparison with all tested CPI scores. Score 2 prevailed in both populations also in this group (39.6%/44.3%).

The analysis of all the participants regarding the presence of CPI score per sextants (data not shown) indicated that 14.7% sextants per person (mean 0.881) had healthy periodontium and 37% (mean 2.226) were affected by gingival bleeding. At the same time, calculus was present in 29.6% (mean 1.773). Periodontitis and the presence of periodontal pockets of 4–5 mm or larger than 6 mm were found in 14.8% (mean 0.888), i.e. 1.3% of sextants per person (mean 0.075).

Analysing the CPI score per sextants with regard to gender, it was shown that women had 1,676 sextants per person with a completely healthy periodontium, as opposed to men who had only 0.791 healthy sextants per person. Gingival bleeding was the most common in both populations. Periodontal pockets (CPI score 3 and 4) were present in an average of 17% sextants per male participants which is significantly higher than in female participants (7.1%) (Table 3, Figure 1).

Regarding the age category, the youngest population had 1.32 sextants per person with the healthy periodontium, but also one half of sextants (50%) had gingival bleeding.

Table 2

Number and percentage of subjects with different CPI scores by gender, age and rank

Parameters	CPI score					
	0	1	2	3	4	Total
Gender						
male	31 (2.4)	145 (11.5)	541 (42.7)*	474 (37.4)	75 (5.9)	1,266 (100.0)
female	12 (8.3)	39 (26.9)	62 (42.8)*	31 (21.4)	1 (0.7)	145 (100.0)
Significance	$\chi^2 = 54.28$; $p < 0.001$; both gender categories, comparison to normal distribution.					
Age category (years)						
≤ 24	10 (6.5)	47 (30.7)	72 (47.1)*	24 (15.7)	0 (0.0)	153 (100.0)
25–34	24 (4.3)	98 (17.8)	275 (49.8)*	152 (27.5)	3 (0.5)	552 (100.0)
35–44	5 (1.1)	32 (6.8)	195 (41.4)	211 (44.8)*	28 (5.9)	471 (100.0)
45–64	4 (1.7)	7 (3.0)	61 (26.0)	118 (50.2)*	45 (19.1)	235 (100.0)
Significance	$\chi^2 = 285.20$; $p < 0.001$; all age categories, comparison to normal distribution.					
Miltary rank						
officer	24 (5.0)	70 (14.5)	191 (39.6)*	176 (36.5)	21 (4.4)	482 (100.0)
corporal	19 (2.0)	114 (12.3)	412 (44.3)*	329 (35.4)	55 (5.9)	929 (100.0)
Significance	$\chi^2 = 13.40$; $p = 0.009$; both military rank categories, comparison to normal distribution.					
Grand total	43 (3.0)	184 (13.0)	603 (42.7)	505 (35.8)	76 (5.4)	1,411 (100.0)

Data are presented as number (%).

CPI – Community Periodontal Index; * – maximal percent value in rows.

Table 3

Mean number and percentage of sextants per person for each score by age category and military rank

Parameters	Inclined sextants/per patients [mean (%)]					
	CPI score					X-excluded sextant
	0	1	2	3	4	
Gender						
male	0.791 (13)	2.198 (37)	1.820 (30)	0.945 (16)	0.084 (1)	0.163 (3)
female	1.676 (28)	2.476 (41)	1.372 (23)	0.393 (7)	0.007 (0.1)	0.076 (1)
Age category (years)						
≤ 24	1.320 (22)	2.980 (50)	1.379 (23)	0.307 (5)	0.000 (0)	0.013 (0.2)
25–34	1.074 (18)	2.621 (44)	1.670 (28)	0.580 (10)	0.005 (0.1)	0.049 (0.8)
35–44	0.696 (12)	1.985 (33)	1.936 (32)	1.132 (19)	0.076 (1)	0.174 (3)
45–64	0.515 (9)	1.294 (22)	1.949 (32)	1.502 (25)	0.289 (5)	0.451 (7)
Military rank						
officer	1.060 (18)	2.212 (37)	1.687 (28)	0.882 (15)	0.058 (1)	0.102 (2)
corporal	0.789 (13)	2.235 (37)	1.819 (30)	0.891 (15)	0.085 (1)	0.181 (3)

CPI – Community Periodontal Index.

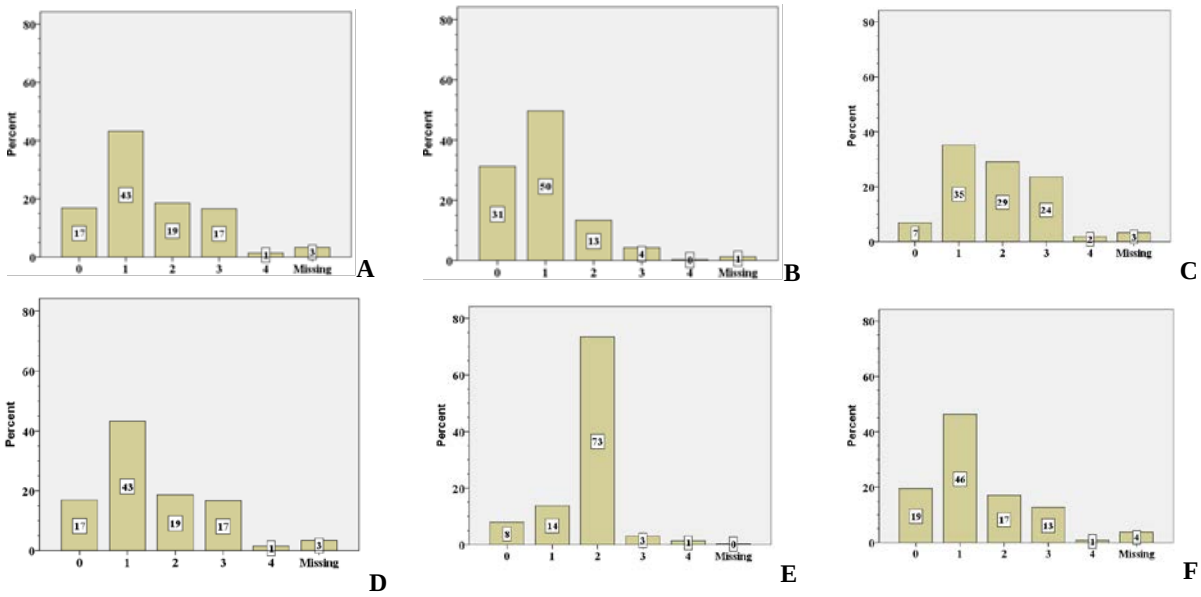

Fig. 1 – Percentage of Community Periodontal Index (CPI) score divided into 6 sextants (A:17-14; B:13-23; C:24-27; D:44-47; E: 33-43; F:34-37).

Except in the oldest group, gingival bleeding was most prevalent in all tested age groups. Interestingly, calculus per sextants was present in approximately the same percentage in all analysed categories (age, gender, military rank). The most visible destructive changes in periodontium were found in the oldest population in which rise in scores 3 and 4 per sextants was detected (Table 3). All CPI scores per sextants had almost identical values in both tested populations (officers and non-commissioned officers) (Table 3).

Upper left and right sextants, which had similar CPI scores, were mostly affected by pathological changes of the periodontium (Figure 1). Periodontal pockets were also present mostly in these sextants (CPI score 3 and 4). Healthy periodontium was mostly found in upper middle sextant (32%).

The results illustrated in Table 4 indicate that giving instruction of oral hygiene was required in 97% of participants, whereas 84% of them needed motivating alongside with scaling and root planning. Finally, 41.2% of subjects needed a complex periodontal treatment. Only 3% of participants had a completely healthy periodontium (no need for any periodontal therapy).

The results also showed that there were numerous gender differences concerning treatment needs. Motivation in the maintenance of oral hygiene and plaque control was necessary in 97.6% of men and 91.7% of women. Scaling and root planning were needed in 86% of men and 64.9% of women. The most significant difference between genders was observed in the need of performing complex periodontal treatments (43.3% in male population and 22.15% in female population). Treatment needs distribution in relation to age had an increasing trend with increasing age, so the youngest population had the smallest need for treatment, especially for the complex periodontal treatment (15.7%) in comparison to the oldest population (64.4%). It is interesting that 50.7% of the most active and the most important population from the

Table 4

Distribution of treatment needs (TNs) in corresponding gender, age and military rank

Parameters	No need	TNs		
	Healthy periodontium	Instruction of oral hygiene	Scaling and root planning	Complex periodontal treatment
Gender				
male	31 (2.4)	1,235 (97.6)	1,091 (86)	549 (43.3)
female	12 (8.3)	133 (91.7)	94 (64.8)	32 (22.1)
Age category (years)				
≤ 24	10 (6.5)	143 (93.5)	96 (62.7)	24 (15.7)
25–34	24 (4.3)	528 (95.6)	430 (77.9)	155 (28.1)
35–44	5 (1.1)	466 (98.9)	434 (92.1)	239 (50.7)
45–64	4 (1.7)	231 (98.3)	224 (95.3)	163 (69.4)
Military rank				
officer	24 (5.0)	458 (95.0)	388 (80.5)	197 (40.9)
corporal	19 (2.0)	910 (97.9)	796 (85.7)	384 (41.3)
Total	43 (3.0)	1,368 (96.9)	1,184 (83.9)	581 (41.2)

All values are expressed as number (percentage).

age of 35 to 44 years had a need for a complex periodontal treatment (Table 4).

With regard to the military rank, it is noticeable that officers in 5% of cases did not need any periodontal treatment, while this percentage was smaller in non-commissioned officers (2%). Regarding treatment needs, both categories showed almost the same treatment needs (Figure 2).

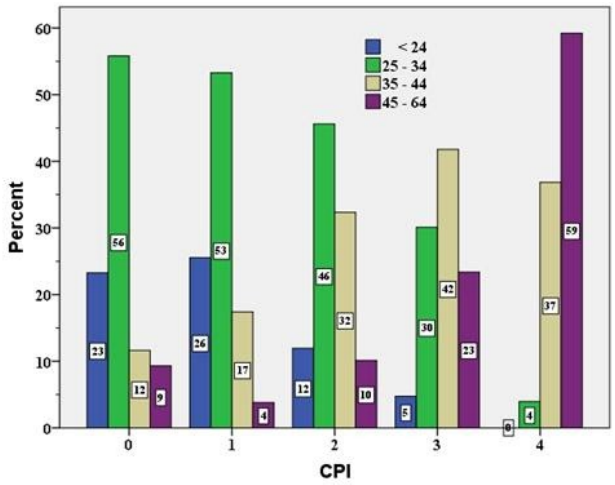

Fig. 2 – Community Periodontal Index (CPI) according to age-groups.

Although the youngest category (aged 15 to 24 years) was present with only 10.8% in the whole group, its participation in CPI categories 0 and 1 was 23% and 26%, respectively. On the other hand, the oldest category was present with 16.7% in the sample, but its presence in CPI categories 3 and 4 was 23% and 59% respectively (Figure 3). Figure 3 illustrates the distribution of genders in relation to CPI. Although there were only 10.3% of the female population in the whole sample, its presence in categories CPI 0 and 1 was 28% and 21%, respectively. In the relatively low percentage of the female population, categories 3 and 4 have been registered. A quite opposite trend was shown in the male population – 99% of the total number of study participants with CPI 4 were of the male gender (Figure 4).

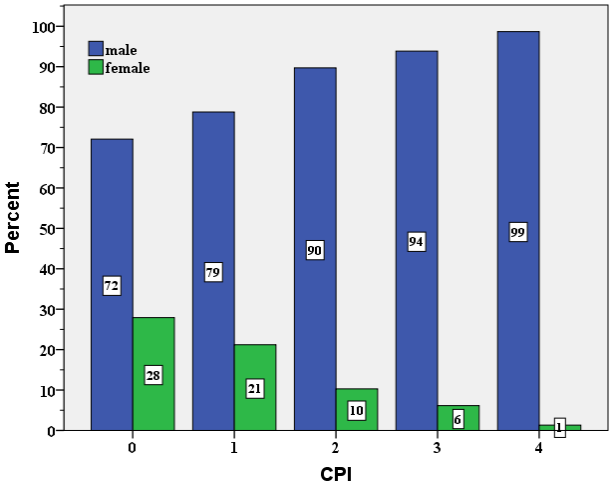

Fig. 3 – Community Periodontal Index (CPI) according to gender category.

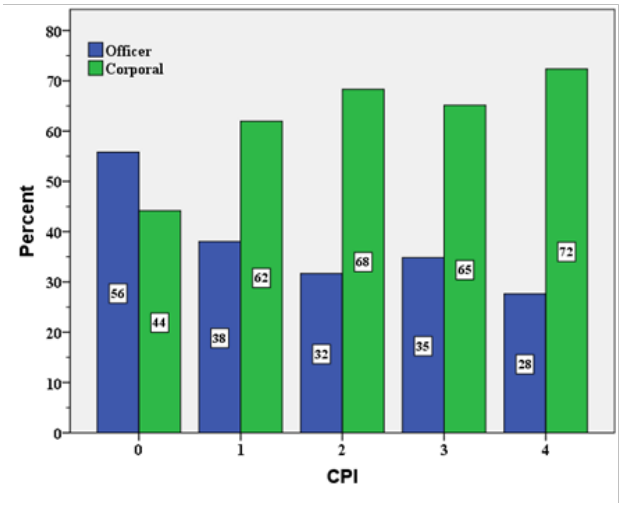

Fig. 4 – Community Periodontal Index (CPI) according to military rank.

Figure 4 shows the distribution of officers and non-commissioned officers in relation to CPI. Although there were 34.2% of officers in the whole sample, their presence in

CPI categories 0 and 1 was 56% and 38%, respectively. This indicates a slightly better condition of oral health regarding the starting CPI categories. In higher CPI categories these differences completely disappeared.

Discussion

According to our study, people with completely healthy periodontal tissues (CPI score 0, average age of 35.2 years) were in minority. A significantly larger percentage of healthy sextants was found in Italian officers (40.95%), in group of cadets (57.95%) whose average age was between 19–25 years [10], in Danish military population whose average age was 25 (53%) [11], as well as in the Spanish military personnel [12]. Also, other authors have shown that the percentage of people with a healthy periodontium depends on age and ranges from 5% to 10% [13–15]. In contrast to these findings, Israeli permanent military force population had 1.19% of healthy periodontium in all sextants [10], and the civilian population in India had 1.7% [16].

In our study the prevalence of the tested military personnel with healthy sextants showed a statistically relevant difference in relation to gender and age. A significantly higher percentage of women had a healthy periodontium – two times higher prevalence of healthy periodontium per sextants compared to men (1.68 *vs* 0.79, respectively). These results are in accordance with the results of other authors [10–12, 17]. It could be explained by good habits and greater awareness of women, as well as their care for maintaining good oral hygiene, but also by the fact that in our study women were statistically significantly younger ($p < 0.001$), accounting for only 10.3% of the total number of study participants.

As expected, the youngest military population (< 25 years) had on average 1.32 healthy sextants and there was a declining trend with the increase age. Namely, the percentage of healthy sextants in tested subjects under 25 years of age was 22%, while among the oldest it was almost three times lower (8.6%). These results were identical to the findings of Katz et al. [1]. However, our findings were not in agreement with the results of Mombiedro-Sandoval and Llena-Puy [12], whose study participants (Spanish military personnel) were significantly younger (average age 27.39), than our subjects (average age 35.2). On the other hand, no difference was observed between groups of officers and corporals, even though other authors have shown that people with a higher level of education have fewer dental ailments than people with lower level of education [1]. In general, a small percentage of healthy sextants in our tested subjects can be explained by inadequate oral hygiene habits, fear from dental interventions, the lower level of awareness for the importance of oral health, as well as numerous professional officers' duties, frequent military drills, operational tasks.

In this research we have shown that the highest percentage of the studied population had the presence of calculus (42.7%), and the mean value of sextants with calculus was 1.77 per person. The percentage of calculus varied in various studies [1, 8, 12, 13, 15, 18]. The presence of calculus per sextants in male subjects was more dominant compared to female subjects (1.82 *vs* 1.37, respectively), and with the increase in age the mean value of sextants with calculus was also increasing and was in range 1.38–1.95. With regard to the military rank, both tested categories (males and females) had very similar mean values of calculus per sextants.

In our study, most of the sextants on average were affected by gingival bleeding (37.1%; mean 2.23). The incidence of gingivitis per sextants among age groups was present as follows: higher proportion in the youngest population (50% of cases) and lower proportion among participants ages 45–64 years (22% of cases). Unlike our results, in other studies, the mean value of sextants with signs of gingival inflammation did not exceed 1.5 sextants per person [10, 19]. Similar results of the presence of gingivitis in female population (37.9%) were obtained by Al Mugeiren [17].

The total prevalence of people with periodontal pockets of both types (CPI score 3 and 4) was around 16%, which was in accordance with a studied American military population (average age 30) [20], as well as with the personnel in the Israeli army [21]. However, the results of many studies, conducted in younger population, have shown a smaller percentage of area affected by periodontal disease [10–12]. When we analysed the age groups, we found that the oldest group had the most frequent and deepest periodontal pockets. Unlike the oldest group, the participants under the age of 25 had on average 0.31 or 0.0 sextants with periodontal pockets. This was in agreement with the results of other authors [1, 14, 21–23]. Analysing data from WHO GODB [24], it can be concluded that in a large number of countries the prevalence of people with deep pockets was less than 22.4%, while in our study 35.8% of participants had the highest CPI score 3 (4–5 mm), and 5.4% of participants had the biggest CPI score 4 (≥ 6 mm).

Concerning the third variable related to the military rank, it was shown that officers and corporals had almost the same values of all tested parameters. These results were in general in accordance with the study of Mombiedro-Sandoval and Llena-Puy [12], except with regard to gingivitis and more shallow periodontal pockets.

Periodontal pockets (CPI score 3 and 4) were mostly present in the upper right (32%) and left (26%) sextant, and also in 3.4% of cases this sextant was missing (edentulous X). Anyway, the lower right sextant (44–47) was the most jeopardised one regarding tooth loss. On average, 3.7% of this sextant was missing, and as a consequence, in the majority of cases this sextant was not included in our study. Correlation between the presence of all periodontal pockets and tooth loss could indicate that the missing teeth were removed due to periodontitis that was insufficiently treated, especially in the upper right and left region. The distribution of risk factors in the onset and development of periodontal disease generally has a significant impact on the severity and frequency of the disease. It is known that there is a positive correlation between the prevalence of attachment and tooth loss [25]. Upper anterior teeth (13–23) had healthy sextants in the highest percentage (31.3%) compared to other sextants, but on average 1.2% of the sextant was missing. The smallest

number of missing teeth was in the lower anterior sextant (0.4% of sextants). Male participants had more missing teeth than female participants (ratio 3 : 1, respectively). Analysing the group of participants with respect to their age, significant difference in the number of missing teeth was found. This finding also fits with the results of research conducted in some European countries [25].

Considering the goal of treatment needs of the military environment, it is necessary to achieve good general health condition (especially oral health) to be able to train and to take part in exercises, manoeuvres and deployments [26]. In our study, the biggest need (over 96% of all participants) was for adequate training and maintenance of oral hygiene. This percentage is almost the same in all tested variables and their categories. Our findings are similar to the findings of other authors [11, 13, 27]. Only scaling and root planning, as well as the removal of all factors that cause dental biofilm accumulation, should be carried out in the smallest proportion in the youngest population (62.7%) and in women (64.8%), but in the largest proportion in the oldest population (95.3%). Complex periodontal treatment required 41.2% of our participants, which is much more compared to results of Mombiedro-Sandoval and Llena-Puy [12]. People aged from 35 to 44 and 45 to 64 had advanced periodontitis and a need for a complex treatment of periodontium in 50.7% and 69.4% of cases, respectively. This kind of interventions should be performed by a periodontist, which is difficult to be achieved in military health centres. Moreover, this kind of treatment takes time and requires several session, which would oblige military officers to be absent from their regular activities. This is something that definitely implies the need for a detailed plan in the resolution of the current situation, and it is definitely necessary to make a lasting and multi-year plan. The plan would emphasize the preventive programme, which would be implemented by general dentists in military health centres, in order to reduce the health, social and economic consequences at a later age.

Conclusion

Unfortunately, a dramatically small number of people have a completely healthy periodontium. Twice as many men suffer from periodontitis compared to women. On the basis of all data, the treatment needs are significant, and diverse and vary depending on age, gender and military rank. It is obvious that there is insufficient information about the importance of oral and periodontal health, as well as of the correct maintenance of oral hygiene. The capacity of dental service should definitely be increased. All military garrisons should be equipped with a dental office and full-time dentists. Dentists should also introduce an obligatory screening for this group of subjects, to hold educational programmes of primary health protection through multimedia presentations and short lectures, in order to increase the awareness of the population about the importance of oral hygiene and early diagnostics of gingival and periodontal diseases. These activities should generally impact the army's capability in performing the most delicate duties in hard conditions.

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Conflict of interest

There are no a conflict of interests.

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The Serbian version of the "Oral Health Questionnaire for Adults"

Srpska verzija „Upitnika za procenu oralnog zdravlja odraslih"

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Abstract

Background/Aim. The assessment of the impact of oral health on the quality of life presents the increasing need in testing oral health of an individual and of the entire population. The aim of the study was to translate the index of "Oral Health Questionnaire for Adults" of the World Health Organization into the Serbian language and to check its reliability in the Serbian Armed Forces professional staff. **Methods.** This study was designed as an observational, epidemiological study. The "Oral Health Questionnaire for Adults" was translated into the Serbian language. The classical test theory was used in the data analysis. The assessed characteristics included internal consistency and construct validity. **Results.** A total of 1,741 participants were included in this analysis. The mean age of the study group was 32.4 ± 9.7 years. In the last 12 months, problems which occurred frequently or very often regarding teeth and mouth were as follows: difficult biting of food (6.2%), difficult chewing (5.1%), difficult speech or difficult pronunciation of certain words (1.9%), dryness of the mouth (2.9%), the feeling of discomfort due to the esthetic appearance of teeth (6.8%). The reliability of the "Oral Health Questionnaire for Adults" (items considering Oral Health Self-Assessment) had high internal consistency (the Cronbach's coefficient was 0.879). The Principal component analysis and Promax rotation revealed 1 factor with Eigenvalue exceeding 1, accounting for 54.3% of the total variance. **Conclusion.** The Serbian version of the „Oral Health Questionnaire for Adults" provides very useful and reliable information on the condition of oral health of the Serbian Armed Forces professional members.

Key words:
attitude to health; habits; military personnel; oral health; serbia; surveys and questionnaires; translating.

Apstrakt

Uvod/Cilj. Procena uticaja stanja oralnog zdravlja na kvalitet života predstavlja sve veću potrebu u ispitivanju oralnog stanja pojedinaca, ali i cele populacije. Cilj rada bio je da se indeks „Upitnik za oralno zdravlje odraslih" Svetske Zdravstvene Organizacije prevede na srpski jezik uz proveru njegove pouzdanosti na uzorku pripadnika oružanih snaga Srbije. **Metode.** U radu je primenjena opservaciona, epidemiološka studija. „Upitnik za procenu oralnog zdravlja odraslih" (*Oral Health Questionnaire for Adults*) preveden je na srpski jezik. Pri analizi podataka korišćena je klasična test teorija. Procenjivane karakteristike uključile su unutrašnju konzistentnost i valjanost konstrukcije. **Rezultati.** U ovu analizu je bio uključen ukupno 1 741 ispitanik. Prosečna starost ispitivane grupe iznosila je 32,4 ± 9,7 godina. U poslednjih 12 meseci od problema koji su se javljali često ili veoma često u vezi sa zubima i usnom dupljom bili su: otežano odgrizanje hrane (6,2%), otežano žvakanje (5,1%), otežan govor ili otežano izgovaranje pojedinih reči (1,9%), suvoća usta (2,9%) i osećaj neprijatnosti usled estetskog izgleda zuba (6,8%). Pouzdanost „Upitnika za procenu oralnog zdravlja odraslih" (stavke koje uzimaju u obzir samoocenjivanje oralnog zdravlja) imala je visoku unutrašnju konzistentnost (Kronbahov koeficijent bio je 0,879). Analiza glavnih komponenti i rotacija Promaks otkrili su 1 faktor sa *Eigenvalue* većim od 1, što čini 54,3% ukupne varijanse. **Zaključak.** Srpska verzija „Upitnik za procenu oralnog zdravlja odraslih" pruža veoma korisne i pouzdane informacije o stanju oralnog zdravlja pripadnika oružanih snaga Srbije.

Ključne reči:
stav prema zdravlju; navike; vojni kolektiv; usta, zdravlje; srbija; ankete i upitnici; prevođenje.

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Introduction

Oral diseases impact around half of the world population and their impact is considered as a very important public health problem in the sense of disease burden and medical expenses [1]. Although oral diseases can be prevented and treated, they still present a problem at the global level [2]. It has been determined that the oral health of individuals in developing countries is getting worse [3]. The problems of public health connected to oral diseases present a serious burden for countries around the world.

World Health Organization (WHO) has a long tradition of epidemiological research in following and monitoring oral health [4]. Studying oral health status, one of the most significant parameters is the number of teeth. A clinical examination is a gold standard for this testing [5]. However, clinical examination has limitations connected to high expenses of personnel, time and resources necessary for this type of research [6]. One of the alternatives to professional clinical testing is questionnaires, which enable the obtaining of basic, but relevant epidemiological information with significantly fewer expenses [7]. In dentistry, self-evaluation is a valid instrument for studying conditions such as oral hygiene, the health of periodontium and the use of dental braces [8]. The studies conducted in several countries point to the fact that self-assessment of the number of teeth was an accurate parameter of high validity [9].

The WHO has a long tradition of epidemiological research, which includes a description of diagnostic criteria that can be easily understood and applied in the programs of public health around the world. The WHO Global Oral Health Data Bank recommends the use of simplified structured questionnaires for the collection of data on self-assessment of oral health and risk factors in adults ("Oral Health Questionnaire for Adults") [4]. The questionnaire has been tested in numerous countries around the world [10, 11]. The language barrier can be the reason to prevent the intensive use of this instrument in countries where English is not a native language. Hence, the purpose of our research was to translate the questionnaire "Oral Health Questionnaire for Adults" of the WHO into the Serbian language and prepare it for the testing of oral health of the Serbian Armed Forces professional members.

Methods

The study was conducted between 2017 and 2019 at the territory of the Republic of Serbia. The study was designed as an observational, epidemiological study, and included 1,741 professional members of the Serbian Armed Forces aged 18–64 years. Based on the data from the literature, a prevalence of 80% was the starting point for sample size calculation in our study [12]. It was necessary to include at least 1,537 participants for a reliable assessment of the oral health of the Serbian Armed Forces professional members, with the strength of the study of 0.8 (80%), mistake type alpha of 0.05%, and foreseen mistake of 2% (0.02).

The approval for the study was obtained by the Ethical Committee of the Military Medical Academy (No. 1/ 15 - 17). Participation in the research was voluntary, and all participants had signed the informed consent for the participation in the research before they were interviewed.

A total of 1,741 participants were included in this analysis; participation rate was 100%, i.e. all of 1,741 respondents who had accepted participation in the study returned fully completed questionnaires. The criteria for entering the study were that the subject was over 18 years old and younger than 64 and that he/she was a professional member of the Serbian Armed Forces. The exclusion criterion for the study was the professional status of a civilian employee in the Serbian Armed Forces, presence of systemic diseases and acute symptoms of dental diseases.

The study used the WHO questionnaire "Oral Health Questionnaire for Adults", published as a part of the "Oral Health Surveys Basic Methods, 5th edition" by the WHO in 2013 [4]. This questionnaire was specifically designed for self-filling of information on the individual oral health. Except for demographic information, other questions refer to risk factors that impact oral health, individual's habits in maintaining oral hygiene and the use of dentistry services. Also, the information on the frequency of sugar intake, alcohol, and tobacco consumption was collected as well. The original questionnaire includes 16 questions. The variables included in the adult questionnaire were as shown in Table 1. We used all the questions that were stated in the WHO questionnaire in the Serbian version of the questionnaire. However, our version contained only last 14 questions because the questions 1 and 2 (ordinal number of participants and age) already existed in the basic questionnaire for social demographic data.

The WHO questionnaire "Oral Health Questionnaire for Adults" has been translated from the original English language into the Serbian language by the two dentists with good knowledge of the English language using internationally accepted recommendations [13]. Then this version was again translated into the English language. Such translation (backward) was additionally assessed by a dentist proficient in the English language. It was determined that there was no difference between the original version of the questionnaire regarding our version. After that, the questionnaire was again translated into the Serbian language and tested in the pilot study. The pilot study was conducted on 20 participants to confirm that the meaning of every single question is well understood and that they do not cause discomfort and displeasure. The participants were asked the questions orally. Upon the successful conduct of the pilot study, the main study was conducted, which included 1,741 participants. Canvassing was conducted by the two trained interviewers who were mutually harmonized. The work standardization in the field was achieved with suitable training of interviewers on the collection of data, data control and data processing [14].

Table 1

The Serbian version of the "Oral Health Questionnaire for Adults"

1. How many natural teeth do you have?/ *Koliko imate svojih zuba?*					
	No natural teeth / *Nemate svoje zube*	→	0		
	1–9 teeth/ *1–9 zuba*	→	1		
	10–19 teeth / *10–19 zuba*	→	2		
	20 teeth or more/ *20 svojih zuba ili više*	→	3		
2. During the past 12 months, did your teeth or mouth cause any pain or discomfort?/ *Tokom poslednjih 12 meseci, da li su Vaši zubi i usta izazivali bilo kakve bolove ili nelagodnosti?*					
	Yes/ *Da*	→	1		
	No/ *Ne*	→	2		
	Don't know/ *Ne znam*	→	9		
	No answer/ *Nema odgovora*	→	0		
3. Do you have any removable dentures?/ *Da li imate bilo kakve mobilne proteze?*		Yes/*Da*	No/*Ne*		
		1	2		
	A partial denture?/ *Parcijalna proteza*	→	→		
	A full upper denture?/ *Totalna gornja proteza*	→	→		
	A full lower denture?/ *Totalna donja proteza*	→	→		
4. How would you describe the state of your teeth and gums? *Kako biste opisali stanje Vaših zuba i desni?*		Teeth/ *Zubi*	Gums/*Desni*		
	Excellent/ *Odlično*	→ 1	→ 1		
	Very good/ *Veoma dobro*	→ 2	→ 2		
	Good/ *Dobro*	→ 3	→ 3		
	Average/*Prosečno*	→ 4	→ 4		
	Poor/ *Loše*	→ 5	→ 5		
	Very poor/ *Veoma loše*	→ 6	→ 6		
	Don't know/ *Ne znam*	→ 9	→ 9		
5. How often do you clean your teeth?/ *Koliko često perete svoje zube?*					
	Never/ *Nikad*	→	1		
	Once a month/ *Jednom mesečno*	→	2		
	2–3 times a month/ *2–3 puta mesečno*	→	3		
	Once a week/ *Jednom nedeljno*	→	4		
	2–6 times a week/ *2–6 puta nedeljno*	→	5		
	Once a day/ *Jednom dnevno*	→	6		
	Twice or more a day/ *Dva ili više puta dnevno*	→	7		
6. Do you use any of the following to clean your teeth?/ *Da li koristite nešto od ponuđenog da bi očistili zube?*		Yes/ *Da*	No/ *Ne*		
		1	2		
	Toothbrush/ *Četkica za zube*	→	→		
	Wooden toothpicks / *Drvene čačkalice*	→	→		
	Plastic toothpicks/ *Plastične čačkalice*	→	→		
	Thread (dental floss) / *Konac za zube*	→	→		
	Interdental toothbrush / *Interdentalna četkica*	→	→		
	Charcoal / *Ugalj*				
	Chewstick / miswak / *Štapić za žvakanje / štapić za čišćenje zuba*	→	→		
	Mouthwash / *Rastvor za ispiranje usta*				
	Other/ *Drugo*	→	→		
	Please specify/ *Molimo navedite šta*	→	→		
		Yes/ Da	No/ Ne		
		1	2		
7. a) Do you use toothpaste to clean your teeth?/ *Da li koristite pastu za zube kada perete iste?*		→	→		
b) Do you use toothpaste that contains fluoride?/ *Da li koristite pastu koja sadrži fluor?*		→	→		
8. How long has it been since you last saw a dentist? / *Koliko je prošlo od vaše poslednje posete stomatologu?*					
	Less than 6 months/ *Manje od 6 meseci*	→	1		
	6–12 months/ *6–12 meseci*	→	2		
	More than 1 year but less than 2 years/ *Više od 1 godine, a manje od 2 godine*	→	3		
	2 years or more but less than 5 years/ *2 godine ili više, ali manje od 5 godina*	→	4		
	5 years or more/ *5 godina ili više*	→	5		
	Never received dental care/ *Nikada nisam bio u poseti stomatologu*	→	6		
9. What was the reason of your last visit to the dentist?/ *Koji je bio razlog vaše poslednje posete stomatologu?*					
	Consultation/advice/ *Konsultacije/savet*	→	1		
	Pain or trouble with teeth, gums or mouth/ *Bol ili problem sa zubima, desnima ili ustima*	→	2		
	Treatment/ follow-up treatment/ *Tretman/ naknadni tretman*	→	3		
	Routine check-up/treatment/ *Rutinski pregled/ tretman*	→	4		
	Don't know/don't remember/*Ne znam/ne sećam se*	→	5		
10. Because of the state of your teeth or mouth, how often did you experience any of the following problems during the past 12 months?/ *U poslednjih 12 meseci da li ste primetili neki od navedenih problema?*					
		Very ofen / *Veoma često*	Fairly often / *Pošteno često*	Sometimes / *Ponekad*	No / *Ne*	Don't know / *Ne znam*
		4	3	2	1	0
	a) Difficulty biting food/ *Otežano odgrizanje hrane*	→	→	→	→	→
	b) Difficulty chewing food/ *Otežano žvakanje*	→	→	→	→	→

c) Difficulty with speech pronouncing certain words / *Otežan govor/otežano izgovaranje pojedinih reči*	✈	✈	✈	✈	✈
d) Dry mouth / *Suva usta*	✈	✈	✈	✈	✈
e) Felt embarrassed due to the esthetic appearance of teeth / *Osećaj neprijatnosti usled estetskog izgleda zuba*	✈	✈	✈	✈	✈
f) Felt tense because of problems with teeth or mouth / *Osećaj nelagodnosti usled problema u usnoj duplji*	✈	✈	✈	✈	✈
g) Avoided smiling because of the esthetic appearance of the teeth / *Izbegavanje osmehivanja usled estetskog izgleda zuba*	✈	✈	✈	✈	✈
h) Had sleep that is often interrupted due to severe pain / *Da li ste se budili tokom noći usled bola*	✈	✈	✈	✈	✈
i) Took days off work / *Da li ste uzimali slobodne dane sa posla*	✈	✈	✈	✈	✈
j) Difficulty doing usual activities/ *Otežano izvođenje svakodnevnih aktivnosti*	✈	✈	✈	✈	✈
k) Felt less tolerant of a spouse or people who are close to you/ *Osećali se manje tolerantno prema supružniku i bližoj okolini*	✈	✈	✈	✈	✈
l) Reduced participation in social activities/ *Da li ste smanjili društvene aktivnosti*	✈	✈	✈	✈	✈

11. How often do you eat or drink any of the following foods, even in small quantities? (Read each item)/ *Koliko često konzumirate navedenu hranu i piće (makar i u manjim količinama)?*

	Several times a day/ *Nekoliko puta dnevno* 6	Every day / *Svaki dan* 5	Several times a week / *Nekoliko puta nedeljno* 4	Once a week / *Jednom nedeljno* 3	Several times a month / *Nekoliko puta mesečno* 2	Seldom/ never / *Retko/ nikad* 0
Fresh fruit/ *Sveže voće*	✈	✈	✈	✈	✈	✈
Biscuits, cakes, cream cakes/ *\Keks, kolači, kremasti kolači*	✈	✈	✈	✈	✈	✈
Sweet pies, buns/ *Slatke pite, lepinje*	✈	✈	✈	✈	✈	✈
Jam or honey/ *Džem ili med*	✈	✈	✈	✈	✈	✈
Chewing gums containing sugar/ *Žvakaće gume sa šećerom*	✈	✈	✈	✈	✈	✈
Sweets/candies/chocolate / *Slatkiši /čokolada*	✈	✈	✈	✈	✈	✈
Lemonade, Coca Cola or other soft drinks/ *Limunada, Koka-Kola, i druga bezalkoholna pića*	✈	✈	✈	✈	✈	✈
Tea with sugar / *Čaj sa šećerom*	✈	✈	✈	✈	✈	✈
Coffee with sugar/ *Kafa sa šećerom*	✈	✈	✈	✈	✈	✈
Characteristic drink for our country / *Karakteristično piće za našu državu*	✈	✈	✈	✈	✈	✈

12. How often do you use any of the following types of tobacco? (Read each item)/ ***Koliko često konzumirate duvanske proizvode?***

	Every day/ Svaki dan 6	Several times a week/ Nekoliko puta nedeljno 5	Once a week/ Jednom nedeljno 4	Several times a month/Nekoliko puta mesečno 3	Seldom/ Retko 2	Never/ Nikad 1
Cigarettes / *Cigarete*	✈	✈	✈	✈	✈	✈
Cigars/ *Tompusi*	✈	✈	✈	✈	✈	✈
A pipe/ *Lula*	✈	✈	✈	✈	✈	✈
Chewing tobacco/ *Duvan za žvakanje*	✈	✈	✈	✈	✈	✈
Use snuff/ *Burmut*	✈	✈	✈	✈	✈	✈
Other/*Drugo*	✈	✈	✈	✈	✈	✈

13. During the past 30 days, on the days you drank alcohol, how many drinks did you usually drink per day? / ***U poslednjih 30 dana, u danima kada ste konzumirali alcohol, koliko pića ste obično pili u toku dana?***

Less than 1 drink/ *Manje od 1 pića*	✈	0
1 drink/ *1 piće*	✈	1
2 drinks/ *2 pića*	✈	2
3 drinks/ *3 pića*	✈	3
4 drinks/ *4 pića*	✈	4
5 or more drinks/ *5 ili više pića*	✈	5
Did not drink alcohol during the past 30 days/ *Nisam pio/pila alkohol u poslednjih 30 dana*	✈	9

14. What level of education have you completed?/ ***Nivo obrazovanja?********

No formal schooling / *Nemam formalno obrazovanje*	✈	1
Less than Primary school / *Osnovna škola*	✈	2
Primary school completed/ *Srednja škola*	✈	3
Secondary school completed / *Fakultet*	✈	4
High school completed/ *Master studije*	✈	5
College/university completed / *Specijalističke studije*	✈	6
Postgraduated degree/ *Doktorske studije*	✈	7
(Insert country-specifi c categories)		

*Level of education in the Serbian version of the "Oral Health Questionnaire for Adults" was adopted according to the education system of the Republic of Serbia.

Statistical analysis

In the descriptive statistics, categorical variables (presented as percentages) and continuous variables (presented as mean ± standard deviation) were calculated. Additionally, we performed analysis to evaluate the reliability and validity of items considering Oral Health Self-Assessment (i.e. question 10 – experience of reduced quality of life due to oral problems).

The classical test theory was used in data analysis. The reliability of the "Oral Health Questionnaire for Adults" was determined by internal consistency coefficient, i.e. Cronbach's coefficient alpha. Cronbach's coefficients > 0.70 were considered acceptable, while values ≥ 0.80 were preferable.

The validity of the "Oral Health Questionnaire for Adults" was evaluated by Principal components analysis. The Promax rotation was used for this analysis, with Kaiser Normalization (delta = 0). The importance of different factors was assessed according to the Kaiser criterion (all factors with eigenvalues greater than 1.0). Value for the Kaiser-Meyer-Olkin measure of sampling adequacy was 0.888, while the value for Bartlett's test of sphericity was highly significant ($p < 0.001$). Statistical analyses were performed using the SPSS 20.0 (IBM SPSS Statistics, Chicago, IL, USA).

Results

The total number of 1,741 participants took part in the survey (Table 2). The average age of the study group was 32.4 ± 9.7 (18–59) years. Out of the total number of participants, 184 participants, i.e. 10.6% were women.

Table 2

Baseline characteristics of the study participants (n = 1,741)

Characteristics	Number (%)
Sex	
male	1,557 (89.4)
female	184 (10.6)
Age groups (years)	
≤ 20	212 (12.2)
21–30	584 (33.5)
31–40	545 (31.3)
41–50	339 (19.5)
≥ 51	61 (3.5)
Marital status	
without a partner	841 (48.3)
with a partner	900 (51.7)

The Serbian version of the "Oral Health Questionnaire for Adults" contained 14 questions (Table 1). The condition of the teeth as good, very good and excellent was described by 57.9% of the participants, while 16% described their condition as bad and very bad. The condition of the gums as good, very good and excellent was described by 60.1% of the participants, while 14.8% described their condition as bad and very bad. The largest number of the participants stated that they brushed their teeth twice or more a day (60.5%), and one third once a day (32.9%). Almost all participants (99.25%) stated they used a toothbrush. The

participants used the following as an aid for maintaining oral hygiene: toothpicks (38.48%), dental floss (27.86%), interdental toothbrush (7.98%) and mouthwash (31.36%). Almost all participants used the toothpaste for brushing their teeth (98.1%), and a toothpaste containing fluoride 82.4%. More than half of the study group (55.3%) visited the dentist less than 6 months ago. 8.3% of the participants did not visit a dentist for more than 2 years. Only one participant never visited the dentist.

As a reason to visit the dentist, 35.4% of the participants stated pain and other oral problems, while 16.3% visited the dentist for consultation and advice. In the last 12 months, out of the mentioned problems, participants cited difficulty in food biting (6.2%), difficulty in chewing (5.1%), difficult speech or difficult pronunciation of certain words (1.9%), mouth dryness (2.9%), feeling of displeasure due to the esthetic appearance of teeth (6.8%), feeling of discomfort due to problems in the oral cavity (4.8%), avoiding smiling due to the esthetic appearance of teeth (6.3%), waking up at night due to pain (2%), taking days off from work (1.1%), difficult performance of daily activities (1%), feeling less tolerance toward a spouse or environment (2.8%) and reduction of daily activities (2.4%).

The participants once or more times a day consumed the following food: fresh fruit (37%), cookies, creams, cakes (14.1%), pies, scones (9.7%), jams or honey (8.5%), chewing gum with sugar (13.2%), candies and chocolate (19.1%), lemonade, Coca-Cola, soda (15.8%), tea with sugar (9.6%), and coffee with sugar (27.4%). Regarding tobacco products, almost one-third of the participants consumed cigarettes (31%), cigars (9%), but only 1 or 2 participants (0.1%) used pipes, Cuban cigars, or chewing tobacco. Every fourth participant (25.5%) did not drink alcohol in the last thirty days. Regarding education, the largest part of the study group had high school education (64.8%), less than a third (26.8%) was university-educated, and only 1% had a PhD degree.

The reliability of the "Oral Health Questionnaire for Adults" (items considering Oral Health Self-Assessment) had high internal consistency: the Cronbach's coefficient was 0.879 (Table 3). The intra-class correlation coefficient was significant (0.874, $p < 0.001$).

Following the Principal component analysis and Promax rotation, 1 factor with Eigenvalue exceeding 1 was observed, accounting for 54.3% of the total variance (Table 4).

Table 3

Descriptive statistics for the "Oral Health Questionnaire for Adults" among the Serbian military personnel (items considering Oral Health Self-Assessment), with corrected item-scale correlations and Cronbach's alpha values, if the item is deleted

Items	Mean ± standard deviation	Corrected item-total correlation	Cronbach's alpha if item deleted	Cronbach's alpha coefficient	Intra-class correlation coefficient
Difficulty biting food	1.3 ± 0.7	0.622	0.861	0.879	0.874 ($p < 0.001$)
Difficulty chewing food	1.2 ± 0.7	0.671	0.856		
Difficulty with speech/ pronouncing words	1.1 ± 0.4	0.569	0.864		
Dry mouth	1.2 ± 0.6	0.415	0.873		
Felt embarrassed due to the appearance of teeth	1.3 ± 0.7	0.670	0.857		
Felt tense because of problems with teeth or mouth	1.3 ± 0.6	0.664	0.857		
Have avoided smiling because of teeth	1.3 ± 0.7	0.662	0.857		
Had sleep that is often interrupted	1.1 ± 0.5	0.590	0.863		
Have taken days off work	1.1 ± 0.4	0.448	0.871		
Difficulty doing usual activities	1.1 ± 0.4	0.602	0.864		
Felt less tolerant of a spouse or people who are close to you	1.1 ± 0.4	0.565	0.865		
Have reduced participation in social activities	1.1 ± 0.5	0.390	0.873		

Table 4

Factor analysis with Promax Rotation Method for the "Oral Health Questionnaire for Adults" among Serbian military personnel (items considering Oral Health Self-Assessment)

Items	Component matrix	Communalities
	1	
Difficulty biting food	0.740	0.542
Difficulty chewing food	0.731	0.588
Difficulty with speech/ pronouncing words	0.731	0.419
Dry mouth	0.728	0.247
Felt embarrassed due to the appearance of teeth	0.696	0.693
Felt tense because of the problems with teeth or mouth	0.693	0.635
Have avoided smiling because of teeth	0.682	0.654
Had sleep that is often interrupted	0.656	0.494
Have taken days off work	0.647	0.515
Difficulty doing usual activities	0.547	0.615
Felt less tolerant of a spouse or people who are close to you	0.497	0.515
Have reduced participation in social activities	0.493	0.593
% variance	54.3	

Discussion

Considering the lack of adequate data on oral health status of professional members of the Serbian Armed Forces, the main goal of our work was to translate the "Oral Health Questionnaire for Adults" from English into Serbian and apply it to the military population.

The WHO manual "Oral Health Surveys – Basic Methods" has encouraged countries to conduct standardized oral health surveys that are comparable internationally. The WHO Global Oral Health Data Bank collates the data gathered through country surveys on the burden of oral disease [5].

Oral health is a key indicator of overall health, well-being, and quality of life. WHO defines oral health as "a state of being free from chronic mouth and facial pain, oral and throat cancer, oral infection and sores, periodontal (gum) disease, tooth decay, tooth loss, and other diseases and disorders that limit an individual's capacity in biting, chewing, smiling, speaking, and psychosocial well-being" [15]. The Global Burden of Disease Study 2016 estimated that oral diseases affected at least 3.58 billion people worldwide, with caries of the permanent teeth being the most prevalent of all conditions assessed. Globally, it is estimated that 2.4 billion people suffer from caries of permanent teeth and 486 million children suffer from caries of primary teeth [16].

The "Oral Health Questionnaire for Adults" has been used in national surveys in many countries [17, 18]. We used the Serbian version of the questionnaire "Oral Health Questionnaire for Adults" in our study according to recommendations of WHO [4]. The recommendation of WHO is to use a single questionnaire to form the global database, but each country has the right and possibility to adjust certain questions to their needs. After the translation of the questionnaire and a successful conduction of the pilot study, we conducted the study of self-assessment of oral health with 1,741 professional members of the Serbian Armed Forces.

All participants who took part in the study responded to each question from the questionnaire, i.e. the response rate was 100% to all questions. This indicates that all questions were clear, concrete and understandable. The items that might be reducing the consistency of the "Oral Health Questionnaire for Adults" among Serbian military personnel (items considering Oral Health Self-Assessment) were: "Dry mouth" and "Have reduced participation in social activities". Also, one item only ("Dry mouth") had low communality (0.247) in our study. However, the questions were asked by 2 dentists trained in the use of this questionnaire, who explained the meaning of the questions to all participants in the best possible way, which minimized the potential impact of information bias.

The largest number of the participants (85.6%) had more than 20 teeth, which is almost identical to the results of the national study made in Chile [19].

In our study, the condition of teeth is described as very good, good and excellent by 57.9% participants. In a study conducted also among the military population, it was found that dental hygiene could be affected by the self-assessment of dental condition. Participants who brush their teeth less than twice a day evaluated their oral health as poor 3.08 times more often compared to those who brush their teeth more than twice a day [20].

In a national study in Canada [21], 84% of respondents rated their dental condition as good or excellent, while in our study only 57.9% of the participants rated their oral health as good or excellent. This is probably because the awareness of the importance of oral health is not yet sufficiently established in our country. Many factors influence how often people use dental services. The reason a person seeks dental care is influenced by the type of care they are likely to receive and the level of untreated problems they may have at any time. People who visit a dental professional for a routine check-up are most likely to benefit from early detection and treatment and receive preventive ser-

vices. Those who seek care for a dental problem may receive less comprehensive treatment and are less likely to receive preventive services [22].

Certain health habits are the main cause of oral disease. Poor or uncontrolled dietary habits, tobacco, unhealthy lifestyle, avoidance of oral health check-ups are all detrimental to the oral well-being of individuals [23].

Toothbrushing is considered a fundamental self-care behavior for the maintenance of oral health, and brushing twice a day has become a social norm, but the evidence base for this frequency may be weak [24]. In many countries, including America and Australia, brushing twice a day has become the social norm [24]. The largest part of our study group brushed teeth 2 or more times a day. A previous study of the Chinese population showed that, in general, a high percentage of the 2,105 respondents reported inadequate oral hygiene practices (i.e. 66.7% or 1,402 of respondents brushed their teeth once a day or less) [25]. More than half of our research group (55.3%) visited the dentist less than 6 months ago, which is similar to a study done in Canada [26]. The frequency of dental visits was also positively associated with dental brushing, toothpaste use, high educational level, being married, having more than 20 teeth, and having dental pain. w[27]. Besides toothbrush, as the basic means for the maintenance of oral hygiene, 27.86% of participants used dental floss, while 31.36% used mouthwash. In the study done in Malaysia, 20.2% of participants used mouthwash, and 18.9% of them used dental floss [28].

5.1% of participants had difficulties chewing food in the last 12 months, which is significantly less regarding the study that included a large number of European countries, where 14% of the study group had difficulty chewing due to teeth and mouth problems [29].

Most food and drinks have little noticeable effects on dental health. Among the drinks that are most likely to damage teeth and restorative materials are sports and energy drinks which contain sugar to feed oral bacteria, and drinks which have a low pH which can erode teeth and increase their sensitivity [30].

Regarding nutrition habits, 19% of participants in the European study drank Coca-Cola and other sodas daily [29], while in our study the percentage was 15.8%. As a reason to visit the dentist, 35.4% of participants in our study stated the pain and problems with teeth, palate or mouth, while only 17% of participants in the European study stated that the reason for the last visit to the dentist was pain and problems with teeth. This difference can be sought in insufficient education, fear from a dentist or even worse financial situation [29].

The limitation of this study is that the sample was made only on a sample of the military population, and it is necessary to do a clinical examination in a subsequent study and compare it with the results of our study.

This study aims to encourage national oral health planners to standardize measurements of oral diseases and conditions that are important for the planning and evaluation of oral health programs, and to ensure the comparability of data collected in the general population.

Conclusion

The findings suggest that the Serbian version of the "Oral Health Questionnaire for Adults" can be used for measuring oral health among the personnel of the Serbian Armed Forces. The questionnaire is short, easy to understand, acceptable to patients and feasible to apply at the clinic.

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Relationships between personality traits, negative affectivity and procrastination in high school students

Relacije između osobina ličnosti, negativnog afektiviteta i prokrastinacije kod srednjoškolaca

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Abstract

Introduction/Aim. Procrastination includes intentional postponing of either starting or finalizing a task and is one of the most important aspects of an academic achievement. The aim of this study was to examine the possibility of procrastination prediction based on the values of certain personality traits and negative affective states (anxiety, depression, stress) in high school students. **Methods.** The research was conducted over a sample of 900 high school students from 9 high schools in Niš attending the 3rd and 4th grade. The research included the following instruments: The Big Five Inventory (BIF), Depression, Anxiety and Stress Scale (DASS-21), as well as the Procrastination Scale. **Results.** The model consisting of personality traits was a statistically meaningful predictor of procrastination. Prediction variables found to be statistically significant were neuroticism, as a positive correlate of procrastination, and conscientiousness, as a negative correlate. None of the negative affectivity dimensions (anxiety, depressiveness and stress) was partially shown to be a significant predictor. **Conclusion.** The lack of conscientiousness can be considered to be the core of procrastination, whereas neuroticism, i.e. some of its facets, such as anxiety, may be encouragement to avoid a task. Obtained results may be used as suggestions to psychological institutions in schools as to which factors are significant for perceiving procrastination problems with students.

Key words:
anxiety; personality assessment; procrastination; students; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Prokrastinacija podrazumeva namerno odlaganje otpočinjanja ili završavanja nekog zadatka i jedan je od bitnih aspekata akademskog postignuća. Cilj ovog rada bio je ispitivanje mogućnosti predviđanja prokrastinacije na osnovu poznavanja vrednosti osobina ličnosti i dimenzija negativnog afektiviteta (stres, anksioznost i depresivnost) kod učenika srednjih škola. **Metode.** Istraživanje je sprovedeno na uzorku od 900 srednjoškolaca iz 9 srednjih škola u Nišu, koji pohađaju treći i četvrti razred. U istraživanju su korišćeni sledeći instrumenti: Inventar velikih pet (*Big Five Inventory* – BFI), Skala depresivnosti, anksioznosti i stresa (DASS-21) i Skala prokrastinacije. **Rezultati.** Model koji čine osobine ličnosti bio je statistički značajan prediktor prokrastinacije. Prediktorske varijable koje su se pokazale kao statistički značajne bile su neuroticizam, kao pozitivni korelat prokrastinacije i savesnost kao negativan korelat. Niti jedna od dimenzija negativnog afektiviteta (anksioznost, depresivnost i stres) nije se parcijalno pokazala kao značajan prediktor. **Zaključak.** Nedostatak savesnosti se može smatrati suštinom prokrastinacije, dok neuroticizam, odnosno pojedine njegove facete, poput anksioznosti, mogu biti podsticaj za izbegavanje zadataka. Rezultati se mogu upotrebiti u svrhu davanja preporuka psihološkim službama u školama o tome koji su faktori značajni u sagledavanju problema prokrastinacije kod učenika.

Ključne reči:
anksioznost; ličnost, procena; aktivnost, odlaganje; studenti; ankete i upitnici.

Introduction

Procrastination or intentional delay is a form of behavior that involves delayed decision-making or/and completing tasks, leaving them for later despite the fact that such behavior can lead to negative consequences. It is one of the more important factors affecting the academic achievement of students. Burka and Yuen [1] believe that the protection of self-

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esteem might be a potential reason for procrastination. Namely, self-esteem depends on the ability assessment, and based on the tasks performed. When the completion of tasks is delayed, it is not easy to assess one's abilities, neither by others nor by oneself, thus the individual's self-esteem is undermined.

Solomon and Rothblum [2] define procrastination as the intentional delay of starting or finishing a task. When we talk about procrastination, the context in which procrastination occurs must also be taken into account. According to one study [3], students who procrastinate at university do not necessarily delay their daily tasks. In this sense, procrastination would entail postponing important and necessary tasks by doing something else which is of lower priority and less essential. Poor time-management, as well as poor self-control and self-reinforcement can be some of the reasons for such behavior [4]. Another possible cause, especially among the students, may be the fear of social exclusion, that is, the need to spend time with peers in order to be accepted by them [3].

Rothblum et al. [5] have defined academic procrastination as a tendency to always or almost always delay academic assignments, and always or almost always experience the anxiety associated with this delay. A positive statistically significant correlation was revealed between academic procrastination and personal anxiety and situational anxiety which is in accordance with the theoretical propositions that procrastination always leads to negative consequences, and is accompanied by negative feelings and anxiety in the learning process [6].

The studies that have analyzed procrastination show a high prevalence of procrastination in university students, although the negative consequences of this behavior are being widely discussed and efforts are being made to reduce it [7]. Steel [8] believes that procrastination is a problematic behavior because around 50% of people who procrastinate experience the negative consequences of such behavior. The data on postponing important tasks by university students say that the frequency of procrastination ranges between 50% and 90% [9].

When examining personality traits, a series of studies have consistently confirmed the association of low conscientiousness and, to some extent, elevated neuroticism with procrastination as a trait [10, 11]. The correlations between conscientiousness and procrastination are often so high that they suggest that procrastination is only one of the facets of conscientiousness [11]. However, despite the relatively high negative correlations between conscientiousness and procrastination, the researchers agreed that these were two separate constructs, with conscientiousness being a higher-order factor and explaining 24% of the variance of procrastination [12].

Procrastination is often linked to an individual's emotional functioning. The authors who report on a positive relationship between anxiety and procrastination point out that procrastinators often emphasize their fear of failure. People prone to procrastination show a greater degree of anxiety than those not prone to procrastination. Some researchers consider anxiety to be the primary motive for chronic procrastination [10]. Lay [13] links procrastination to negative affective outcomes such as high levels of depression and anxiety and low levels of self-esteem. Van Eerde [11] reports a moderate relationship between procrastination and depression. The abovementioned author suggests that there is not enough clear empirical evidence regarding the direction of causality between procrastination and mood variables: whether procrastination is a consequence of depression and/or anxiety, or procrastination itself plays a role in stimulating these conditions. Either way, avoidance behavior is a central component in the conceptualization and treatment of depressive and anxiety disorders.

Studies on procrastination in our country and region indicate the average level of procrastination among the students [14, 15] and a low but statistically significant relationship between procrastination and anxiety [16], as well as the negative correlation with average grade point as the quantitative measure of academic success.

By conducting an analysis on a random sample of students (n = 240) attending the University of Rijeka, the authors found that students who repeat the year procrastinate more than students who complete their studies on time [3]. According to the same research, there is a positive correlation between procrastination and the symptoms of depression, and a negative correlation between procrastination and life satisfaction, self-satisfaction as a student and the average grade during studies.

This study tended to identify some of the determinants of procrastination in order to shed light on this topic, without going into the microanalysis of the cause and effect relationships of individual correlates. Particular emphasis was placed on personality traits and negative affectivity dimensions as some of the correlates of procrastination analyzed so far.

The overall aim of the study was to examine the relationships between personality traits (neuroticism,extraversion, openness to experience, agreeableness and conscientiousness), dimensions of negative affectivity (depression, anxiety and stress) and procrastination in a sample of high school students. More specifically, the study examined the possibility of predicting procrastination based on personality traits and negative affectivity in the sample. To be more precise, this study intended to establish the possibility of procrastination prediction based on personality traits as well as negative affectivity on the aforementioned sample. In addition to that, differences in the level of procrastination expressiveness with regards to the gender and class of the examinees were examined.

Methods

Sample

The sample consisted of 900 students from 9 high schools in Niš. The call for participation was sent to all high schools in Niš. Nine schools responded, and their principles provided their consent for research realization. Third-grade and fourth-grade high school students were examined. The schools surveyed were: four grammar schools (400 students in total); High School of Economics (100 students); School

of Catering and Tourism (100 students); Trading School (100 students); Food Processing and Chemistry School (100 students); Medical School (100 students). Quota sampling was used. The quotas were based on the number of respondents by school and grade attended (100 students from each school and 50 students from each grade, that is, 450 students from each grade in total). Gender-wise, the sample included 332 boys and 568 girls.

Survey procedure

The students were surveyed during the class, and they were previously explained the purpose of the research, as well as the anonymity of the data. All students agreed to participate in the survey. The survey was conducted during the 2017/18 school year. The research was conducted within the project "Help and Support in Creating a Professional Profile – Choose the Right Path" financed by the City of Niš, organized by the Students Psychological Counseling within the Students Cultural Center and Center for Students Support within the University of Niš. The University of Niš and the Faculty of Philosophy in Niš provided their support as well as consent for the research realization. Particular consents for research conducting within the examined schools have been signed by the principles of all schools participating in the research.

Research variables and instruments

Personality traits (neuroticism, extraversion, openness to experience, agreeableness and conscientiousness) were operationalized with scores from the Big Five Personality Inventory subscales (Big Five Inventory – BFI) [17]. This instrument represents the operationalization of the Big Five personality traits. It contains 44 items used to evaluate five dimensions. All subscales have a satisfactory level of reliability: in a sample of 599 members of the general population (348 females) between 16 and 72 years of age, the Cronbach's alpha is 0.69 for extraversion, 0.70 for agreeableness, 0.66 for conscientiousness, 0.65 for neuroticism, and 0.81 for openness to experience [18]. The scale is available for use. In a sample of 900 high school students, the Cronbach's alpha was as follows: 0.67 for extraversion, 0.73 for agreeableness, 0.70 for conscientiousness, 0.81 for neuroticism, and 0.78 for openness to experience.

Depression, anxiety, and stress – these variables were operationalized with scores from the Depression, Anxiety, and Stress Scale subscales (DASS-21) [19]. The scale consists of three subscales: Depression (e.g., "I felt I had nothing to hope for"), Anxiety (e.g., "I felt scared for no reason"), and Stress (e.g., "I noticed I was getting annoyed"). The respondents gave the responses by using the 4-level Likert-type scale (from 0 – not at all, to 3 – mostly or almost always), in order to say how they had felt in the past week. The study used the official translation of the DASS-21 scale into Serbian [19]. The reliability of the entire scale expressed by the Cronbach's alpha is high and amounts to 0.92. The reliability of the scale for the tested sample was 0.91.

Procrastination was operationalized with the score from the Procrastination Scale [20]. It was designed to assess the tendency to procrastinate in general across a range of tasks. It contains twenty statements, twelve of which relate to obligation delay and eight to non-avoidance behavior. The scale is the five-level Likert type, with a range from 1 – extremely uncharacteristic to 5 – extremely characteristic. Adding up all the items gives a sum whose higher value indicates a greater tendency to procrastinate. The Cronbach's alpha is 0.82 which indicates the scale is reliable. The authors of this paper obtained permission to use the scale for research purposes. After obtaining permission to use the scale, a reverse translation was performed and the scale was used in a sample of high school students in Niš. The reliability of the scale on the sample tested was 0.78.

Questionnaire on sociodemographic data designed for the purposes of this research contained the questions regarding student gender, grade, high school attending, and parents' education level.

Data analysis

In order to examine the possibility of procrastination prediction based on personality traits as well as negative affectivity dimensions, descriptive statistics technique and technique of hierarchy linear regression were used.

Results

Table 1 shows the descriptive parameters related to the procrastination.

During the first set of correlation analysis, the relationship between personality traits, negative affectivity dimensions and procrastination were examined (Table 2). As for the results relevant to this research, significant relationship between neuroticism, extraversion and conscientiousness on the one hand, and procrastination on the other were identified. Namely, the findings showed that neuroticism had a positive correlation with procrastination, while the remaining four personality dimensions had a negative correlation with the phenomenon in question. With respect to the correlation between anxiety, depression and stress, they were also significant and positive, that is, the higher the values of a person's negative affectivity dimensions, a person is more likely to procrastinate.

Intercorrelation values among the dimensions of the

Table 1

Descriptive indicators of high school students' procrastination

Variable	Min–Max	Mean ± SD	Variance	Skewness	S.E	Kurtosis	S.E
Procrastination	22.00–98.00	55.45 ± 11.39	129.72	0.04	0.09	0.45	0.17

Min – minimum; Max – maximum; SD – standard deviation; S.E. – standard error.

Five-Factor Model ranged from 0.024 ($p > 0.05$) between neuroticism and extraversion, to 0.266 ($p < 0.01$) between agreeableness and conscientiousness. With regard to the intercorrelation between the different negative affectivity dimensions, the results indicated that the highest correlation exists between stress and anxiety ($r = 0.671$, $p < 0.01$), while the lowest correlation observed was between stress and depression ($r = 0.605$, $p < 0.01$).

Hierarchical regression analysis was used to identify the significant effects of personality traits and negative affectivity dimensions on the level of procrastination. Procrastination was the criterion variable. In order to control the effect of personality traits on procrastination, the extraversion, neuroticism, conscientiousness, agreeableness, and openness to experience were introduced as predictors in the first step, while the affectivity dimensions were added in the second step. Table 3 presents the data related to the possibility of procrastination prediction based on certain psychological variables (including personality traits, as well as negative affectivity traits).

Table 3 clearly showed that both prediction models were statistically significant. In the first model, personality traits (extraversion, agreeableness, conscientiousness, neuroticism, and openness to experience) were a statistically significant predictors of procrastination, and their predictive power was medium in size – it explained 32.7% of the criterion variance. The common variance of personality traits and negative affectivity dimensions were 33.6%, while the change in the percentage of explained variance, after anxiety, depression and stress were introduced to the model, were significant. After introducing the dimensions of negative affectivity into the analysis, extraversion, as a personality trait, lost its statistical significance, while other traits identified as significant predictors were neuroticism, which had a positive correlation with procrastination, and conscientiousness which had a negative correlation with procrastination. None of the dimensions of negative affectivity (anxiety, depression, and stress) were shown to be a significant predictor in the second model.

The independent samples *t*-test was used to verify the significance of differences in procrastination rate in relation to the respondents' gender and grade they attended.

In regard to the gender of the respondents, no statistically significant difference was found between male high school students [mean (M) = 54.90, standard deviation (SD) = 10.72) and female high school students (M = 55.75, SD = 12.06) in terms of procrastination rate ($t = -1.015$; df = 898; $p > 0.05$). The results were similar with regard to the grade the respondents attended. Namely, there was no significant difference found ($t = 1.208$, df = 898, $p > 0.05$) in procrastination between 3rd grade high school students (M = 55.94, SD = 11.48) and 4th grade high school students (M = 54.97, SD = 11.28)

With regards to differences in procrastination expressiveness between the students enrolled in different schools, the research was shown that the highest rate of procrastina-

Table 2

Intercorrelation between personality traits, negative affectivity dimensions and procrastination

Variables	Stress	Anxiety	Depression	Procrastination
Extraversion	0.003	-0.067*	-0.182**	-0.135**
Agreeableness	-0.178**	-0.184**	-0.262**	-0.182**
Conscientiousness	-0.210**	-0.253**	-0.318**	-0.542**
Neuroticism	0.580**	0.474**	0.403**	0.300**
Openness to experience	0.033	-0.039	-0.055	-0.010
Procrastination	0.236**	0.280**	0.311**	–

***Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).**

Table 3

The results of hierarchical regression analysis

Model	Predictors	Model summary	Independent contribution of predictors (ß ; *p*)
1	Extraversion	R = 0.572; R^2 = 0.327; ΔR^2 = 0.324 $F_{(5,894)}$ = 87.003; $p < 0.01$	-0.069 ; < 0.05
	Agreeableness		-0.029 ; > 0.05
	Conscientiousness		-0.485 ; < 0.01
	Neuroticism		-0.169 ; < 0.05
	Openness to experience		-0.058 ; > 0.05
2	Extraversion	R = 0.580; R^2 = 0.336; ΔR^2 = 0.330 $F_{(8,891)}$ = 56.469; $p < 0.001$ Change to R^2 = 0.009; $p < 0.01$	-0.054 ; > 0.05
	Agreeableness		-0.015 ; > 0.05
	Conscientiousness		-0.467 ; < 0.01
	Neuroticism		-0.133 ; < 0.01
	Openness to experience		-0.060 ; > 0.05
	Stress		-0.035 ; > 0.05
	Anxiety		-0.069 ; > 0.05
	Depression		-0.075 ; > 0.0

R – the coefficient of multiple correlation; R^2 – the coefficient of determination; ΔR^2 – the adjusted coefficient of determination; ß – standardized regression coefficient.

tion value was observed in medical school students (M = 58.4, SD = 11.24), while the lowest procrastination rate was recorded in Food Processing and Chemistry School (M = 52.39, SD = 10.90).

The statistical significance of differences in procrastination rate with regard to the school attended by respondents was verified by using the Brown–Forsythe test (Table 4), since Levene's test for homogeneity of variance (Levene's test result was 3.014, $p < 0.01$) showed that it was not statistically justified to use the F-test value because there was no homogeneity of variance across groups.

Based on the results shown in Table 4, we concluded that there were no statistically significant differences in the level of procrastination among students attending different schools.

Table 4

The statistical significance of differences in procrastination rate with regard to the school attended by respondents (Robust Test of Equality of Means)

Test	Statistic[a]	df1	df2	*p*
Brown-Forsythe	1.768	8	718.400	0.08

a – Asymptotically F distributed.

Discussion

It is almost impossible that any of us has not at least once delayed an obligation even though there was no rational reason for it. Although almost all age groups are familiar with the procrastination phenomenon, the majority of authors have devoted their research attention to procrastination among the high school and university students [21]. When it comes to the dominant reasons why procrastination occurs in high school students, authors such as Özer [22], as well as by Ebadi and Shakoorzadeh [23] indicate that more than half of high school students choose to procrastinate for reasons such as fear of failure, difficulty in decision making, laziness and fear of risk-taking. In addition to that, as additional procrastination factors in high school students, Asri et al. [24] listed the following: too burdensome, and difficult to work, insufficient knowledge, too perfectionist, bad management of learning, lack of self-regulation, stress and fatigue, lack of social support and indiscipline teachers. Academic procrastination, most importantly, leads to low learning achievement [24].

With regards to procrastination expressiveness on the examined sample of 900 high school students of both genders, average values (AS = 55.45) were below the theoretical average values (AS = 60), taking into consideration the examined scale.

Our main findings were related to examine the possibility of procrastination prediction based on knowledge of the values of personality traits and dimensions of negative affectivity. The results confirmed the possibility of predicting the level of procrastination based on a set of predictor variables consisting of personality traits such as extraversion, agreeableness, conscientiousness, neuroticism, and openness to experience, as well as on the negative affectivity dimensions (stress, anxiety, and depression). Neuroticism and conscientiousness stand out as significant predictors of procrastination as well: respondents with higher values of neuroticism and lower values of conscientiousness delay their obligations more often, which fully coincides with some foreign studies [5]. Many studies have confirmed a positive relationship between neuroticism and procrastination [8, 10, 12, 13, 25]. Individuals who score high on neuroticism tend to procrastinate more. People with higher negative emotion had weak adaptation rate and low impulse control. Anxiety management training for neurotic persons may result in increase in educational motivation and then decrease in educational procrastination [26]. A study conducted by Johnson and Bloom [25] confirmed the significant predictor role of neuroticism in predicting procrastination. This particularly relates to the facets of impulsivity and vulnerability.

The same situation is observed concerning the conscientiousness dimension. There are also many foreign studies that confirm and support the negative relationship between conscientiousness and procrastination [8, 10, 12, 27]. According to the study which examined academic procrastination among international graduate students, conscientiousness was the only factor that made a unique contribution to the prediction of academic procrastination. Extraversion, neuroticism, openness and agreeableness did not make significantly unique contributions to the prediction of academic procrastination [28]. By emphasizing the general theoretical agreement among most authors on the strong negative relationship between procrastination and conscientiousness, Steel [8] suggests that procrastination should be conceptually defined as poor conscientiousness and self-regulation failure. Conscientiousness, agreeableness, and intellect also showed an indirect effect on reducing academic procrastination, mediated through the strategy of environmental control [29].

Although theoretically and conceptually procrastination is "diametrically opposed" to conscientiousness (especially its aspects such as – responsibility, commitment to hard work, self-discipline), the relationship between these two psychological variables is not so simple and straightforward. This relationship becomes particularly interesting when observed in the broader context of the intercorrelation between the particular dimensions of the Five-Factor Model, which have proven to be significant predictors of procrastination. For example, an interesting model for examining the relationship between neuroticism, conscientiousness, and procrastination is suggested by Lee et al. [12]. Namely, the aforementioned authors conducted a research in a sample of 354 students at the Midwestern University (USA), and received the empirical confirmation of their model, which predicts that conscientiousness is, in fact, a mediating variable between procrastination and neuroticism. However, since this paper was focused solely on the predictor role of personality dimensions in relation to the procrastination, we will not analyze in detail the interrelationship between the variables mentioned in the previous model.

With regard to the relationship between procrastination and certain sociodemographic variables, there were no significant differences in procrastination rates between the re-

spondents of different gender, age, or between those attending different high schools.

The data showing no significant differences in procrastination rate between male and female respondents is not consistent with some studies conducted in the region [16]. Considering that men and women have equal rights in our country in this modern age, and therefore the social expectations of women and men are quite the same, the results obtained showing the lack of differences in procrastination between male and female respondents are not surprising. The absence of differences in procrastination between male and female subjects is consistent with the results of some foreign studies [2].

Considering the structure of the sample, that is, the fact that the sample included the high school students attending the 3rd and 4th grade, the fact that there was no statistically significant difference in procrastination between the students attending different high school grades was expected and logical.

A large number of studies indicated the importance and pervasiveness of the procrastination, especially among college students and high school students [30, 31], and at the same time, they indicated the need to approach this phenomenon in a systematic, empirical manner with the clear intention of minimizing the negative effects of procrastination while, at the same time, acting preventatively in order to further reduced task delays and preserved mental health.

The main limitation of this study could be the sample, which only consisted of the high school students from Niš. In order to produce more generalizable results, high school students from other parts of Serbia should be included as well, since the local environment may be one of the factors directly or indirectly affecting procrastination. Furthermore, it would be useful to include some other important variables that may affect procrastination, such as self-esteem, self-efficacy, and factors related to academic achievement and school environment, etc. Finally, maybe some future studies should consider the interrelationship of the variables studied as well as the possible mediator and/or mediation model in order to identify the direction of influence of the different variables on procrastination.

Conclusion

The results confirmed the possibility to predict procrastination based on the values of personality traits obtained by the Five-Factor Model, as well as based on the values of negative affectivity states. From the aspect of personality traits, neuroticism and conscientiousness were identified as partially significant predictors, where neuroticism had a positive correlation and conscientiousness had a negative correlation with procrastination.

Depression, anxiety, and stress combined with personality traits did not turned out to be significant predictors of procrastination, although all these three concepts had a positive correlation with procrastination. Gender, grade, as well as the high school students attended did not prove to be significant factors that affect procrastination.

Obtained results may be used as suggestions to psychological institutions in schools which factors are significant for perceiving procrastination problems with students. In addition to that, this research might be beneficial to institutions in charge of offering psychological support to students within the University, as the results could be used for preparing prevention programs (recognizing, facing and overcoming early signs of procrastination) for final-year high school students planning to continue their education and become academic workers.

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The research of health education programme efficiency in changing the attitudes and behaviours of dental students in the field of oral health

Ispitivanje efikasnosti zdravstveno-vaspitnog programa u korigovanju stavova i ponašanja studenata stomatologije u oblasti oralnog zdravlja

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Abstract

Introduction/Aim. The interest in oral health quality and the tendency to evaluate, provide, improve and compare it, are constantly improving. The main aim of this research was to examine the effectiveness of health education programme to change habits, attitudes and behaviours of dental students. **Methods.** The research was conducted at the Faculty of Dentistry in Pančevo. The first stage was related to the survey, the measurement of oral health status and the implementation of targeted health education. The second stage was conducted after the health education intervention, as a survey of attitudinal and behavioural changes and clinical measurement of oral health change. The sample consisted of 65 first-year and 54 fourth-year students, a total of 119 students. The following were defined as research instruments: the research record, questionnaires [the original questionnaire, Hiroshima University Dental Behavioural Inventory (HU-DBI) questionnaire] and health education intervention for modelling factors that determine attitudes and behaviours related to oral health. The Decayed-missing-filled (DMF) index was used to evaluate oral health and hard dental tissue conditions. **Results.** At the beginning of the research we asked the students: "Do you think your mouth and teeth condition is good?" Contrary to our expectations, 15.4% of the first-year students and 37% of the fourth-year students did not know the answer, or thought they had a problem. 80% of the first-year and 85.2% of the fourth-year students had positive attitude about the statement that once in six months they should go to the dentist for a check-up. 7.7% of the first-year students did not remember the last time they had visited a dentist. The implemented health education intervention led, to some extent, to changes in habits, attitudes and behaviours of students related to oral health. **Conclusions.** Students of dental medicine do not have sufficiently clear attitudes and safe behaviour regarding oral health. Targeted health education intervention represents the method of choice to take care of one's own health.

Key words:
attitude to health; dental students; knowledge; oral health; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Nedovoljno se zna o uticaju kliničke obuke o oralnom zdravlju na ponašanje studenata stomatologije. Interesovanje za kvalitet oralnog zdravlja i težnja da se ono proceni, obezbedi, unapredi i poredi, stalno je u značajnom porastu. Cilj istraživanja je bio da se ispita efikasnost zdravstveno-vaspitnog programa u korigovanju navika, stavova i ponašanja studenata stomatologije. **Metode.** Istraživanje je sprovedeno na Stomatološkom fakultetu u Pančevu. Prva etapa odnosila se na anketno istraživanje, merenje stanja oralnog zdravlja i implementaciju ciljane zdravstvene edukacije. Druga etapa sprovođena je nakon zdravstveno-vaspitne edukacije, kao anketno istraživanje promene stavova i ponašanja i kliničko merenje promena oralnog zdravlja. Uzorak istraživanja činilo je 65 studenata prve i 54 studenta četvrte godine, ukupno 119 studenata. Kao istraživački instrumenti definisani su: istraživački karton, anketni upitnici (originalni anketni upitnik i *Hiroshima University Dental Behavioural Inventory* – HU-DBI upitnik) i zdravstveno vaspitna intervencija za modelovanje faktora koji određuju stavove i ponašanje u odnosu na oralno zdravlje. Za procenu oralnog zdravlja i stanja tvrdih zubnih tkiva korišćen je *Decayed-missing-filled* (DMF) indeks. **Rezultati.** Na početku istraživanja pitali smo studente da li misle da je stanje njihovih usta i zuba dobro. Suprotno našem očekivanju, 15,4% studenata prve i 37% studenata četvrte godine nije znalo odgovor ili su smatrali da imaju problem. Pozitivno mišljenje o tome da jednom u šest meseci treba otići na kontrolu kod stomatologa imalo je

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80% studenata prve i 85,2% studenata četvrte godine, a 7,7% studenata prve godine studija nije se sećalo poslednje posete stomatologu. Sprovedeni zdravstveno-vaspitni program doveo je, u izvesnoj meri, do poboljšanja u navikama, stavovima i ponašanju studenata u vezi sa oralnim zdravljem. **Zaključak.** Studenti stomatologije nemaju u dovoljnoj meri jasne stavove i bezbedno ponašanje u pogledu oralnog zdravlja. Ciljana zdravstveno-vaspitna edukacija predstavlja metod izbora za preuzimanje brige o sopstvenom zdravlju.

Ključne reči:
stav prema zdravlju; studenti stomatologije; znanje; usta, zdravlje; ankete i upitnici.

Introduction

The importance of oral health is not emphasized enough and that represents the risk factor in terms of the occurrence of oral diseases [1, 2]. Mouth and tooth diseases, though preventable, are very common in economically less developed countries and have a significant impact on both the individuals and society as a whole [3]. The knowledge of oral health is considered one of the basic preconditions for the development of healthy habits. The prevention of oral disease is the most acknowledged and efficient method of ensuring oral health. Oral health is now considered to be equally important in relation to general health [4]. Even though a research in the related study groups indicates that only the existence of knowledge is not sufficient to make changes in people's behaviour, the link between knowledge and better oral health is essential.

Health education, as a part of the oral health promotion, contributes to raising awareness of oral and dental health importance and development of certain skills that can enable the change of risky behaviour. Unfortunately, health promotion activities in Serbia are not systematically and consistently implemented and the health care system is oriented towards treatment rather than prevention of oral diseases [5]. Due to insufficient health sector resources, it is of high importance to select the most effective prevention strategies, such as intervention health programmes that, with reasonable costs, can significantly influence behavioural change, which is relevant for students' health.

For a number of reasons, young students represent appropriate population of respondents to investigate behaviours and attitudes that affect oral health. They are a homogeneous group of a similar educational level and at the age when the aesthetic moment, conditioned by healthy mouth and teeth, is a very important social value. In many countries, students occupy important positions in public life and think as the leaders of the future. Therefore, patterns of students' health behaviour and trust in them can be of particular benefit and it is very important because they are soon to become (or already are) parents, whose role is crucial in forming healthy habits of the population [6].

Going through their studies, students of dental medicine should be able to become a positive model of behaviour towards oral health. They are expected to have a higher level of knowledge, skills and positive attitudes, as well as to possess and take better care of oral health and to be able to have higher influence on their environment, family and society in general. Likewise, their knowledge, behaviour and attitudes should change positively over the years of studies. Because professional (dental) students specialize in preventive information and health promotion, it is important that their own oral health knowledge, attitude, and practice are adequate [7, 8].

Restructuring of dental health care system, as well as the poor realization of continuous education in preventive fields and implementation of prescribed measures of health care programme of the population endanger human oral health. That implies a need for more intensive promotion of oral health in the wider community [9]. A study that evaluates the dental students' application of dental knowledge to enhance their own oral care could be of great benefit, because students will be the ones who will convey the same knowledge and practices to their patients [10].

During their education, students meet patients of different age, different background and therefore, with the right knowledge and their own healthy habits, they can play an important role in the health education of both individuals and groups, and they could be role models to their patients and a wider community as well [4].

The aim of this study was to identify habits, attitudes and behaviours of dental students in relation to oral health, to find mechanisms of changing risk factors that affect oral health, as well as to examine the effectiveness of a health education programme to change habits, attitudes and behaviours of the students of dentistry.

Methods

Research method and plan

The research was conducted as a longitudinal cross-sectional study. Research preparations and the research itself were conducted at the Faculty of Dentistry in Pančevo in two stages: the first stage was conducted before health education intervention (during September and October of the academic year 2018) and was related to a survey, conducted through interviews and dental examination. The measurement of oral health condition was performed at the Dental Clinic of the Faculty of Dentistry in Pančevo. Health education intervention was conducted afterwards in all the examined students of the first and fourth year of studies, with a term of six months. The second stage was conducted with all students of the research sample, after the health education intervention (in March 2019), as a survey of attitude and behaviour changes and clinical measurement of oral health changes.

The research sample consisted of the students of dentistry in the first and fourth year of studies at the Faculty of Dentistry in Pančevo, a total of 119 students, out of which 65 were the first-year and 54 were the fourth-year students. Stu-

dents were selected as a compact group for the observation unit, by simple random choice.

The implementation of this study was approved by the Ethics Committee of the Faculty of Dentistry in Pančevo. Only those respondents having sent written consent in relation to the voluntary participation in the study were included in the mentioned study.

Research instruments

The following were defined as research instruments: research records of oral health condition in respondents, adjusted to the World Health Organization (WHO) parameters [6]; for the evaluation of selected oral health parameters, the Decayed missing (extracted) filled (DMF) index, was used.

For the research of habits, attitudes and behaviours, the following were used: a) the original questionnaire to record respondents' attitudes and behaviours regarding oral health, and b) Hiroshima University Dental Behavioural Inventory (HU – DBI) questionnaire, which was developed by Kawamura and is used to assess behaviour related to oral health [9].

Habits regarding oral health were tested through the group of questions where respondents were asked to express their agreement or disagreement with the provided answers, or possible reasons for certain habits. Habits were measured through two levels of agreement with the given reasons/ claims, and possible answers were affirmative or negative. The accessibility of habits was evaluated on the basis of a three-stage scale, as: health reasons, cleanliness reasons and personal hygiene reasons.

Attitudes regarding oral health were assessed through answers to a group of questions where students were asked to express their opinion about the impact of a particular attitude on behaviour in the field of oral health. Students' attitudes were measured using three levels of agreement with offered statements, and possible answers were affirmative, negative or uncertain. The acceptability of the attitudes was evaluated on the basis of three-step scale, as an acceptable, unacceptable and indefinite attitude. As acceptable attitude was considered opinion that "oral hygiene is important for oral health maintenance", that "healthy teeth and mouth affect the appearance and impression we leave", while as unacceptable or uncertain attitude was considered answer "I am not sure" with stated claims.

Health education intervention for modelling the factors that determine attitudes and behaviour in relation to oral health for the purposes of this research was defined in three stages through three complementary fields as: the importance of oral health, oral hygiene, health-safe habits, and included: *method* (group health education work and communication methods – live demonstrations, creative workshops); *means of research* (visual, audio-visual and demonstration models); *content* (characteristics of good oral health, the importance of oral health for overall health, preventability of oral diseases, control and preventive examinations at the dentist, definition and explanation of terms such as dental plaque, decay, gingivitis, concretions, periodontal disease, oral hygiene, oral hygiene accessories, toothbrush technique, an individual goal in achieving good oral health); practical work (training in proper oral hygiene, training in the use of oral hygiene aids – dental floss, dental floss holder, proximal brush, mouthwashes, oral and dental hygiene control – dental biofilm staining method, toothpaste selection criteria for daily use – interpretation of fluoride composition declaration on toothpaste).

Statistical analysis

The statistical analyses were performed using the SPSS 19.0 software. The obtained data for numerical characteristics were presented in the tables. Out of the methods of descriptive statistics, the arithmetic mean, standard deviation, coefficient of variation and standard error were used. Out of the methods of differential statistics, parametric tests of an independent sample were used in the research (confidence interval for probability $p = 0.95$, ANOVA, Levene's test, Student's *t*-test), parametric test of dependent samples (Paired samples *t*-test), nonparametric tests of independent samples (Pearson's chi-square test, Fisher's exact tests).

Results

Students' assessment of their own oral health at the beginning of the research

Self-assessment of oral health – at the beginning of the research, we asked students if they think the condition of their mouth and teeth was good. With a statistical significance of $\chi^2 = 8.410$, at the level of $p < 0.015$, contrary to our expectations, we received the answer that 15.4% of first-year students, and 37% of fourth-year students did not know the answer to that question, or thought they had a problem (Table 1).

Referring to hard dental tissue condition – the average number of healthy teeth was 20.5, the number of decayed teeth for the entire sample was 0.58, the number of extracted teeth was 0.84, while the average number of filled teeth was 5.94. There was no significant difference between the first and the fourth-year students. The average DMF index in the study groups was 7.36 (Tables 2–5).

Table 1

Self-assessment of oral health - Answers to the question: Do you think your mouth and teeth condition is good at the moment?

Answers	Before the health education intervention			After the health education intervention		
	Year of studies		Total number	Year of studies		Total number
	first	fourth		first	fourth	
Yes, n (%)	55 (84.6)	34 (63.0)	89 (74.8)	54 (84.4)	37 (68.5)	91 (77.1)
No or I have a problem, n (%)	8 (12.3)	12 (22.2)	20 (16.8)	8 (12.5)	11 (20.4)	19 (16.1)
I don't know, n (%)	2 (3.1)	8 (14.8)	10 (8.4)	2 (3.1)	6 (11.1)	8 (6.8)
Total, n (%)	65 (100.0)	54 (100.0)	119 (100.0)	64* (100.0)	54 (100.0)	118 (100.0)
	$\chi^2 = 8.410$;	$p < 0.015$		$\chi^2 = 4.837$;	$p > 0.05$	

***The total number is different because not all students answered the questions from the interview.**

Table 2

Condition of hard dental tissues /DMF

Statistical parameters	Before the health education intervention			After the health education intervention		
	Year of studies		Total number	Year of studies		Total number
	first	fourth		first	fourth	
Number	65	54	119	65	54	119
Minimum	0	0	0	0	0	0
Maximum	15	16	16	15	16	16
Mean	7.569	7.111	7.361	7.277	7.111	7.202
SEM	0.583	0.561	0.407	0.580	0.561	0.405
SD	4.704	4.119	4.435	4.679	4.119	4.416
95% Confidence interval						
lower bound	6.43	6.01	6.56	6.12	5.99	6.40
upper bound	8.71	8.21	8.16	8.44	8.24	8.00
Significance of differences						
t-test (independent samples)	$t = 0.559$; $p > 0.05$			$t = 0.203$; $p > 0.05$		

DMF – decayed, missing, filled; SEM – standard error of the mean; SD – standard deviation.

Table 3

Decayed teeth

Statistical parameters	Before the health education intervention			After the health education intervention		
	Year of studies		Total number	Year of studies		Total number
	first	fourth		first	fourth	
Number	65	54	119	65	54	119
Minimum	0	0	0	0	0	0
Maximum	4	3	4	3	2	3
Mean	0.692	0.444	0.580	0.569	0.241	0.420
SEM	0.122	0.114	0.085	0.116	0.083	0.075
SD	0.983	0.839	0.925	0.935	0.612	0.818
95% Confidence interval						
lower bound	0.45	0.22	0.41	0.34	0.07	0.27
upper bound	0.93	0.67	0.75	0.80	0.41	0.57
Significance of differences						
t-test (independent samples)	$t = 1.484$; $p > 0.05$			$t = 2.216$; $p < 0.05$		

SEM – standard error of the mean; SD – standard deviation.

Table 4

Extracted teeth

Statistical parameters	Before the health education intervention			After the health education intervention		
	Year of studies		Total number	Year of studies		Total number
	first	fourth		first	fourth	
Number	65	54	119	65	54	119
Minimum	0	0	0	0	0	0
Maximum	4	8	8	4	8	8
Mean	0.696	0.685	0.840	0.696	0.685	0.840
SEM	0.183	0.199	0.135	0.183	0.199	0.135
SD	1.479	1.464	1.473	1.479	1.464	1.473
95% Confidence interval						
lower bound	0.60	0.29	0.57	0.60	0.29	0.57
upper bound	1.34	1.08	1.11	1.34	1.08	1.11
Significance of differences						
t-test (independent samples)	$t = 1.048$; $p > 0.05$			$t = 1.048$; $p > 0.05$		

SEM – standard error of the mean; SD – standard deviation.

Table 5

Filled teeth

Statistical parameters	Before the health education intervention			After the health education intervention		
	Year of studies		Total number	Year of studies		Total number
	first	fourth		first	fourth	
Number	65	54	119	65	54	119
Minimum	0	0	0	0	0	0
Maximum	13	14	14	13	14	14
Mean	5.908	5.981	5.941	5.908	6.185	6.034
SEM	0.472	0.476	0.335	0.481	0.482	0.341
SD	3.803	3.499	3.653	3.880	3.545	3.719
95% Confidence interval						
lower bound	4.98	5.05	5.28	4.95	5.22	5.36
upper bound	6.83	6.91	6.60	6.87	7.15	6.71
Significance of differences						
t-test (independent samples)	$t = 0.109$; $p > 0.05$			$t = 0.404$; $p > 0.05$		

SEM – standard error of the mean; SD – standard deviation.

In connection to brushing there was a statistically significant difference in parameters in the study groups at the level of $p < 0.05$. Also, in the sense of a habit as a part of face washing, or personal hygiene maintenance, there was a statistically significant difference at the level of $p < 0.001$ (Table 6).

The frequency of oral hygiene maintenance in the studied groups of students was evaluated through three offered modalities: more than twice a day, once or twice a day, and not every day. It was found that: 77% of first-year students brushed their teeth more than twice a day, 42.6% of fourth-year students brushed their teeth once or twice a day, and 2% of students from both groups did not brush their teeth every day. There was a statistically significant difference in the studied population ($\chi^2 = 6.226$; $p < 0.05$).

Behaviour regarding oral health – students were asked to express their opinions regarding different behavioural modalities in oral health and the factors that may influence oral health. Students' behaviour in the field of oral health and the selected factors were evaluated according to their answers to the questions asked. We found that students in the total sample did not have acceptable or safe behaviour regarding oral health (Table 7). 80% of the first-year students and 85.2% of the fourth-year students had positive opinion about the statement that once every six months they should go for dental examination. 7.7% of the first-year students did not remember the last time they had visited a dentist. However, in daily practice, students behave in the opposite way, since half of the respondents visited a dentist a year ago.

The results of the examination of attitudes regarding oral health are shown in Table 8.

Changes in oral health of the respondents as a result of health education intervention

The implemented health education intervention led, to some extent, to changes in habits, attitudes and behaviours of students (Tables 1 and 6–8).

68.5% of the fourth-year students and 84.4% of the first-year students, after the health education intervention, considered their mouth and teeth condition to be good (Table 1).

After the health education intervention, 87.7% of the first-year students and 100% of the fourth-year students stated that dental health was their reason for regular oral hygiene, and there was a statistically significant difference between the studied groups of students $\chi^2 = 6.486$ at the level of $p < 0.001$ (Table 6).

57.8% of the first-year students and 96% of the fourth-year students, as a reason for regular oral hygiene, cited the fact that they saw it as a part of hygiene in general, and there was a statistically significant difference between the studied groups of students $\chi^2 = 12.158$ at the level of $p < 0.001$ (Table 6). 76.9% of the first-year students and 94.3% of the fourth-year students visited their dentist for a regular check-up, and there was statistically a significant difference between the studied groups of students $\chi^2 = 4.861$ at the level of $p < 0.05$ (Table 8).

Table 6

Reasons for the habit of oral hygiene practicing before and after the health education intervention

Answers to the questions	Before		After	
	Year of studies		Year of studies	
	first	fourth	first	fourth
To remove food debris, n (%)				
yes	63 (96.9)	37 (37.0)	63 (96.9)	47 (100.0)
no	2 (3.1)	0 (0)	2 (3.1)	0 (0)
Total, n (%)	65 (100.0)	37* (100.0)	65 (100.0)	47* (100.0)
	$\chi^2 = 1.161$; $p > 0.05$		$\chi^2 = 1.472$; $p > 0.05$	
To stay healthy, n (%)			57	49
yes	56 (86.2)	50 (98.0)	87.7%	100.0%
no	9 (13.8)	1 (2.0)	8 (12.3)	0 (0)
Total, n (%)	65 (100.0)	51* (100.0)	65 (100.0)	49* (100.0)
	$\chi^2 = 5.125$; $p < 0.05$		$\chi^2 = 6.486$; $p < 0.001$	
Out of habit, as a part of face washing, n (%)				
yes	33 (53.2)	22 (91.7)	37 (57.8)	24 (96.0)
no	29 (46.8)	2 (8.3)	27 (42.2)	1 (4)
Total, n (%)	62* (100.0)	24* (100.0)	64* (100.0)	25* (100.0)
	$\chi^2 = 11.091$; $p < 0.001$		$\chi^2 = 12.158$; $p < 0.001$	

***the total number is different because not all students answered the questions from the interview.**

Table 7

Behaviour regarding oral health before and after the health education intervention

Answers to the questions	Before		After	
	Year of studies		Year of studies	
	first	fourth	first	fourth
Gums often bleed while i am brushing my teeth, n (%)				
yes	5 (7.7)	4 (7.4)	10 (15.4)	4 (7.4)
no	60 (92.3)	50 (92.6)	55 (84.6)	50 (92.6)
Total, n (%)	65 (100.0)	54 (100.0)	65 (100.0)	54 (100.0)
	$\chi^2 = 0.003$; $p > 0.05$		$\chi^2 = 1.808$; $p > 0.05$	
I worry/check if i have a bad breath, n (%)				
yes	61 (93.8)	47 (87.0)	61 (93.8)	45 (83.3)
no	4 (6.2)	7 (13.0)	4 (6.2)	9 (16.7)
Total, n (%)	65 (100.0)	54 (100.0)	65 (100.0)	54 (100.0)
	$\chi^2 = 1.630$; $p > 0.05$		$\chi^2 = 3.350$; $p > 0.05$	
I delay visiting a dentist until my tooth starts to hurt, n (%)				
yes	13 (20.0)	9 (16.7)	13 (20.0)	15 (27.8)
no	52 (80.0)	45 (83.3)	52 (80.0)	39 (72.2)
Total, n (%)	65 (100.0)	54 (100.0)	65 (100.0)	54 (100.0)
	$\chi^2 = 0.217$; $p > 0.05$		$\chi^2 = 0.992$; $p > 0.05$	
I used plaque staining methods to check if my teeth are clean, n (%)				
yes	10 (15.4)	17 (31.5)	10 (15.4)	18 (33.3)
no	55 (84.6)	37 (68.5)	55 (84.6)	36 (66.7)
Total, n (%)	65 (100.0)	54 (100.0)	65 (100.0)	54 (100.0)
	$\chi^2 = 4.357$; $p < 0.05$		$\chi^2 = 5.281$; $p < 0.01$	
I use dental floss every day, n (%)				
yes	17 (26.2)	19 (35.2)	16 (24.6)	18 (33.3)
no	48 (73.8)	35 (64.8)	49 (75.4)	36 (66.7)
Total, n (%)	65 (100.0)	54 (100.0)	65 (100.0)	54 (100.0)
	$\chi^2 = 1.140$; $p > 0.05$		$\chi^2 = 1.098$; $p > 0.05$	
I use mouthwash regularly, n (%)				
yes	33 (50.8)	29 (53.7)	33 (50.8)	25 (46.3)
no	32 (49.2)	25 (46.3)	32 (49.2)	29 (53.7)
Total, n (%)	65 (100.0)	54 (100.0)	65 (100.0)	54 (100.0)
	$\chi^2 = 0.102$; $p > 0.05$		$\chi^2 = 0.236$; $p > 0.05$	

Discussion

The most important task of modelling behavioural factors through health education programmes is to form sustainable habits and safe behaviour for both the individuals and population as a whole.

During their basic studies, students acquire knowledge and skills that are necessary for positive attitude towards prevention. It is expected that there is a difference in oral health of the society as a whole and that of dental students, since students chose dental medicine as their profession and therefore should be able to voluntarily change their hygiene habits and accept themselves as health workers who are or will be motivated enough to improve both themselves and the community. Dental students, the future oral health professionals, play an important role in educating and promoting public oral health. Dental students' oral health attitudes reflect their understanding of the importance of disease prevention and their commitment to improving their patients' oral health. Dental students, in general, have been found to have a positive attitude towards oral health, but their own oral health behaviour must improve if they are to serve as positive models for their patients, families, and friends [11]. These habits are particularly changing after continuous health education programs [12]. However, for some of the items in our questionnaire, a rather high number of unexpected answers were obtained. This indicates that personal attitudes towards prevention are greatly influenced by academic level and gained academic knowledge. Our results demonstrate not only the obstacles, but also the need to break deep-seated prejudices and preconceptions that prevent elementary students from realizing the value and efficiency of prevention. Unsatisfactory answers were registered in students of the fourth year of studies, and this indicates that preventive education is insufficient through the curriculum of basic studies, at least in the first year of studying dental medicine.

This research has shown that personal care for oral health maintenance among students of dental medicine is low at the beginning of health education intervention, and that further educational efforts are needed to achieve an overall

Table 8

Attitudes regarding oral health before and after the health education intervention

Answers to the questions	Before		After	
	Year of studies		Year of studies	
	first	fourth	first	fourth
Oral hygiene is important for oral health, n (%)				
agree	62 (95.4)	53 (98.1)	57 (93.4)	54 (100.0)
don't agree	3 (4.6)	1 (1.9)	1 (1.6)	0 (0.0)
not sure	0 (0.0)	0 (0.0)	3 (4.9)	0 (0.0)
Total, n (%)	65 (100.0)	54 (100.0)	61* (100.0)	54 (100.0)
	$\chi^2 = 0.693$; $p > 0.05$		$\chi^2 = 3.420$; $p > 0.05$	
Healthy mouth and teeth affect the appearance and impressions i leave, n (%)				
agree	63 (96.9)	51 (96.2)	56 (91.8)	53 (98.1)
not sure	2 (3.1)	2 (3.8)	5 (8.2)	1 (1.9)
Total, n (%)	65 (100.0)	53* (100.0)	61*(100.0)	54 (100.0)
	$\chi^2 = 0.043$; $p > 0.05$		$\chi^2 = 2.332$; $p > 0.05$	
I brush my teeth regularly, n (%)				
yes	64 (98.5)	50 (100.0)	64 (98.5)	54 (100.0)
no	1 (1.5)	0 (0.0)	1 (1.5)	0 (0.0)
Total, n (%)	65 (100.0)	50* (100.0)	65 (100.0)	54 (100.0)
	$\chi^2 = 0.772$; $p > 0.05$		$\chi^2 = 0.838$; $p > 0.05$	
I visit the dentist for regular check-ups, n (%)				
yes	50 (78.1)	30 (88.2)	50 (76.9)	33 (94.3)
no	14 (21.9)	4 (11.8)	15 (23.1)	2 (5.7)
Total, n (%)	64 (100.0)	34* (100.0)	65 (100.0)	35* (100.0)
	$\chi^2 = 1.514$; $p > 0.05$		$\chi^2 = 4.861$; $p < 0.05$	

***the total number is different because not all students answered the questions from the interview.**

improvement of oral health, habits, attitudes and behaviour of students [8, 13].

Political, cultural and socioeconomic factors have a great influence on the formation of certain attitudes regarding the oral health of each individual [14–17]. The study of oral health, behaviour and habits, which is influenced by different environments, is difficult because there are overlaps of cultural influences and other factors, such as the knowledge of oral health, socioeconomic status and personal experiences [18, 19].

This study has shown that 15.4% of the first-year students and 37% of the fourth-year students do not know the answer to the question: "Do you think that the condition of your mouth and teeth is good?", or think they have a problem. The results of Swedish study of 20 to 25-year-olds have shown that 59% of respondents are satisfied with the appearance of their teeth [20], while in the Jordanian study that percent was higher – 69% of students were satisfied with the condition of their mouth and teeth [11]. This indicates the need for serious and scientifically proven approaches to promote oral health in order to raise students' level of health awareness. It is the moral responsibility of all the health care personnel to provide an adequate oral care for those patients in need at primary health care institutions [21, 22].

The higher prevalence of tooth decay in our students is related to the lack of implementation of preventive measures and organized health education programmes from an early age, which is specific for most East European countries [23]. Regarding the frequency of oral hygiene maintenance, it was found that 77% of the first-year students brushed their teeth more than twice a day, and 42.6% of the fourth-year students brushed their teeth once or twice a day; however, 2% of students from both groups did not brush their teeth every day. A much higher percentage of Lithuanian dentistry students (92%) brush their teeth twice a day, while the percentage of Indian, Jordanian and Turkish students is much lower [11, 24, 25].

The students of dental medicine were expected to demonstrate good knowledge of oral health in this study, since this is an important content of their professional education and this knowledge is essential for them to educate patients and the community when they begin working in the health care system. However, it seems that there are things that have to be improved, such as practicing regular visits to the dentist, using dental floss, etc [4, 26].

Inadequate student behaviour has also been reported in other studies. This is confirmed by the statements on the existence of bleeding gums in 56% of the first-year students and 44% of the fourth-year students. 45% of Finnish students have bleeding gums, while the results of Australian, Lithuanian, Japanese or Greek students are much better [7, 27, 28].

Students often tend to underestimate the receptivity of soft and hard dental deposits to caries and periodontal disease, and they do not consider it to be a serious health problem like some other chronic medical conditions [1, 29]. Poor

periodontal status is also indicated by Japanese study, where students need dental treatment [30].

Similar to the findings in other studies [31, 32], this study also found positive attitudes regarding oral hygiene and prevention of oral diseases after a health education intervention, the importance of regular oral hygiene for oral health maintenance, healthy teeth and mouth, as well as regular check-ups at the dentist, which affects appearance and impression they leave in community.

In this research, we expected that students of higher study years will express more positive attitudes and more responsible behaviour in relation to oral health than students at the beginning of the studies, but it was completely refuted, because the opposite was the case, which was quite an unexpected finding. The more so, this requires reconsideration of basic study programmes of dental medicine. Obviously, insufficient attention is paid to the preventive risk factors for endangering oral health, i.e. the importance of preventive and promotive health behaviour when it comes to oral health and its applications in daily practice.

After the conducted program, certain changes in oral health were measured. Several studies have confirmed that oral health attitudes become more positive with age and educational level [18, 33]. The results of this research show that the awareness of dental health, as measured by the HU-DBI results, increases with health education intervention. Changes in students' habits, attitudes and behaviour have been observed, which is in accordance with other authors' results [33–36]. Similar studies were conducted at different faculties and in different environments and they all showed the same – that constant professional development of students affects their oral hygiene [37, 38]. To serve as a good model, the improvement of oral health-related behaviour and attitude should start from the 1st year of education [39].

Despite the improvements obtained in our study, six months seems to be a short period to reach definite conclusions.

Conclusion

The established self-assessment of oral health in dental students at the beginning of this research indicates a low level of awareness of their own oral health. The implemented health education intervention led, to some extent, to changes in habits, attitudes and behaviours of students related to oral health. This requires some revisions in the practical training programmes of dental students.

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Individual-psychological factors and perception of social support in burnout syndrome

Individualno-psihološki faktori i percepcija socijalne podrške kod sindroma izgaranja

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Abstract

Background/Aim. Burnout syndrome is a psychological phenomenon that occurs as a response to chronic interpersonal stressors at work. It is manifested by emotional exhaustion, depersonalization and a sense of reduced personal accomplishment. The aim of the study was to examine the correlation between burnout syndrome, individual-psychological factors and social support among special and regular education teachers, as well as to determine differences of burnout syndrome dimensions between groups of teachers with different educational backgrounds. **Methods.** This non-experimental, cross-sectional correlation study included 317 teachers (122 special education teachers and 38 other teachers in special education for children with intellectual disabilities and 157 teachers from regular primary schools) from Belgrade. Maslach Burnout Inventory (MBI), Assertiveness Assessment Questionnaire Scale (A-Scale), Rosenberg Self-Esteem Scale (RSS), Teacher Self-Efficacy Scale (TSES), and Multidimensional Perceived Social Support Scale (MSPSS) were used in the study. **Results.** The highest prevalence of high levels of burnout were recorded on the emotional exhaustion subscale and were 38% for special education teachers, 47% for other teachers in special education, and 39% for teachers in regular education. The most important predictors of burnout in special education teachers were: for emotional exhaustion (self-esteem, efficiency of class management and seniority); for depersonalization (self-esteem, efficiency of class management and social support); for a sense of lower personal accomplishment (assertiveness, effective student learning and class management). The most important predictors of burnout in regular education teachers were: for emotional exhaustion (assertiveness, effective class management, social support and seniority); for depersonalization (self-esteem, effective student learning and seniority); for a reduced personal accomplishment (self-esteem, effective student learning and social support). **Conclusion.** Individual-psychological factors have emerged as an important predictor of burnout syndrome in the teaching profession, indicating the importance of examining the impact of these factors in other professions that are characterized by the specific demands for a deeper emotional investment during the work process.

Key words:
burnout, psychological; education; education, special; educational personnel; psychology; schools; students; social support; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Sindrom izgaranja je psihološki fenomen koji nastaje kao odgovor na hronične izvore stresa na radnom mestu. Ovaj sindrom se manifestuje emocionalnom iscrpljenošću, depersonalizacijom i osećajem smanjenog ličnog postignuća. Cilj rada bio je da se ispita povezanost sindroma izgaranja na poslu sa individualno-psihološkim faktorima i socijalnom podrškom kod nastavnika specijalnog i redovnog obrazovanja, kao i da se utvrdi razlika u stepenu izraženosti dimenzija sindroma izgaranja na poslu između grupa nastavnika različitog obrazovnog profila. **Metode.** U ovoj neeksperimentalnoj, korelacionoj studiji preseka učestvovalo je 317 nastavnika (122 defektologa-nastavnika i 38 nastavnika drugih usmerenja u specijalnim školama za intelektualno ometenu decu i 157 nastavnika iz redovnih osnovnih škola) iz Beograda. U istraživanju su korišćeni: Maslač upitnik sindroma izgaranja (MBI), Upitnik

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za procenu asertivnosti (A-skala), Rozenbergova skala samopoštovanja (RSS), Skala samoefikasnosti nastavnika (TSES) i Multidimenzionalna skala opažene socijalne podrške (MSPSS). **Rezultati.** Najveća zastupljenost visokih nivoa izgaranja na poslu zabeležena je na području emocionalne iscrpljenosti i iznosila je 38% kod defektologa, 47% kod nedefektologa u specijalnim školama i 39% kod nastavnika u redovnom obrazovanju. Najznačajniji prediktori sindroma izgaranja defektologa bili su: za emocionalnu iscrpljenost (samopoštovanje, efikasnost upravljanja odeljenjem i radni staž); za depersonalizaciju (samopoštovanje, efikasnost upravljanja odeljenjem i socijalna podrška); za osećaj smanjenog postignuća (asertivnost, efikasno učenje učenika i upravljanje odeljenjem). Najvažniji prediktori izgaranja na poslu nastavnika redovnog obrazovanja bili su: za emocionalnu iscrpljenost (asertivnost, efikasno upravljanje odeljenjem, socijalna podrška i radni staž); za depersonalizaciju (samopoštovanje, efikasno učenje učenika i radni staž); za osećaj smanjenog postignuća (samopoštovanje, efikasno učenje učenika i socijalna podrška). **Zaključak.** Individualno-psihološki faktori su se izdvojili kao bitan prediktor sindroma izgaranja na poslu u profesiji nastavnika, što ukazuje na značaj ispitivanja uticaja ovih faktora i u drugim profesijama koje se odlikuju specifičnim zahtevima za dubljim emotivnim angažovanjem tokom radnog procesa.

Ključne reči:
sagorevanje na radu, sindrom; obrazovanje; obrazovanje, specijalno; osoblje, obrazovno; psihologija; škole; studenti; socijalna podrška; ankete i upitnici.

Introduction

Burnout syndrome at work is manifested by emotional exhaustion, depersonalization, and a sense of reduced personal accomplishment [1]. It occurs in response to workplace related long-term emotional and interpersonal stressors [2]. In the eleventh edition of the International Classification of Diseases, burnout syndrome was included in the "Employment or Unemployment Problems" (QD85) section [3], and defined thoroughly. This means that the World Health Organization is also considering the risk of burnout syndrome in the workplace in general.

Studies dealing with mental health research indicate the increasing prevalence of burnout syndrome among teachers, at all levels of education [4]. Teachers in their everyday work are focused on the various communication relationships and on problem solving, both of which often lead to increased sense of responsibility, emotional effort and stress. This condition, if lasting long enough, can cause latent or manifest disorders, especially anxiety, but also the development of burnout syndrome [5, 6]. The most common sources of stress in the teaching profession arise from: coping with learning difficulties and behavior of students; conflicts with colleagues; lack of administrative support; problematic relationships with parents; short deadlines and unfulfilled expectations [4, 6].

Intercultural studies on the prevalence of burnout syndrome in teachers show diverse results. The general conclusion is that teachers experience higher levels of stress than the general population, and that special education teachers (SET) are at particular risk [6, 7]. Studies of burnout syndrome among regular education teachers (RET) indicate that primary school teachers have higher burnout than high school teachers [8]. There are also some differences with regard to the type of student's special education needs, so a study in Iran found that those teaching students with autism exhibit significantly higher levels of burnout than teachers of children with intellectual disabilities and hearing impairment [9]. Other study highlights the negative consequences of working with children with emotional disturbance [6]. We could search for a possible explanation for all these findings in the differences that exist in the work positions of teachers and in the social position of the profession [7].

The changes in educational reform that have taken place with the introduction of inclusive education in our country in 2009 led to changes in the educational approach and in setting priorities. This has increased the demands on teachers and their approach to teaching, and can have a stressful effect on both RET and SET. The developmental advancement of children in need is very slow, so SET may feel less successful and have lower sense of personal accomplishment [10]. This may also occur with RET, given that educational reforms in the Republic of Serbia are addressing deinstitutionalization and inclusion of an increasing number of children with developmental disabilities in regular classrooms [11].

The most significant predictors of burnout syndrome are thought to be personality factors, followed by interpersonal relationships, communication skills and social support [12–14]. Some studies indicate that lower self-esteem is associated with greater susceptibility to burnout syndrome in teachers [15]. It has also been confirmed that teachers who have high levels of self-efficacy successfully resist the challenges they face during the work process (problems related to students behavior, learning, communication with parents, etc.), and they do not perceive them as stressful, unlike teachers with low levels of self-efficacy [16, 17]. Various sources of social support may also be closely correlated with burnout syndrome, since the support of loved ones in stressful situations can significantly reduce psychological stress, anxiety and burnout [18, 19].

The importance of individual-psychological factors such as assertiveness, self-esteem and self-efficacy in burnout syndrome can also be understood through the construct of resilience, that is, resistance to stress. Resilience is defined as the ability of an individual to return to its original level of functioning after the action of a stressor or to resist stressor negative impact [20]. Resilience, as a complex construct, is significantly influenced by external factors as well as internal factors, such as self-esteem and self-efficacy [21, 22]. Also, individuals who develop mental, physical and social resources which contribute to their resilience and direct them towards assertive behavior in decision-making, are better prepared to deal with stressors in their working environment [23, 24]. Assertiveness, self-esteem and self-efficacy develop throughout life, through learning, experience and interaction with others, so it is very important to focus on factors that we

can influence in order to prevent burnout and promote positive mental health of employees.

Research into the impact of individual-psychological and environmental factors on the emergence and development of teacher burnout syndrome in our country is scarce. In regard to this, the first aim of the study was to examine the association between burnout syndrome and individual-psychological characteristics: assertiveness, self-esteem, self-efficacy, and perceived social support. The second aim was to examine the potential differences in the severity of burnout syndrome dimensions between special education teachers working with intellectually disabled children and teachers working in regular education.

Methods

This cross-sectional correlation study was conducted from May to July 2018 at the territory of Belgrade Municipality. The study was conducted after obtaining the approval of the Ethics Committee of the Faculty of Medical Sciences in Kragujevac and written consent of the principals and participants of all schools included in the study. Each participant was informed of the basic purpose of the research and signed consent to participate in the research.

Participants

The study group consists of teachers working with children with intellectual disabilities in eight special elementary schools (special education teachers and other teachers in special education, i.e. teachers trained in other fields), and teachers who teach in four regular elementary schools. The selection of regular schools that were included in the study was conducted on the basis of their territorial proximity to the special primary schools included in the survey.

The respondents included in the study were special education teachers in special education (SET group) or other teachers in special education (OTSE group) or regular education teachers (RET group), aged 25 to 60 years with at least one year of professional experience. Teachers with less than one year of professional experience were not selected, in order to avoid the impact of workplace adjustment stress during the first year of employment.

Of the 388 surveyed, only the participants who fully responded to the given battery of instruments (n = 317) entered the final sample. The group of teachers in special education included 122 SET and 38 OTSE participants, while 157 participants were in regular education group. Groups of SET and RET were dominated by women (91% and 84.1%, respectively), while in the group of OTSE this percentage was lower (60%).

There were no differences in age [F (2) = 0.096, $p = 0.909$] between the groups, and the average age of the subjects was 41.56 ± 8.92. The average length of service was 14.04 ± 8.87 years, with no differences between the groups [F (2) = 2.602, $p = 0.076$]. Regarding the level of education, certain differences were obtained [χ^2 (2) = 10.678, $p = 0.005$], primarily because there are almost no special education teachers who have completed only higher education or vocational studies, while in groups of OTSE and RET, this percentage was around 10%. There is no difference between groups in work status [χ^2 (2) = 1.317, $p = 0.251$], because only about a quarter of respondents in each group work part-time. Also, there was no difference in marital status [χ^2 (3) = 3.216, $p = 0.392$].

Instruments

Assertiveness was assessed with the Assertiveness Assessment Scale Questionnaire – A Scale [25]. The scale consists of items describing reactions and behaviors typical of (non)assertiveness. The subject was instructed to use a five-point Likert-type scale (ranging from the constant absence of reactions or behavior to their constant presence), to evaluate their potential reactions in social situations requiring assertiveness. Of the 27 items in the A scale, 13 are positive (assertive), while 14 are negative. The score range is from 27 to 135 points, with a higher score indicating a greater assertiveness [25]. The internal reliability of the scale in our study was high ($\alpha = 0.92$).

Self-esteem is expressed through the score on the Rosenberg Self-Esteem Scale (RSS) [26]. It is a one-dimensional scale that measures a person's global self-esteem or general value orientation toward themselves. The scale contains 10 statements, five in the positive and five in the negative direction, and the respondents answer them on a scale from 0 – "I strongly disagree", to 3 – "I strongly agree". The range of scores is from 0 to 30 where a higher score means a higher degree of self-esteem. The reliability of the scale in our sample was good ($\alpha = 0.81$).

Teacher self-efficacy has been operationalized through the Teacher Self-Efficacy Scale (TSES) [27]. The TSES is designed to identify the difficulties that teachers most commonly encounter in their daily school activities. The scale contains of 12 questions grouped into three subscales: efficacy in instructional strategies, efficacy in classroom management, and efficacy in student engagement. The task of the respondents was to express their degree of agreement with the statement on a five-point Likert scale (from 1 – "not at all", to 5 – "at large"). A higher score indicates greater efficacy. The internal consistency of the scale, in our study was high ($\alpha = 0.91$).

Social support is expressed through the Multidimensional Scale of Perceived Social Support (MSPSS) [28], Serbian version of the scale [29]. This instrument provides a subjective assessment of the support a respondent perceives receiving from family, friends and other important persons. The questionnaire contains 12 items, and the responses range from a complete disagreement with a statement (1), through neutral position (4), to a complete agreement with a statement (7). Only the total score was used in the interpretation of the results, the maximum value of which is 84 and indicates the highest degree of perceived social support [29]. The internal reliability of the scale in our study was high ($\alpha = 0.93$).

Finally, burnout syndrome is expressed through scores on the Maslach Burnout Inventory – Human Service Survey or MBI-HSS [1], its Serbian version [30]. The scale measures the frequency and intensity of work-related burnout in people working in the human services or helping professions. The questionnaire consists of 22 statements, which are quantified on a scale from 0 – "never", 3 – "several times during the month", to 6 – "every day", and contains three subscales that measure: the feeling of

emotional exhaustion (EE) – experience of overexertion and exhaustion of emotional/physical resources; depersonalization (DP) – a negative, overly indifferent reaction to various aspects of work and the experience of alienation from other people in the workplace; a sense of reduced personal accomplishment (PA) – declining sense of competence and successful achievement in working with people. High scores on the emotional exhaustion and depersonalization subscales contribute to burnout syndrome, while high scores on the personal achievement subscale reduce it [1, 30]. The total score range is 0–132. The internal consistency of the scale was good ($\alpha = 0.79$).

Statistical analysis

The sample size was determined by the current number of full-time teachers employed in elementary schools for intellectually disabled children in Belgrade [11]. The distribution of categories for alpha error 0.05, beta error 0.10, and study strength of 90% for two-way testing were calculated. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to test for normal distribution. Arithmetic mean, median, standard deviation, Mann-Whitney-Wilcoxon test, and χ^2 test were used in the analysis. The methods of parametric correlation and regression, as well as non-parametric correlation depending on the distribution, were used in the analysis of the correlation. In all the methods used, the significance level was 0.05. The IBM SPSS Version 21 software package was used.

Results

The results on the work-related emotional exhaustion subscale, in the SET and RET groups showed slightly lower scores than the average theoretical values (Table 1). In contrast, depersonalization scores deviated significantly toward lower values. Finally, on the scale of reduced personal accomplishment, the scores in these two groups deviated significantly toward higher values. In the OTSE group, scores on all dimensions did not deviate significantly from the average.

Distribution of the respondents according to the levels of burnout (Table 2) shows that for emotional exhaustion just over one third of the respondents in the SET and RET groups fell into the high burnout category, while in the OTSE group the number of those respondents was almost 50%. In depersonalization, most subjects were in the low burnout category,

Table 1

Descriptive data for the burnout scale in all groups

Parameter	Group	n	Mean ± SD	Min-Max	Skewness	Kurtosis	*p*
Emotional exhaustion	SET	122	22.60 ± 13.997	1–54	0.313	-0.925	0.064*
	RET	157	22.99 ± 12.197	0–54	0.278	-0.619	0.067*
	OTSE	38	25.50 ± 10.321	0–44	-0.320	-0.224	0.730†
Depersonalisation	SET	122	5.03 ± 5.236	0–24	10.321	10.370	0.000*
	RET	157	5.73 ± 4.985	0–21	10.056	0.648	0.000*
	OTSE	38	5.87 ± 5.126	0–21	0.934	0.657	0.057*
Personal accomplishment	SET	122	38.18 ± 7.355	18–48	-0.645	-0.210	0.015*
	RET	157	38.20 ± 6.779	16–48	-0.956	0.855	0.000*
	OTSE	38	34.34 ± 10.055	10–48	-0.556	-0.354	0.051*

***Kolmogorov-Smirnov test; †Shapiro-Wilk test.**
SET – Special education teachers; RET – Regular education teachers; OTSE – Other teachers in special education; SD – standard deviation.

Table 2

Percentual distribution of burnout categories

Parameter	SET	RET	OTSE
Emotional exhaustion			
low (0–16)	37.7	18.4	35.6
moderate (17–26)	24.6	34.2	25.6
high (27+)	37.7	47.4	38.8
Depersonalisation			
low (0–6)	73.0	57.9	65.6
moderate (7–12)	15.6	28.9	23.8
high (13+)	11.5	13.2	10.6
Personal accomplishment			
low (0–31)	Skewness.9	42.1	13.1
moderate (32–38)	27.0	18.4	29.4
high (39+)	54.1	39.5	57.5

For abbreviations see under Table 1.

although this percentage was slightly higher in the SET group. In terms of personal accomplishment, just over half of the respondents in the SET and RET groups fell into the category of the high accomplishment, while the percentage in the OTSE group was around 40%.

There were no differences in scores on burnout dimensions between SET and RET groups. Although the results for the OTSE group have to be taken with caution due to the small number of subjects, when comparing the results in this group with other two groups, it can be seen that it stands out with higher scores on the dimension of emotional exhaustion [F (2.314) = 1.775, $p = 0.048$] and lower scores on the personal accomplishment dimension [F (2.314) = 4.454, $p = 0.012$], although both differences are low.

Individual-psychological factors

As for the demographic variables, women were found to be slightly more emotionally exhausted (t (277) = 2.164, $p < 0.05$). Similar was shown for those who were divorced [F (3.275) = 5.869, $p < 0.001$]. With the increase in age (r = 0.294, $p < 0.001$), and especially the length of service (r = 0.359, $p < 0.001$), emotional exhaustion increases, while with the increase of seniority, the depersonalization increases (r = 0.202, $p < 0.001$). Finally, the more emotionally drained the subjects were (r = 0.547, $p < 0.001$), with a higher sense of depersonalization (r = 0.415, $p < 0.001$) and low accomplishment (r = 0.422, $p < 0.001$), the more they think about changing jobs.

Regarding determination, the relationship between the intensity of burnout syndrome and these psychological factors, Table 3 presents descriptive data for all three scales for assessment of assertiveness, self-esteem and self-efficacy.

There was no difference between the three groups of respondents at the assertiveness [F (2.314) = 2.620, $p = 0.074$], and self-esteem scale [F (2.314) = 1.439, $p = 0.239$]. There is a difference when it comes to assessing teacher's efficacy: efficacy for learning strategies [F (2.314) = 8.762, $p < 0.001$], and classroom management efficacy [F (2.314) = 8.330, $p < 0.001$], while for student engagement efficacy, this difference is at the border of significance [F (2.314) = 2.846, $p = 0.060$]. All these differences were found to be the result of lower scores in the OTSE group compared to the other two groups (Table 4).

Due to the fact that there were some differences be-

Table 3

Descriptive data for assertiveness, self-esteem and self-efficacy

Parameter	Mean ± SD	Min–Max	Skewness	Kurtosis	*p**
Assertiveness					
SET	97.88 ± 14.332	58–127	-0.101	-0.449	0.129
RET	94.64 ± 14.470	49–130	-0.307	0.326	0.074
OTSE	94.37 ± 13.179	75–126	0.514	-0.493	0.132
Self-esteem					
SET	23.75 ± 4.568	12–30	-0.686	-0.110	0.000
RET	23.93 ± 3.852	9–30	-0.644	0.742	0.000
OTSE	22.58 ± 4.104	15–29	-0.280	-0.958	0.072
Efficacy in instructional strategies					
SET	17.94 ± 2.001	12–20	-0.687	-0.366	0.000
RET	17.65 ± 2.038	11–20	-10.10	10.01	0.000
OTSE	16.29 ± 2.799	12–20	-0.111	-10.279	0.005
Efficacy in classroom management					
SET	16.34 ± 2.781	9–20	-0.494	-0.344	0.000
RET	16.29 ± 2.569	9–20	-0.491	-0.343	0.000
OTSE	14.37 ± 3.258	8–20	-0.051	-0.735	0.181
Efficacy in student engagement					
SET	15.40 ± 2.917	7–20	-0.309	-0.135	0.018
RET	15.74 ± 2.816	9–20	-0.366	-0.653	0.000
OTSE	14.45 ± 2.947	10–20	0.531	-0.797	0.013

***Kolmogorov-Smirnov test.**

For abbreviations see under Table 1.

Table 4

Differences between groups in assertiveness, self-esteem and self-efficacy scores

Group	Efficacy in instructional strategies	Efficacy in classroom management	Efficacy in student engagement
OTSE	16.29	14.37	14.45
RET	17.62	16.27	15.68
SET	17.94	16.34	15.40

tween the groups, the correlations with burnout syndrome were evaluated for each group separately (Table 5).

A moderate to high positive correlation was found in the SET and RET groups between sense of personal accomplishment and all psychological variables. The negative correlation was found between the other two aspects of burnout syndrome and the mentioned variables. Similar data were obtained in the group of OTSE, only with lower coefficients.

Social support

As shown in the Table 6 (the relationship between the intensity of burnout syndrome and the perceived social support), the scores deviate towards higher values in all three groups, as respondents estimate that they receive relatively high levels of social support from their social environment.

No significant differences in scores were obtained on the social support scale between the groups [F (2.314) = 0.172, $p > 0.05$], so the correlation between the aspects of burnout syndrome and social support was analyzed for the whole sample. Spearman's correlation coefficient ranged from moderately positive in personal accomplishment (r = 0.440, $p < 0.001$), to moderately negative in depersonalization (r = -0.473, $p < 0.001$), and highly negative in emotional exhaustion (r = -0.573, $p < 0.001$).

Prediction of burnout syndrome

In the last part of the statistical analyses, a regression analysis was performed. For the SET and RET groups, those variables which showed at least moderate correlations with the burnout dimensions, and without high inter-correlation, were included in the predictive models. The model for a group of OTSE was not tested due to small number of subjects, the number of outliers, and quite low correlations [31].

In the group of SET, several variables which significantly predicted the variance of the criterion variable stood out. The model is significant [F (3.118) = 36.132, $p < 0.001$] and explains as much as 48% of variance (Adjusted R Square is 46.6%). Table 7 shows the values for the predictor variables that were found to be significant.

Emotional exhaustion in the RET group was significantly predicted by the model shown in Table 8 [F (4.152) = 31.129, $p < 0.001$]. This model predicts 45% of variance (Adjusted R Square is 43.6%). In Table 8, the values for the predictor variables that were found to be significant were given.

Table 5

Correlations of assertiveness, self-esteem and self-efficacy scores with MBI-HSS Scores (by groups)

Groups	EE	DP	PA
SET			
assertiveness	-0.513**	-0.443**	0.501**
self-esteem	-0.613**	-0.635**	0.430**
efficacy in instructional strategies	-0.322**	-0.484**	0.485**
efficacy in classroom management	-0.509**	-0.479**	0.450**
efficacy in student engagement	-0.475**	-0.317**	0.336**
RET			
assertiveness	-0.461**	-0.337**	0.375**
self-esteem	-0.438**	-0.413**	0.487**
efficacy in instructional strategies	-0.187*	-0.320**	0.417**
efficacy in classroom management	-0.445**	-0.385**	0.493**
efficacy in student engagement	-0.437**	-0.429**	0.506**
OTSE			
assertiveness	-0.248	-0.426**	0.432**
self-esteem	-0.077	-0.550**	0.533**
efficacy in instructional strategies	-0.044	-0.421**	0.535**
efficacy in classroom management	0.304	-0.287	0.342*
efficacy in student engagement	-0.503**	-0.364*	-0.460**

***$p < 0.05$; **$p < 0.01$.**

EE – emotional exhaustion; DP – depersonalization; PA – personal accomplishment; MBI-HSS – Maslach Burnout Inventory-Human Service Survey.

For other abbreviations see under Table 1.

Table 6

Descriptive data for the Social support scale

Group	n	Mean ± SD	Min–Max	Skewn.	Kurtosis	*p**
SET	122	73.97 ± 12.408	29–84	-1.729	2.748	0.000
RET	157	74.71 ± 8.941	36–84	-1.665	3.628	0.000
OTSE	38	73.68 ± 9.112	51–84	-0.722	-0.317	0.007

***Kolmogorov-Smirnov test.**

For abbreviations see under Table 1.

In the SET group, depersonalization is predicted by the model shown in Table 9 [F (3,118) = 37.811, $p < 0.001$], with 48.3% of the variance (Adjusted R Square is 47%).

Depersonalization in the group of RET is significantly predicted by the model shown in Table 10 [F (3.153) = 18.581, $p < 0.001$]. This model predicts 26.7% of the variance (Adjusted R Square stands at 25.3%).

Finally, personal accomplishment in the SET group is significantly predicted by the model shown in Table 11 [F (3.118) = 21.348, $p < 0.001$], with 35.2% of the variance

Table 7

Model of emotional exhaustion (dependent variable) in the special education teacher (SET) group

Model	Unstandardized coefficients		Standardized coefficients	*t*	*p*
	B	Standard error	Beta		
(Constant)	78.197	6.679		11.708	0.000
Self-esteem	-1.364	0.230	-0.445	-5.937	0.000
Efficacy in classroom management	-1.665	0.369	-0.331	-4.514	0.000
Seniority	0.267	0.118	0.155	2.260	0.026

Table 8

Model of emotional exhaustion (dependent variable) in the regular education teacher (RET) group

Model	Unstandardized coefficients		Standardized coefficients	*t*	*p*
	B	Standard error	Beta		
(Constant)	71.860	7.411		9.697	0.000
Seniority	0.464	0.078	0.365	5.966	0.000
Assertiveness	-0.235	0.059	-0.270	-3.994	0.000
Efficacy in classroom management	-0.954	0.340	-0.202	-2.807	0.006
Social support	-0.238	0.093	-0.175	-2.567	0.011

Table 9

Depersonalisation (dependent variable) model in the special education teacher (SET) group

Model	Unstandardized coefficients		Standardized coefficients	*t*	*p*
	B	Standard error	Beta		
(Constant)	29.635	2.515		11.785	0.000
Self-esteem	-0.465	0.104	-0.406	-4.459	0.000
Efficacy in classroom management	-0.453	0.138	-0.241	-3.280	0.001
Social support	-0.083	0.038	-0.197	-2.197	0.030

Table 10

Depersonalisation (dependent variable) model in the regular education teacher (RET) group

Model	Unstandardized coefficients		Standardized coefficients	*t*	*p*
	B	Standard error	Beta		
(Constant)	20.355	2.694		7.556	0.000
Efficacy in student engagement	-0.512	0.138	-0.288	-3.698	0.000
Self-esteem	-0.324	0.103	-0.248	-3.157	0.002
Seniority	0.080	0.037	0.154	2.144	0.034

Table 11

Model of personal accomplishment (dependent variable) in the special education teacher (SET) group

Model	Unstandardized coefficients		Standardized coefficients	*t*	*p*
	B	Standard error	Beta		
(Constant)	-1.285	5.187		-0.248	0.805
Assertiveness	0.147	0.046	0.287	3.195	0.002
Efficacy in student engagement	0.961	0.327	0.262	2.936	0.004
Efficacy in classroom management	0.478	0.239	0.181	2.001	0.048

Table 12

Model of personal accomplishment (dependent variable) in the regular education teacher (RET) group

Model	Unstandardized coefficients		Standardized coefficients	*t*	*p*
	B	Standard error	Beta		
(Constant)	5.142	3.996		1.287	0.200
Efficacy in student engagement	0.778	0.180	0.322	4.331	0.000
Self-esteem	0.495	0.134	0.279	3.705	0.000
Social support	0.121	0.055	0.161	2.217	0.028

(Adjusted R Square is 33.5%).

In the RET group, dimension of personal accomplishment is significantly predicted by the model in Table 12 [F (3.153) = 28,867, $p < 0.001$], with 36.1% of the explained variance (Adjusted R Square was 35%).

Discussion

The highest levels of burnout were observed on the emotional exhaustion dimension. They range from almost half in the OTSE group to around one third in the other two groups. This finding is in line with research conducted in similar studies [32].

If we compare all three groups, we see that OTSE working in special schools, in comparison to others, exhibit slightly higher levels of burnout symptoms like emotional exhaustion and reduced personal accomplishment. Some studies support this finding, indicating that the type of education, or qualification for work, affect burnout syndrome [6, 33]. Lack of teacher training for work with children needing intensive development support can lead to maladaptation to the work process, which affects adversely both the teacher and the student [33]. The teacher may develop negative attitudes towards the student or the process itself, due to misunderstandings of problems, unrealistic expectations or time constraints, which can further lead to emotional exhaustion [34]. In schools for children with developmental disabilities in Serbia, in addition to special education teachers, classes can be delivered by teachers of other educational profiles, who have appropriate professional qualifications for the subjects they teach, but are not specially educated for working with children with disabilities. In this context, we can assume that their psychological and academic preparation for special education work was shorter, and that emotional investment and expectations in working with these children may be less adaptable.

This view is supported by the further results of our study, where we find that there is a difference when it comes to assessing teachers' self-efficacy. The OTSE group assessed their efficacy for learning strategies, classroom management, and student engagement lower compared the other two groups. Education specializing in working with children with disabilities provides special education teachers with a set of competencies for the effective choice of learning strategies, classroom management, and teaching of this type of students, unlike education in other college settings, where the focus of study is more on the study of specific subjects in the domain of typical child development. When these teachers work in regular education, the lack of the competencies is not visible to a greater extent, but in special education this obviously becomes a problem, perceived by the teachers themselves.

The main aim of our study was an attempt to identify a set of predictors of burnout syndrome from demographic, psychological factors, and social support variables. Numerous studies have examined the link between psychological factors, social support and burnout syndrome [12, 13, 35, 36], but a very limited number of studies have focused on simultaneous examination of the predictive values of these factors, with teachers working in different types of education. As expected, in all three groups of teachers, a negative correlation of all psychological factors with emotional exhaustion, depersonalization and reduced personal realization was confirmed. Prediction models have additionally focused our attention on individual factors.

The most significant predictors of emotional exhaustion of special education teachers were the self-esteem and efficacy of classroom management, and to some extent the work experience. It is already known that these factors are positively correlated with each other, or that positive belief about one's own efficacy increase one's sense of self-worth [37]. Other studies have confirmed the correlation between self-efficacy and burnout syndrome, too [6, 35, 36]. Self-esteem enhances teacher resilience, enabling them to manage critical events and stressful situations they are often exposed to, while working with children with special needs [4]. Considering that emotional exhaustion, as the first stage of burnout syndrome, actually arises in response to stress, work problems and unfulfilled expectations [38], we can assume that special education teachers who have a sense of self-confidence, self-esteem and work-related efficacy more easily protect themselves from exhaustion.

In the group of regular education teachers, assertiveness as well as social support emerged as the most important predictors of emotional exhaustion, in addition to work experience and effective classroom management. For regular education teachers, the ability to communicate adequately and assertively is a basic tool for working with students, given that communication is the basis of good interpersonal relationships and of effective management for each group of people. In order for the teacher to direct and control the class functioning, it is necessary for them to have a developed skill of clear and effective communication. Assertiveness involves establishing clear rules and boundaries with respect to others, and its im-

portance in achievement of good interactions with students and parents is undeniable. Assertiveness enhances adaptability to stress and helps a person to cope with obstacles, while protecting them from emotional exhaustion [13]. Social support is an important resource that helps teachers deal with the emotional demands of teaching and has a significant impact on teacher engagement. Family and friends are very important because an individual relies on them for social, emotional and material support, and this is an important sociocultural aspect traditionally rooted in the people who live in these territories.

In the group of special education teachers, social support stood out as a significant predictor of depersonalization, in addition to self-esteem and effective classroom management, which were protective factors in emotional exhaustion too. Considering that depersonalization is the second stage of the burnout syndrome, related to the interpersonal dimension, characterized by a cynical attitude and experience of alienation from people at work [39], we can assume that effective classroom management and self-esteem are no longer sufficient at this stage, additional support and external gratification are thus necessary. Because depersonalization is rooted in the realm of interpersonal relationships, it seems likely that teachers who have more satisfying interpersonal relationships will also exhibit lower depersonalization. In addition to family and friends support, in the context of burnout prevention, the perception of social support from supervisors and colleagues is also important. Administrative support and team efficacy have previously shown strong connection with special education teachers' job satisfaction [40].

Important protective factors for depersonalization in regular education teachers are efficacy in student engagement, self-esteem and seniority. Depersonalization is one of the ways in which, through reduced engagement with others, employees try to reduce the emotional burden or feeling of dissatisfaction at work, so the connection to efficacy and self-esteem is also expected here. People with high levels of self-efficacy believe in their abilities, they approach demanding tasks as challenges to overcome, not as threats to be avoided [22].

Assertiveness, effective student engagement, and classroom management stood out as predictors of personal accomplishment in the special education teachers group. The reduced accomplishment is also reflected in the negative evaluation of personal competences and productivity, and in the experience of diminished self-efficacy [41]. The experience of effective teaching and classroom management produce enhanced sense of personal accomplishment. Assertiveness plays a significant role in the social integration of children with developmental disabilities, since difficulties in performing various activities often condition them to develop passive or aggressive behavior [42].

The factors that enhance achievement in the RET group are efficacy of student engagement, self-esteem and social support. Effective teaching is the key to every teacher's success. Unlike special education teachers, regular education teachers are more likely to seek external confirmation of student performance through grades, competitions, discipline, so in this case the environmental recognition and social support can influence the development of personal realization.

Finally, we should not forget that work experience stood out as a significant predictor of emotional exhaustion and depersonalization. Although the opposite is often confirmed [6], our findings are in line with some national and international research [43, 44]. Older teachers in Serbia have experienced numerous changes in their field in recent years. Those changes require new knowledge and skills, and such experience can lead to the accumulation of long-lasting demands and the high degree of difficulty in dealing with new demands, all of which can act as stressors for the teaching profession.

Limitation of the study

This study has several limitations. It is a cross-sectional study that included respondents only from Belgrade. Future research could also include practical indicators of burnout syndrome (absenteeism, fluctuation, presence of psychophysical symptoms). Further research could also point to potential differences in teacher burnout syndrome in relation to the number of students in the class, the age of the students, the number of students taught according to an individual educational program, etc.

Conclusion

This study indicates the need for a deeper understanding of the individual-psychological and environmental factors that may influence strategies to overcome burnout syndrome. Teachers who exhibit lower levels of self-esteem and self-efficacy, who are less assertive, are more likely to develop burnout syndrome at work. The social support of family and friends is also an important predictor of burnout, especially regarding emotional exhaustion. As assertiveness, self-esteem, and self-efficacy are acquired, developed, refined, and changed throughout one's life, it would be important to strengthen employees' psychological mechanisms through organized and continuous interventions aimed at enhancing personal resources and reducing the sense of stress in working with students. This would also be important for human resource management in general, given that every organization's goal is to increase productivity and efficiency of its employees with a high degree of personal satisfaction.

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Functional and histological changes of the pancreas and the liver in the rats after the acute and subacute administration of diazinon

Funkcionalne i histološke promene pankreasa i jetre kod pacova posle akutne i subakutne primene diazinona

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Abstract

Background/Aim. Organophosphate pesticides (OPs) are used extensively worldwide in agriculture and forestry, and their application represents a major health problem for humans and animals. The aim of this study was to investigate the possibility of the adaptation of an organism to the prolonged administration of a low dose of diazinon. **Methods.** The study was conducted on a total of 60 male Wistar rats. The first 30 rats were divided into four equal diazinon groups (n = 6) and the control one (corn oil). Diazinon was orally administered once at doses: 200, 400, 600, 800 mg/kg (one dose – one group). The concentration of glucose, the activity of α-amylase and the relative activity of LDH1-LDH5 isoenzymes in the blood were measured 24 hours after the application. The remaining 30 rats were divided into two equal diazinon groups (n = 10) and the control one (corn oil). The first group was treated during 7 days, and the second during 14 days with 55 mg/kg of diazinon (1/10 of previously determined LD_{50} value). The histopathology of the pancreas and the liver, as well as the relative activities of LDH isoenzymes in the blood, were determined after the completion of both time periods. **Results.** Single administration of increasing doses of diazinon resulted in a significant increase in the concentrations of glucose, activity of α-amylase and LDH isoenzymes. Subacute application of a low diazinon dose induced histopathological changes in the pancreas manifested by acinar cell necrosis, and in the liver in the form of portal hepatitis and multifocal necrosis. The cumulative doses resulted in statistically significantly lower activities of LDH isoenzymes compared with the single administration of these doses, indicating a lower degree of the cells damage after the subacute diazinon administration. **Conclusion.** Subacute administration of a low dose of diazinon leads to a different adaptation degree of organs and organ systems to toxic effects caused by this organophosphate.

Key words:
diazinon; histological techniques; liver; organophosphorus compounds; pancreas; rats; toxicity test, subacute.

Apstrakt

Uvod/Cilj. Organofosfatni pesticidi (OP) se intenzivno koriste širom sveta u poljoprivredi i šumarstvu, a njihova primena predstavlja značajan zdravstveni problem kod ljudi i životinja. Cilj ove studije bio je da se ispita mogućnost adaptacije organizma na prolongiranu primenu niskih doza diazinona. **Metode.** Studija je sprovedena na ukupno 60 pacova muškog pola Vistar soja. Prvih 30 pacova je podeljeno u četiri jednake grupe tretirane diazinonom (n = 6) i kontrolnu grupu (kukuruzno ulje). Diazinon je primenjivan jednokratno peroralno u dozama: 200, 400, 600, 800 mg/kg (jedna doza – jedna grupa). Koncentracija glukoze, aktivnost α-amilaze i relativna aktivnost LDH1-LDH5 izoenzima u krvi, određivani su 24 sata nakon aplikacije. Preostalih 30 pacova je podeljeno u dve jednake diazinon grupe (n = 10) i kontrolnu grupu (kukuruzno ulje). Prva grupa je tretirana 7 dana, a druga 14 dana sa 55 mg/kg diazinona (1/10 prethodno određene vrednosti LD_{50}). Histopatologija pankreasa i jetre i određivanje relativne aktivnosti LDH izoenzima u krvi urađeni su po završetku oba vremenska perioda. **Rezultati.** Jednokratna primena rastućih doza diazinona rezultirala je statistički značajnim povećanjem koncentracije glukoze, aktivnosti α-amilaze i LDH izoenzima. Subakutna primena niske doze diazinona indukovala je histopatološke promene u pankreasu manifestovane acinarnom nekrozom, a u jetri promene su se ispoljile u vidu portalnog hepatitisa i multifokalne nekroze. Kumulativne doze diazinona rezultirale su statistički značajno nižom aktivnošću LDH izoenzima u poređenju sa jednokratnom primenom tih doza, što ukazuje

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na niži stepen oštećenja ćelija posle subakutne primene diazinona. **Zaključak.** Subakutna primena niske doze diazinona dovodi do različitog stepena adaptacije organa i organskih sistema na toksične efekte izazvane tim organofosfatom.

Ključne reči:
diazinon; histološke tehnike; jetra; organofosforna jedinjenja; pankreas; pacovi; toksičnost, subakutna, testovi.

Introduction

In the order to enhance food production and because of their broad-spectrum insecticidal activity organophosphate pesticides (OPs) are used extensively worldwide in agriculture and forestry. However, only a very small amount of the applied pesticides reaches the target pests, and the rest spreads through water, soil, and food [1]. Therefore, their application represents a major environmental, as well as a health problem for humans and animals.

In humans and animals, diazinon is metabolized to the more toxic metabolite − diazoxone. Its anticholinesterase (AChE) activity leads to the accumulation of acetylcholine at nerve endings, resulting in overstimulation of the nicotinic and muscarinic receptors. Other mechanisms by which diazinon induces toxic effects in the organism are the oxidative stress and inflammation, leading to a histopathological lesions in the liver, pancreas, kidney and brain [2–5]. There are studies that suggest a correlation between oxidative stress and the AChE mechanism of action of OPs. Ranjbar et al. [6] proved that in OPs manufacturing workers, there is a strong correlation between inhibition of AChE in erythrocytes and increased concentration of thiobarbituric acid-reactive substances (TBARS), as an indicator of lipid peroxidation. In addition, intoxication with diazinon results in increased activity of total lactate dehydrogenase (LDH) [7, 8]. Increase in total LDH is rather nonspecific parameter, and because of these, we conducted measurement of its isoenzymes. LDH is an intracellular enzyme, biomarker of energy metabolism, which exists in the 5 isoforms, localized particularly in the heart, erythrocytes and brain (LDH-1), reticuloendothelial system (LDH-2), lungs (LDH-3), pancreas and kidneys (LDH-4), liver and striated muscle (LDH-5). When the cells damaged, there is a "leaking" of LDH from the cells to the bloodstream, where its elevated level is identified. Therefore, LDH isoenzymes are useful biomarkers because they serve as indicators of disturbances integrity of the cells in the different tissues and organs induced by pathological conditions [9–11].

The aim of this study was to investigate the possibility of the adaptation of an organism to the prolonged administration of a low dose of diazinon (1/10 of LD_{50} value).

Methods

In this study we used diazinon (Makhteshim Chemical Works Ltd., Israel) minimum purity of 95%, and corn oil (Uvita, Serbia) as a diazinon solvent. All animal procedures were conducted in accordance with the Directive 2010/63/EU on the protection of animals used for study and other scientific purposes and in accordance with the requirements of the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade.

The study was conducted on a total of 60 male Wistar rats, weighing 200 ± 20 g. Maximum volume of all substances administered perorally to the rats did not exceed 0.1 mL/100 g of rat bw.

The first 30 rats were divided into four equal diazinon groups, containing 6 animals each and the control one (corn oil). Diazinon was orally administered at increased single doses: 200, 400, 600 and 800 mg/kg (one dose – one diazinon group). Twenty-four hours after the application of diazinon, the concentration of glucose, the activity of α-amylase and the relative activity of LDH1-LDH5 isoenzymes in the blood of the rats were measured, in relation to a series of increasing doses of diazinon.

The glucose concentration in the plasma was determined using glucose assay kit (Linear Chemicals S.L., Spain), in the reaction of glucose oxidation by the glucose oxidase (GOD) and the concentration was expressed in mmol/L [12].

The activity of α-amylase in the plasma was assayed using α-amylase assay kit (Linear Chemicals S.L., Spain) with 2-chloro-p-nitrophenyl-α-D-maltotrioside (CNP-G3) as a substrate. The enzyme activity was expressed in U/L.

Isoenzymes LDH1–LDH5 in the blood plasma were detected by PAGE technique using Tris–glycine buffer (25 mM Tris, 192 mM glycine pH 8.3) and sodium lactate as a substrate in the presence of nitroblue tetrazolium chloride 13. LDH1–LDH5 isoenzyme bands intensity was analyzed using TotalLab TL 120 and the activity of each isoenzyme was expressed as band intensity [13].

The remaining 30 rats were divided into two equal diazinon groups, containing 10 animals each and the control one (corn oil). The group I was orally treated (by gastric tube) during 7 days, and the group II during 14 days with 55 mg/kg of diazinon (1/10 of previously determined LD_{50} value). The control group was administered with corn oil with the same procedure. After the completion of treatments, the animals were anesthetized by diethyl ether and sacrificed immediately. The pancreas and the liver were removed and fixed by immersion in 10% neutral buffered formaldehyde (NBF) for histopathology. After fixation, the samples of the pancreas and the liver were dehydrated and embedded in paraffin. Paraffin tissue blocks were cut into 5 μm thick sections, routinely processed and stained with hematoxylin and eosin (HE). Histological preparations were examined using a microscope Olympus BX51 (Tokyo, Japan). Semiquantitative scoring of the severity and the incidence of histopathological lesions in the pancreas and the liver of the rats was performed in accordance with Ramos et al. [14] and Gülçubuk et al. [15], respectively.

In addition, in the rats from this part of the experiment, the relative activity of LDH1-LDH5 isoenzymes in the blood plasma was determined on the 7th and 14th day.

The statistical analysis was performed using a two-way (ANOVA) followed by Tukey's multiple comparisons test. Values $p < 0.05$ were considered significant. All experimental results are shown as the mean ± standard error of the mean (SEM).

Results

The influence of diazinon on the parameters of the pancreatic function

The results showed that the increase in glucose concentration and α-amylase activity are dose-dependent (Table 1). All four tested doses of diazinon resulted in a significant increase in both parameters relative to the control ($p < 0.001$), and the highest tested dose (800 mg/kg) led to a significant increase compared to the previous doses (200, 400, 600 mg/kg) ($p < 0.001$). The effects of diazinon on the concentration of glucose and the activity of α-amylase between doses of 400 and 600 mg/kg did not reach statistical significance.

The influence of diazinon on the activity of LDH1-LDH5 isoenzymes

Diazinon at doses of 400, 600 and 800 mg/kg significantly increased the relative activity of all five isoenzymes of LDH compared to the control ($p < 0.001$), except for the LDH3 isoenzyme, where the dose of 400 mg/kg achieved a lower statistical significance compared with the control ($p < 0.01$) (Figure 1). For the LDH4/5 isoenzymes, which indicate the damage of the pancreas and the liver, the effects of the doses of 400, 600 and 800 mg/kg were also statistically significantly higher than the dose of 200 mg/kg ($p < 0.001$).

At the dose of 800 mg/kg, the activity of LDH4/5 was significantly higher than the activity recorded with the doses of 400 mg/kg and 600 mg/kg ($p < 0.001$) (Figures 1 and 2).

In the group of rats that was treated with diazinon at the

Table 1

The concentrations of glucose and the activity of α-amylase in the blood plasma of the rats treated one time *per os* with increasing doses of diazinon

Treatment	Glucose (mmol/L)	α-amylase (U/L)
Corn oil (control group), 1 mL/kg	3.86 ± 0.16	534 ± 124
Diazinon (mg/kg)		
200	5.14 ± 0.28[a]	1,354 ± 125[a]
400	6.85 ± 0.14[a,b]	1,711 ± 153[a,b]
600	6.93 ± 0.25[a,b]	1,728 ± 109[a,b]
800	8.79 ± 0.32[a,b,c,d]	1,835 ± 120[a,b,c,d]

Data are expressed as mean ± standard error of the mean (SEM) (Two-way ANOVA/Tukey).
[a]$p < 0.001$ compared with the control group; [b]$p < 0.001$ compared with the 200 mg/kg; [c]$p < 0.001$ compared with the 400 mg/kg; [d]$p < 0.001$ compared with the 600 mg/kg.

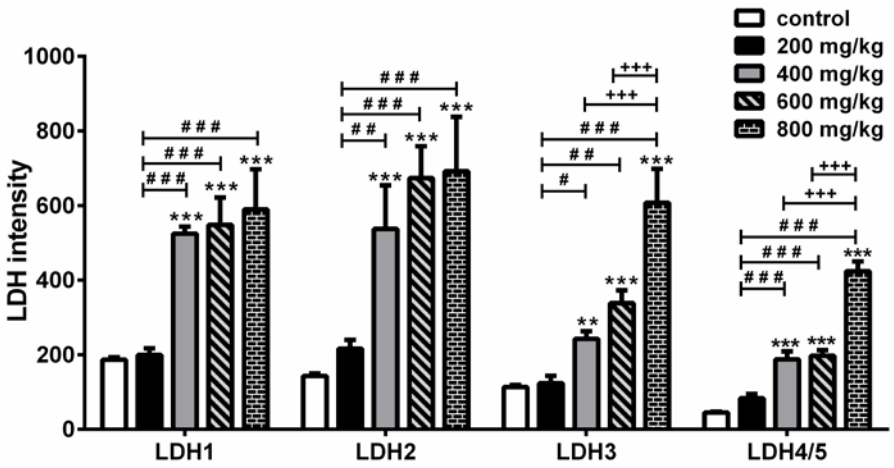

Fig. 1 – Distribution of LDH1-LDH5 isoenzymes in the rats treated with increased single doses of diazinon (200, 400, 600, 800 mg/kg).
Data are expressed as mean ± standard error of the mean (SEM).
****p < 0.01, *****p < 0.001 vs. control; #p < 0.05, ##p < 0.01, ###p < 0.001 and +p < 0.05, ++p < 0.01, +++p < 0.001 between different doses.**

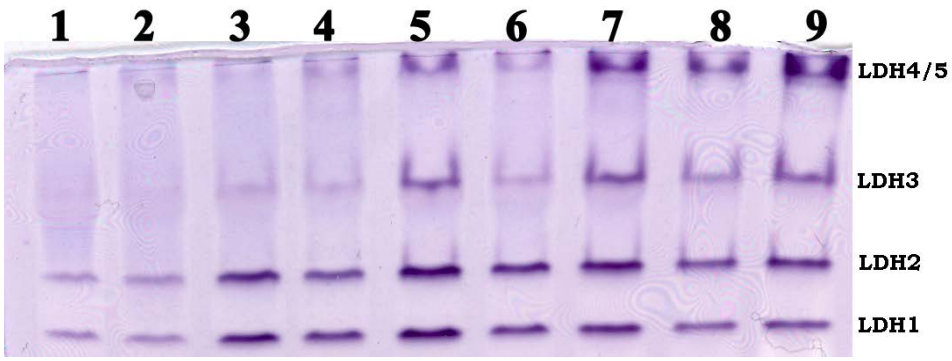

Fig. 2 – Representative polyacrylamide gel electrophoresis (PAGE) of isoenzymes LDH1-LDH5 in relation to a series of increasing doses of diazinon.
Column 1, control rats; Column 2, 200 mg/kg; Columns 3 and 4, 400 mg/kg; Columns 5 and 6, 600 mg/kg; Columns 7– 9, 800 mg/kg of diazinon.

dose of 55 mg/kg for 7 and 14 days, the activity of isoenzymes LDH1-LDH5 was significantly higher than that of the control group on the 7th ($p < 0.001$) and 14th day ($p < 0.001$, $p < 0.01$) (Figure 3). However, the activity of isoenzymes LDH4/5 and LDH3 (indicates damage to the lungs) on the 14th day was statistically significantly lower than their activity on the 7th day ($p < 0.001$). The activity of isoenzyme LDH2 on day 14 of the treatment was not statistically significantly different from the activity on the 7th day. Only the LDH1 isoenzyme activity on the 14th day was statistically significantly higher than the activity on the 7th day of the treatment ($p < 0.001$) (Figures 3 and 4).

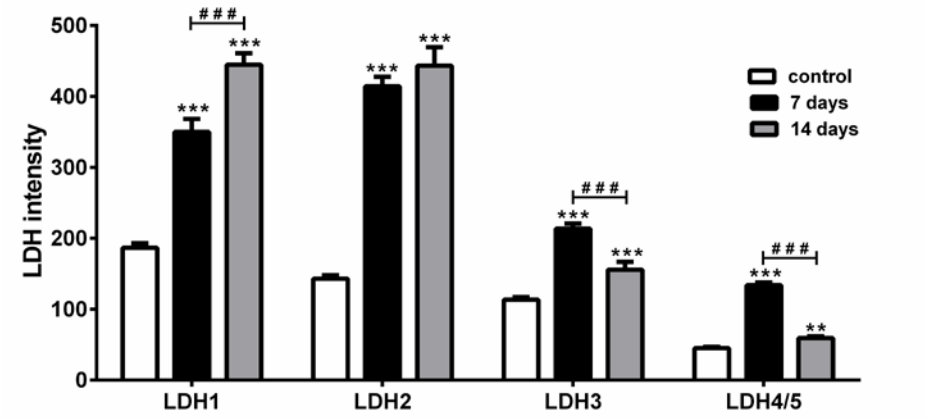

Fig. 3 – Distribution of LDH1-LDH5 isoenzymes in the rats treated with 55 mg/kg of diazinon during 7 and 14 days.
Data are expressed as mean ± standard error of the mean (SEM).
****p* < 0.01, *****p* < 0.001 vs. control; ###*p* < 0.001 between the 7th and the 14th day.**

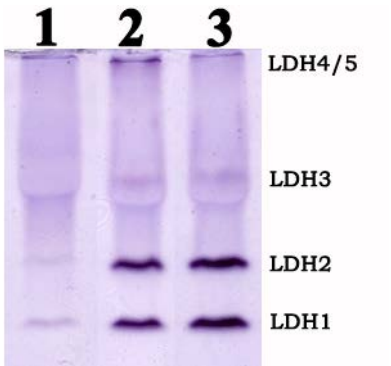

Fig. 4 – Representative polyacrylamide gel electrophoresis (PAGE) of isoenzymes LDH1-LDH5 in relation to the 7th and the 14th day of the treatment with 55 mg/kg of diazinon.
Column 1, control rats; Column 2, 7 days treatment; Column 3, 14 days treatment.

The activities of LDH1-LDH5 isoenzymes after a cumulative diazinon dose of approximately 800 mg/kg (within 14 days) were statistically significantly lower than the activities after the single dose of 800 mg/kg: LDH1 ($p < 0.05$), LDH2 ($p < 0.01$), LDH3 ($p < 0.001$) and LDH4/5 ($p < 0.001$) (Figure 5). In addition, the activities of LDH1 and LDH4/5 isoenzymes after a cumulative diazinon dose of approximately 400 mg/kg (within 7 days) were statistically significantly lower than the activities after the single dose of 400 mg/kg ($p < 0.01$) (Figure 5).

Histopathology of the pancreas and the liver

Histopathological findings of the pancreas revealed necrosis of acinar cells in both analyzed periods: on the 7th (Figure 6a) and 14th day (Figure 6b), but slightly more pronounced on the 14th day (total score 3, range 1–2), which is associated with larger number of macrophages (Table 2). Edema and hemorrhage were discrete in all experimental group samples, while fat necrosis and fibrosis were not noted at all. Also, some discrete degenerative changes in cells within Langerhans

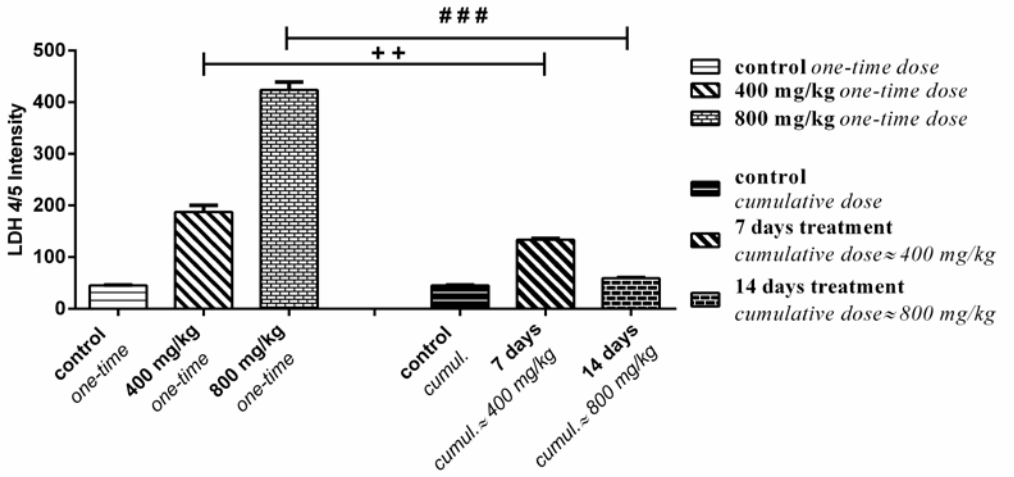

Fig. 5 – Comparison of the distribution of the LDH1-LDH5 isoenzymes in the rats treated with single doses of diazinon (400, 800 mg/kg) (left) and the rats treated with 55 mg/kg of diazinon during 7 days (cumulative dose of ≈ 400 mg/kg) and 14 days (cumulative dose of ≈ 800 mg/kg) (right). Data are expressed as mean ± standard error of the mean (SEM). ###$p < 0.001$ between single and cumulative dose of ≈ 800 mg/kg; ++$p < 0.01$ between single and cumulative dose of ≈ 400 mg/kg.

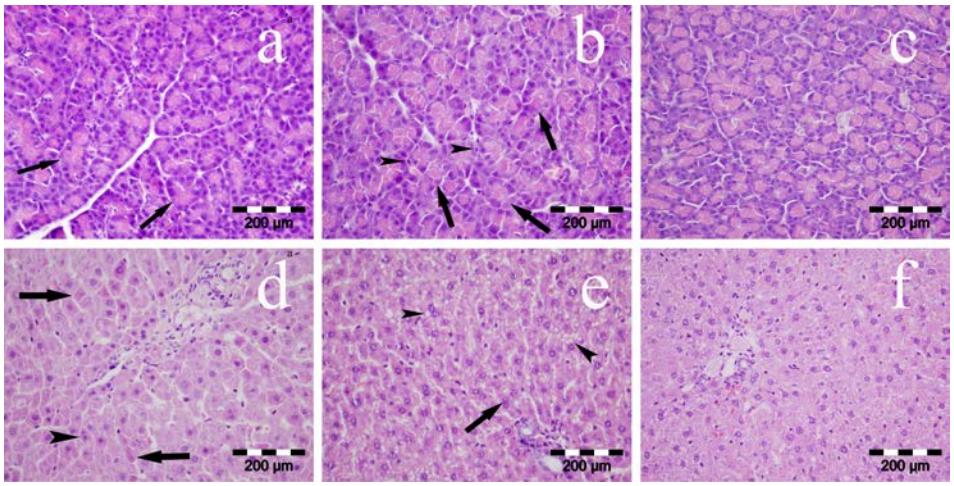

Fig. 6 – Histopathological changes of the pancreas and the liver in the rats treated with diazinon (hematoxilin-eosin staining, 400x). a) Pancreas on the 7th day of the treatment: necrosis of acinar cells (loss of nuclei) (arrows); b) Pancreas on the 14th day of the treatment: necrosis of acinar cells (arrowheads) and numerous macrophages (arrows); c) Pancreas in the control group (corn oil): normal histological pattern; d) Liver on the 7th day of the treatment: necrosis of hepatocytes (arrows) and regenerative changes represented with binucleated hepatocytes (arrowhead); e) Liver on the 14th day of the treatment: hepatocytes show prominent microvesicular fat change (arrowheads) and regenerative changes represented with binucleated hepatocytes (arrow); f) Liver in the control group (corn oil): normal histological architecture, presence of a small number of lymphocytes in the portal spaces.

islets were noted on the 14th day. All morphological features of the pancreas showed normal histological pattern in the control group treated with corn oil (Figure 6c). The results of semiquantitative scoring of histopathological changes in the pancreas are presented in the Table 2.

Liver histopathology showed moderate mononuclear cell infiltration of the portal spaces, both on the 7th (Figure 6d) and 14th day (Figure 6e), with the median of degree of portal inflammation 2 (range 1–2) and 1 (range 0–2), respectively (Table 3). A slightly higher proportion of macrophages were present within the portal spaces on day 14. Normal histological architecture and the presence of a small number of lymphocytes in the portal spaces were features of the liver in the control group with corn oil (Figure 6f). Hepatocytes damage revealed hydropic degeneration, multifocal necrosis and apoptosis on the 7th day (Figure 6d), while microvesicular steatosis and hydropic degeneration were more common histopathological findings on the 14th day (Figure 6e). The median of degree of necroinflammatory activity was 2 (range 1–2) on the 7th day and 1 (range 0–1) on the 14th day (Table 3). The regenerative response of the liver (binucleated hepatocytes) was more pronounced on the 14th day, while in the control group it was not prominent. Fibrosis was noted neither in the experimental groups nor in the control one. The results of semiquantitative scoring of histopathological changes in the liver are presented in Table 3.

Table 2

Degree of leukocyte infiltration and acinar necrosis of the pancreas

Groups	Degree of leukocyte infiltration, median (range)	Degree of acinar necrosis, median (range)	Total histopathological score, median (range)
Group I 7th day of diazinon treatment (55 mg/kg)	1 (0-1)	1 (0-1)	2 (0-1)
Group II 14th day of diazinon treatment (55 mg/kg)	2 (1-2)	1 (1-2)	3 (1-2)
Control group (corn oil, 1 mL/kg)	0	0	0

Table 3

Degree of portal inflammation and necroinflammatory activity of the liver

Groups	Degree of portal inflammation, median (range)	Degree of necroinflammatory activity, median (range)	Total histopathological score, median (range)
Group I 7th day of diazinon treatment (55 mg/kg)	2 (1-2)	2 (1-2)	4 (1-2)
Group II 14th day of diazinon treatment (55 mg/kg)	1 (0-2)	1 (0-1)	2 (0-2)
Control group (corn oil, 1 mL/kg)	0 (0-1)	0	0 (0-1)

Discussion

The pancreas and the liver are among the main targets of the toxic effects of OPs, which lead to their damage and dysfunction. The degree of damage to these organs, besides the OPs dose level, also depends crucially on the exposition period. Therefore, we examined whether the adaptation to toxic effects occurs during subacute diazinon poisoning. In this context, we compared the activities of LDH1-LDH5 isoenzymes after a single and cumulative administration of approximately the same dose, and compared the activities of LDH1-LDH5 isoenzymes for the cumulative doses on the 7th and 14th day of the treatment. Also, we compared our histopathological findings of the pancreas and the liver (7 and 14 days) with the histopathological findings of these organs after the acute diazinon administration, obtained by other authors in test protocols similar to ours.

Acute toxicity studies of diazinon in rats, after single oral administration of increasing doses (25, 50, 100, 200, 300 mg/kg) showed that histopathological changes were not recorded up to a dose of 200 mg/kg, when expressed pancreatitis occurred. In the histopathological finding for the dose of 200 mg/kg, fat necrosis, cellular and glandular degeneration, and congestion were observed [7]. In our study, the rats treated with diazinon at a dose of 55 mg/kg, received a cumulative dose of approximately 400 mg/kg after 7 days, and approximately 800 mg/kg after 14 days of the treatment. Histopathological findings showed that the subacute administration of diazinon (55 mg/kg) caused damage to both exocrine and endocrine pancreas in the rats. We noted necrosis of acinar cells both on the 7th and on the 14th day of the treatment (Table 2; Figures 6a and 6b), but the total histopathological changes were more pronounced on the 14th day (total score 3, range 1-2) compared with the 7th day (total score 2, range 0-1) (Table 2). Linking the results of the previous and our study, the progressive character of pancreatic changes is notable in the function of time. It suggested that pancreatic butyrylcholinesterase (BChE) 7 and AChE 8 are target enzymes for OPs toxicity. The inhibition of pancreatic cholinesterases causes cholinergic overstimulation, resulting in ductular hypertension. Pancreas is a very vulnerable gland and any increased internal pressure can consequently cause severe tissue damage, which leads to acute pancreatitis and dysfunction [16, 17].

When we applied a series of increasing doses of diazinon to a separate group of rats, as a result, we obtained a

significant increase in α-amylase activity with all doses compared to the control value (activity increased in the range from 2.5 to 3.5 times) (Table 1). Acute pancreatitis is diagnosed when the α-amylase is 3 or more times higher than physiological values [16]. The administration of increasing doses of diazinon in our case also resulted in a dose-dependent increase in the serum α-amylase activity (Table 1). This finding is consistent with the results of Gokcimen et al. [7].

Another mechanism by which diazinon induces pancreatitis may be oxidative/nitrosative stress, resulting in the destruction of Langerhans islets cells [18–20]. In our study, discrete degenerative changes in Langerhans islets were observed on the 14th day of the treatment. Chronic administration of diazinon (10 mg/kg) to the rats for 2 months causes a significant increase in the levels of malondialdehyde (MDA), the activity of myeloperoxidase (MPO), as an indicator of inflammation, and the serum glucose levels. In the same study, histopathological examination showed destruction in pancreatic tissues, and the β-cells were the most affected cells among the injured islets [21]. At increasing doses of diazinon, as well as in the case α-amylase, we recorded a dose-dependent manner increase in the serum glucose level, with statistically significant differences between the doses (Table 1). Our highest tested dose of diazinon (800 mg/kg) increases the serum glucose level over 2 times compared to the control value. For the purpose of comparison, in the rats receiving a cumulative dose of diazinon of 980 mg/kg within 14 days, the glucose levels were increased by about twofold compared with the control rats [18]. During the four weeks of application, diazinon (70 mg/kg) induced instability in glucose homeostasis and diabetes in rats [22]. The limitation of our study is that the plasma levels of α-amylase and glucose were not determined after the subacute administration of diazinon. However, based on the higher histopathological score on the 14th day compared to the 7th day, we assume that these values would still be statistically significantly higher than those in the control group.

Histopathological findings of the liver in our study indicate portal hepatitis both on the 7th and on the 14th day (Figures 6d and 6e). However, it was observed that the intensity of inflammation and necrotic changes in the liver were more pronounced on day 7 (median of degree 2) compared to day 14 (median of degree 1) (Table 3). This is supported by the finding that on the 14th day, the activity of LDH4/5 isoenzymes was significantly lower compared to day 7 (Figure 3), implying a lower degree of hepatocyte damage on the 14th day. The same significant decrease in the activity on day 14 compared to day 7 of the treatment was observed in the activity of LDH3 isoenzyme (Figure 3), indicating reduced lung damage even though the cumulative dose was doubled. The finding that the LDH2 isoenzyme activity (biomarker for reticuloendothelial system) was not statistically significantly different on days 7 and 14 (Figure 3) also indicates some form of adaptation of the organism to subacute diazinon poisoning. This result did not exist in the case of the LDH1 isoenzyme, which indicates the damage to the heart, erythrocytes and brain.

Comparing the activities of LDH1-LDH5 isoenzymes, a statistically significant difference is observed between a single and cumulative dose of 400 mg/kg (LDH1, LDH4/5) and between a single and cumulative dose of 800 mg/kg (all five isoenzymes) (Figure 5). The findings that LDH isoenzyme activity was statistically significantly lower at the cumulative dose of 400 mg/kg, and at the cumulative dose of 800 mg/kg, especially for LDH3 and LDH4/5, indicates the adaptation of the organism to the prolonged administration of low doses of diazinon.

We did not perform the examination of pathological changes in the liver after single doses of 400 and 800 mg/kg, in order to compare them with the changes after the subacute administration of diazinon (7 and 14 days). However, hepatotoxicity and pathohistological changes are described by different authors after the acute [23, 24], subacute [25–27] and subchronical [2] exposure of rats to diazinon. The protocol and methodology that are most similar to our experimental conditions were conducted by Beydilli et al. [23]. In that study, diazinon (in the corn oil) was administered orally to rats in a single dose of 335 mg/kg. For the assessment of histopathological changes in the liver, was used a range of 3 grades (1–3), based on the intensity and prevalence of lesions. The liver tissue was significantly damaged, and assessed with the maximal grade 3. The histopathological findings were dominated by: severe sinusoidal dilatations, moderate disrupt radial alignment of hepatocytes, severe vacuolization of hepatocyte cytoplasm and centrilobular necrosis. If we make an analogy with our results, we can say that the pathohistological changes of the liver after the cumulative dose ≈ 800 mg/kg (median of degree of portal inflammation 1, range 0–2 and necroinflammatory activity 1, range 0–1), have a lower intensity compared to the single dose of 335 mg/kg (grade 3, range 1–3). This finding also suggests the existence of the adaptation of the organism to the subacute administration of diazinon. Ivanović et al. [28] have proved that during subchronic administration of diazinon in rats there is a downregulation of nicotinic and muscarinic receptor functions, indicating the adaptation of the peripheral cholinergic system.

In summary, single administration of increasing doses of diazinon in rats results in a significant increase in the concentrations of glucose, activity of α-amylase and LDH1-LDH5 isoenzymes in the blood plasma. Subacute application of diazinon (7 and 14 days) at a low dose (55 mg/kg) induces histopathological changes in the pancreas manifested by acinar cell necrosis, and in the liver in the form of portal hepatitis and multifocal necrosis of the hepatocytes. Histopathological findings of the pancreas were more pronounced on the 14th day. Contrary to that, the histopathological changes in the liver were less pronounced, and the activity of the LDH4/5 isoenzymes was statistically significantly lower on day 14 compared to day 7. Also, a decrease in the activity on day 14 compared to day 7 of the treatment was observed in the activity of the LDH3 isoenzyme, indicating a reduced lung damage, even though the cumulative dose was doubled. Furthermore, the cumulative doses of ≈ 400 and ≈ 800 mg/kg resulted in lower activities of LDH1-LDH5 isoenzymes

compared with the single administration of these doses, indicating a lower degree of the cells damage after the subacute diazinon administration.

Conclusion

Subacute administration of a low dose of diazinon leads to a different adaptation degree of organs and organ systems to toxic effects caused by this organophosphate.

Conflict of interest

The authors declare no conflict of interest.

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The effects of experimentally irradiated pituitary gland on the growth of rats' tibia, skull, maxilla and mandible

Uticaj eksperimentalno zračene hipofize na rast potkolenice, lobanje, gornje i donje vilice pacova

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Abstract

Background/Aim. The reaction of an organism to radiation depends on the level of irradiation and the sensitivity of the affected tissue cells. The biological effects on the cells and tissues are proportional to the absorbed radiation energy. The aim of our study was to examine the effects of hypofunction of the pituitary gland, previously irradiated with x-rays, on the growth of rat's craniofacial and stomatognathic system as well as rat's tibia. **Methods.** A total dose of x-rays of 27.92 Gy was applied in eight sessions in the period from 8 to 63 days of animal age on their heads and the effects of irradiation-induced hypofunction of the pituitary gland on the growth of rat's craniofacial and stomatognathic system and tibia were examined. In order to differentiate the effects of the irradiated pituitary gland from the direct effects of x-rays on the head, the experiment was set as a double study. One group of animals had the pituitary gland protected with a lead plate set beneath the projection of the gland, while the second group was irradiated with the same dose, but without the protection. The control group of animals were non-irradiated. **Results.** Growth measurements of numerous parameters of the craniofacial and stomatognathic system as well as tibia in rats with the entire locally irradiated head showed a statistically significant delay, compared to both the non-irradiated control group and the group with the protected pituitary gland. **Conclusion.** The damage of the stomatognathic and craniofacial system was greater in animals with irradiated head without pituitary gland protection compared to those with the gland protection whereby the growth of the craniofacial system was most affected. Irraduition-induced pituitary gland hypofunction and irradiation itself showed a stronger effect on the lower jaw growth rate impairment compared to the upper jaw. Similarly, the delay in tibia growth was more pronounced in animals without pituitary gland protection during irradiation of their heads compared to non-protected ones.

Key words:
pituitary gland; x-rays; rats; growth; tibia; skull; maxilla, mandible.

Apstrakt

Uvod/Cilj. Reakcija organizma na zračenje zavisi od doze zračenja i osetljivosti ćelija tretiranog tkiva. Biološki efekti zračenja na ćelije i tkiva proporcionalni su apsorbovanoj radijacionoj energiji. Cilj istraživanja je bio da se ispitaju efekti hipofunkcije prethodno x-zracima ozračene hipofize na rast kraniofacijalnog i stomatognatnog sistema pacova. **Metode.** Primenjena doza x-zračenja od 27,92 Gy aplicirana je na dve grupe pacova u 8 seansi tokom perioda od 8 do 63 dana njihove starosti, uz praćenje efekata zračenja na rast kraniofacijalnog i stomatognatog sistema. U nameri da u merenju brojnih parametara pomenutih sistema razdvojimo efekte ozračene hipofize od direktnih efekata x zraka koje smo primenili na glavu, postavljen je eksperiment sa ozračivanjem glave dvema grupama životinja istim dozama x zraka, ali je jednoj zaštićena hipofiza olovnom pločicom postavljenom iznad hipofiza. **Rezultati.** Zaostajanje u rastu stomatognatog sistema kod pacova kojima je lokalno ozračena cela glava u odnosu na kontrolne životinje predstavlja zbirni efekat delovanja zračenjem izazvane hipofunkcije hipofize i direktnog delovanja x zračenja, dok zaostajanje u rastu stomatognatog sistema kod pacova sa ozračenom glavom i hipofizom zaštićenom od zračenja predstavlja isključivo efekat x zraka. **Zaključak.** Oštećenja stomatognatog i kraniofacijalnog sistema bila su veća kod životinja kojima hipofiza tokom zračenja glave nije bila zaštićena u odnosu na one sa zaštitom žlezde prilikom zračenja, pri čemu je rast kraniofacijalnog sistema u sagitalnom pravcu bio najviše pogođen. Veći zaostatak u rastu usled kombinovanoig dejstva hipofunkcije hipofize izazvane zračenjem i samog zračanje ustanovljen je na donjoj vilici u odnosu na gornju vilicu. Slično ovome, zao

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stajanje u rastu tibije bilo je izraženije kod životinja kojima tokom zračenja cele glave hipofiza nije bila zaštićena u odnosu na neštićene životinje.

Ključne reči:
hipofiza; x zraci; pacovi; rast; tibija; lobanja; gornja vilica; donja vilica.

Introduction

The reaction of an organism to radiation depends on the level of irradiation and the sensitivity of the affected tissue cells. The biological effects on the cells and tissues are proportional to the absorbed radiation energy. A marked damage, with the same dose, is evident in tissues made up of cells with a high replication index, or it occurs at an undifferentiated embryonic level. This is one of the milestones of radiobiology, set up by Tribondeau and Reaminer [1] in 1905.

One of the most marked negative effects of the irradiation of the neck and head region is pituitary hypofunction. There is a number of opposed opinions regarding the sensitivity of the pituitary gland to radiation [2]. In some studies, it was considered that the pituitary gland of adults is resistant to radiation [3] and that high doses are needed in order to induce hormonal changes. Nowadays, it is thought that the hypothalamic pituitary unit is a particularly radiosensitive region, but due to a high hormonal reserve, the negative effects become evident after a longer latent period. It is established that the growth hormone secreting acidophilic pituicytes are sensitive at a single dose greater than 30 Gy [4, 5], and this is often associated with the impact on growth, sexual function and physical and psychological health [6]. Consequently, hypopituitarism develops after radiotherapy for sellar neoplasms, brain tumors and head and neck tumors [7].

Postradiation therapy hypopituitarism shows more damage in younger patients [8]. Growth failure develops after the radiotherapy tumor doses of 46 Gy [9]. Growth hormone insufficiency of 50–100% is usually the only abnormality after irradiation of hypothalamic-pituitary axis with the doses < 30 Gy [10]. Children irradiated for the head and neck tumors have significant alterations in some skeletal measurements, such as asymmetry, potential deformity [11] and deviations in craniofacial structures [12]. The mandible is more sensitive to radiation compared to the maxilla, and especially the condylar region as the center of growth. Doses of 30 Gy to 40 Gy and above are particularly harmful regarding facial bone growth [13, 14].

The majority of bones of rat's neurocranium grow by apposition along the sutures, and except for the frontonasal bone, this ends by the age of 34 days. The scull must be observed as a whole, as each part affects the development not only of adjoining structures, but also of distant structures. Differential growth along the sutures gives the final shape to the scull [15].

Not many authors have researched the scull and jaw growth in rats [16–18], although in examining the adverse effects of various *noxae* on rat's head and jaw growth many authors also recorded values from control, untreated groups [19–21].

The pituitary gland in rat is located ventral to the diencephalon and caudal to the *chiasma optici* in a shallow groove at the base of the sphenoid bone [22]. The adenohypophysis is a heart-shaped disk joined to the neurohypophysis at the medial line. The *pars intermedia* separate these two parts. The horizontal cleft of the hypophyseal space is located between the neurohypophysis and the *pars intermedia* and it persists throughout the lifespan [23].

Since the 1980s, extensive studies have been carried out on the effects of irradiation on the development and growth of rats. Some researchers irradiated whole animals, and some only certain parts of their bodies. The studies on the influence of limb irradiation on the growth of long bones showed a significant, but reversible delay in the growth of femur or tibia as early as at doses of 5 to 7 Gy [24, 25]. At a dose of 17.5 Gy, a greater delay in tibial growth in width than in length was observed [26]. Severe damage in femur osteogenesis was caused by a dose of 20 Gy, while three doses of 10 Gy each caused mild, but significant changes [27].

Different doses were also used to irradiate rat's head in examining the effect on the skull and jaw growth. Neurological and cognitive impairments have also been reported [28, 29], as well as a decline in immunity [30].

Sedlecki et al. [31] and Demajo [32] have studied the effects of irradiation on the growth of the scull and teeth in rats. Their results indicate that doses above 4.8 Gy (maximal dose 9.6 Gy) result in measurable effects on the decrease in jaw parameters. A dose of 20 Gy caused a significant delay in the growth of the lower jaw [33].

The irradiation of the rat's head with the doses of 20, 22, and 24 Gy caused a significant decrease in the secretion of the pituitary gland hormone, the most sensitive being the growth hormone [34]. A dose of 10 Gy of γ-radiation applied to the whole body, abdomen and head caused increased hypothalamic-pituitary axis activity and greater hormone secretion in order to protect the body from radiation [35].

The aim of our study was to examine the effects of hypofunction of the pituitary gland, previously irradiated with a total dose of x-rays of 27.92 Gy applied in eight sessions in the period from the 8 to 63 days of animal age, on the rats' growth, as well as on their growth of the craniofacial and stomatognathic system. In order to differentiate the effects of the irradiated pituitary from the direct effects of x-rays on the head, the experiment was set as a double study. One group of rats had the pituitary gland protected with a lead plate set beneath the projection of the gland. The second group was irradiated with the same dose, but without the protection with a lead plate.

Methods

Experimental animals

In the experiment, Wistar rats (n = 45) with a genetic uniformity of 98% were studied, thus ensuring a uniform response to irradiation. The rats were introduced in the trial at the age of eight days. Up to the age of 30 days, they were kept with their mothers in groups of 6–8 animals per cage. They were weaned at the age of 30 days and set in separate individual cages. All animals were kept under a uniform light regime (light from 5.00 AM to 7.00 PM). Room temperature was 22 ± 2 °C, and they were fed with standard food chaw and watered *ad libitum*.

The first group was not irradiated (control group, n = 15). In the second group (n = 15) the rats' heads were irradiated, but their bodies were shielded and thus protected. This group is described as the irradiated group. The rats in the third group (n = 15) had not only their bodies protected, but the region of the pituitary gland as well. This group is described further in the paper as the protected group. During the experiment, a number of rats died, making the final number of animals 30. Thus, 10 animals in each group were finally studied.

Irradiation

The heads were irradiated with a Philips roentgen, usually used for radiotherapy. The conditions were as follows: 240 kV; 7.5 mA; filter 10 mm Al; speed 18.321 Gy/min and time 19 min 2 sec. The field width was 14 cm and the distance of the focus from the skin was 87.5 cm.

The overall irradiation dose of 27.92 Gy was divided into eight single doses of 3.49 Gy each. The animals were treated twice a week (Friday and Tuesday), every alternate week. The rats were treated for the first time at the age of eight days. After that, they were treated on the 12th, 22nd, 26th, 36th, 40th, 50th and 54th day of age. During each session, rats were irradiated and the room temperature in which the irradiation treatment was carried out was 18 °C. Only the heads were irradiated, as a 6 mm thick lead plate of fitting dimensions protected the rest of the bodies. In the protected group, the pituitary gland was also protected with 6 mm thick lead plate.

The rats were set in a specially constructed wooden stand. In order to stop frantic movements, the extremities were fixed by rubber tubing. The head was fixed in such a way that a thin wire was placed on one side behind the incisors and on the other side, in order to immobilize them in such a fashion as not to hurt the soft tissues.

Measurements of the tibia

The right tibia was prepared [36] and its weight was determined on an automatic Mettler scale. The length was measured with a precise measuring instrument with a precision of a tenth of a millimeter.

Measurements of the skull

The heads and the jaws were cleaned of all soft tissues [36], with an orthodontic measuring instrument Dentaurum, with a precision of a tenth of a millimeter, and the following parameters were taken: 1. skull length – from the tip of the nasal bone to the most distal point of the occipital bone (Figure 1); 2. skull width – the distance between the most prominent points of parietal bones (Figure 1); 3. skull front height – from the mid coronary suture between the frontal and parietal bones up to the middle of the suture between the sphenoid bone and the sphenoid base (Figure 2); 4. skull hind height – from the midsuture between the intraparietal and occipital bones down to the midline of the lower Foramen magnum edge (Figure 2); 5. length of maxilla – the distance between anterior and posterior nasal spines (Figure 3); 6. length of mandible – from the posterior edge of the angulus to the apex of the alveolar calyx of the incisor on the vestibular side (Figure 4); 7. height of mandible – from the point between ramus and corpus on the upper edge of mandible to the most convex point of incisura premaseterica on the lower edge of the corpus. (Figure 4); 8. length of ramus – from the tip of the condylar extension to the lower edge of the angulus (Figure 4); 9. width between the condyles – distance between most lateral points of condylar extensions (Figure 5).

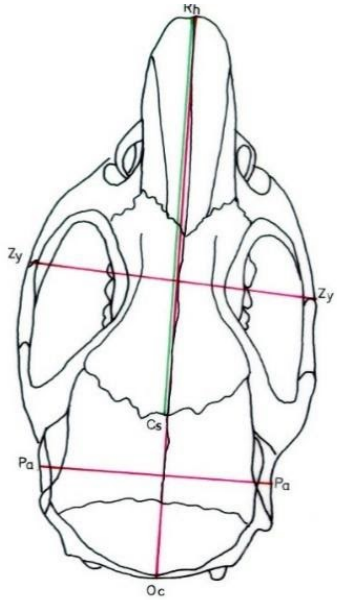

Fig. 1 – Skull length (Rh–Oc), skull width (Pa–Pa).

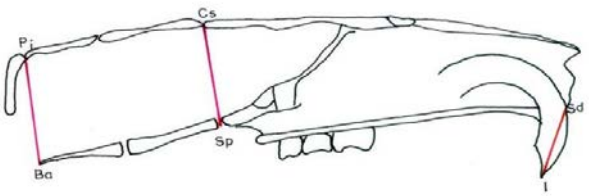

Fig. 2 – Frontal (Cs–Sp) and hind (Pi–Ba) skull height.

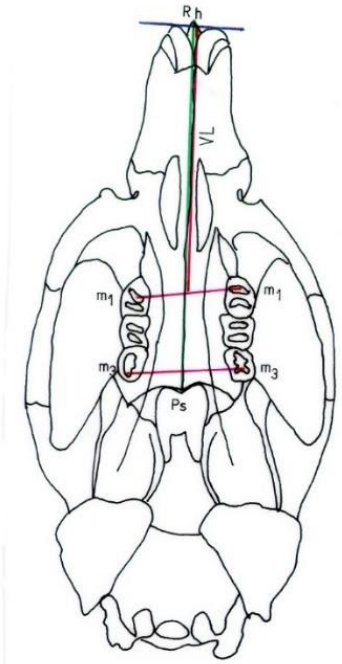

Fig. 3 – Length of maxilla (Rh-Ps).

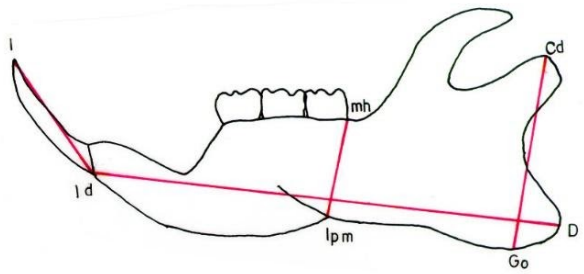

Fig. 4 – Length (Id-D) and height (mh-Ipm) of mandible and length of ramus (Cd-Go).

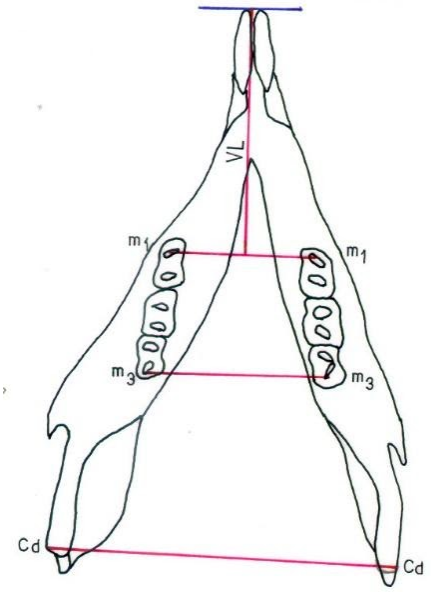

Fig. 5 – Width between the condyles (Cd-Cd).

Results

The irradiation of rats' heads with multiple doses of x-rays results in impaired growth. The protection of the pituitary gland during irradiation decreased the negative effects of radiation on the growth of rats, and on the growth of rats' jaws and teeth. The first few doses in the protected group even showed a stimulative effect on the overall growth.

Body length and mass growth, as well as the pituitary weight in the completely irradiated group, were significantly impaired ($p < 0.001$) as it had been shown earlier [37, 38].

During the experiment, some changes that could be attributed to the effects of radiation were also recorded. However, further studies of these changes were not planned, but we did consider it of interest to make a few comments on them. The epilation of hair on the head started at the age of 25–29 days (after the fourth irradiation treatment) and was evident all the time during the experiment. In the protected group, hair loss was obvious after the fourth treatment only on the exposed parts of the head. The protected areas had normal fur. Some of the irradiated rats by the 60th day of age had inflamed eyes with concurrent bleeding, apathy, weakness (the rats were somnolent and they gathered at the bottom of the cage). Water consumption increased and feed consumption decreased. There were no cases of spontaneous teeth fallout.

At the age of 60 days, the rats reach sexual maturity and secondary sexual characteristics are evident. The most prominent sexual feature is large testicles, which are lacking fur and are easily seen. The irradiated rats at the age of 60 days had testicles, which were atrophic and furry. When decapitated, the bleeding was very scarce and the blood was dark and dense.

Length and weight of the tibia

In our experiment, tibia was measured in order to determine the effect of pituitary gland irradiation on the growth of long bones, which were not in the radiation field.

The average weight of the tibia was the highest in the control group (403.37 mg), slightly lower in the protected group and the lowest in the irradiated group. Analysis of variance and the Student's test showed a statistical significant differences in the weight and length of the tibia of irradiated non-protected rats compared to the control and the protected group ($p < 0.001$). There was no significant difference between the protected and the control group (Table 1).

The results showed that the tibiae were the longest in the control group and the shortest in the irradiated group. The difference was statistically significant between the control and the irradiated group. The length of the tibia was shown to be the most variable within the irradiated group.

Within the control and the protected group, there was no significant correlation between the weight and the length of the tibia. Within the irradiated group of rats, the correlation was negative; however, this was not up to a significant degree. The negative value of correlation in the irradiated

group of animals was the result of a more intensive tibia weight loss compared to the length.

Skull length

The longest average length was measured in the control group of animals. This value was significantly lower in the protected group ($p < 0.05$). The lowest results ($p < 0.001$) were recorded within the irradiated group (Table 2).

Skull width

The same as for the skull length, the largest average values of skull width were recorded in the control group. In the protected group, the values were significantly lower ($p < 0.05$). The lowest values for the skull width were in the irradiated non-protected group of rats. The values for the irradiated, non-protected group were significantly lower compared to the control group ($p < 0.001$) (Table 2).

There was no significant correlation between the measured parameters in all observed groups.

A greater delay in the width than in the length of the skull in the protected group compared to the control group is probably due to the protection of the pituitary gland by the lead, which also included the bones of the roof of the skull. The protection did not cover the parietal bones where the width of the skull was measured, so the irradiation had a greater effect on the transverse than on the sagittal dimension.

Frontal and hind skull height

In the control group, the frontal skull average height was significantly higher ($p < 0.05$) compared to the protected group. In the irradiated, non-protected group, this difference was even more impressive ($p < 0.001$). The variations of the measured values were moderate (Table 3).

From the obtained readings for the hind height it can be clearly seen that the average readings were significantly lower in the irradiated, protected group ($p < 0.05$) and the irradiated non-protected group ($p < 0.001$) (Table 3).

In the control and in the protected group, there was no significant correlation between the skull front and hind height. The correlation values were almost identical, as in the irradiated, non-protected group the lack of frontal and hind skull height was comparable. Within the non-protected group, the correlation between the measured parameters was high. Based on this finding, it can be concluded that in the irradiated, non-protected group, the radiation resulted in a significant inhibition of the growth of both the frontal and hind portions of the skull.

The significantly low values for the skull length and width in the irradiated, non-protected group are the result of the joined effects of radiation and pituitary hypofunction on the growth of the skull.

Table 1

Tibia length and weight in irradiated (IRR) rats

Group	Tibia length (mm)		Tibia weight (g)	
	mean ± SD	CV(%)	mean ± SD	CV(%)
Control (non-IRR)	31.74 ± 0.25	0.7886	403.37 ± 2.89	0.7164
IRR-protected	31.52 ± 0.15	0.4914	400.78 ± 0.89	0.2225
IRR	26.74 ± 1.22*†	4.5535	366.12 ± 8.10*†	2.2133

***$p < 0.001$ v.s. Control; †$p < 0.001$ v.s. IRR-protected (*t*-test).**
CV – coefficient of variation; SD – standard deviation.

Table 2

Skull length and width in irradiated (IRR) rats

Group	Skull width (mm)		Skull length (mm)	
	mean ± SD	CV(%)	mean ± SD	CV(%)
Control (non-IRR)	15.48 ± 0.10	0.6673	39.81 ± 0.98	2.4710
IRR- protected	15.34 ± 0.12*	0.7653	38.88 ± 0.29*	0.7454
IRR	14.15 ± 0.39**†	2.7922	33.78 ± 1.63**†	4.8197

***$p < 0.05$, **$p < 0.001$ v.s. Control; †$p < 0.001$ v.s. IRR-protected (*t*-test).**
CV – coefficient of variation; SD – standard deviation.

Table 3

Frontal and hind skull height in irradiated (IRR) rats

Group	Frontal skull height (mm)		Hind skull height (mm)	
	mean ± SD	CV(%)	mean ± SD	CV(%)
Control (non-IRR)	10.96 ± 0.37	3.3923	9.79 ± 0.30	3.0245
IRR-protected	10.60 ± 0.19*	1.8340	9.53 ± 0.12*	1.2172
IRR	9.03 ± 0.33**†	3.6932	8.32 ± 0.37**†	3.6971

***$p < 0.05$, **$p < 0.001$ v.s. Control; †$p < 0.001$ v.s. IRR-protected (*t*-test).**
CV – coefficient of variation; SD – standard deviation.

In the protected group, these parameters were lower compared to the control group, but significantly higher compared to the irradiated, non-protected group. Based upon statistical analysis, the difference between the protected group and the control group was smaller than between the irradiated, non-protected group and the protected group. These data highlight the fact that the greater negative effect on the sagittal and transversal growth of the skull was due to the irradiated pituitary gland hypofunction, compared to the direct effects of radiation. The decreased growth of the skull width, compared to skull length in the protected group and compared to the control group, was probably due to the protection of the pituitary by a lead plate that covered the bones of the skull. However, the plate did not cover the parietal bones. Thus, the radiation affected more the transversal dimension than the sagittal one.

In the irradiated, non-protected group, the sagittal dimension was more affected as more bones and sutures are present in this direction compared to the transversal. Hence, it is more probable that the lack of the growth hormone affects more the sagittal dimension. In the protected group, the height of the skull was smaller compared to the control group, but significantly higher compared to the irradiated, non-protected group. This difference was the direct consequence of the effects of radiation. Within the irradiated, non-protected group, there was a simultaneous effect of radiation on the bones and insufficient growth hormone synthesis due to the radiation damage of the pituitary gland. The results in the irradiated, protected group could have been affected by the lead protection as the plates covered also the hind portions of the skull. Thus, the differences in frontal skull height were a more reliable indicator for the studied groups.

Maxillary length

Maxillary length in the control group was significantly longer than in both irradiated the protected and non-protected group, but the level of significance was different. In regard to the protected group, it amounted $p < 0.05$ and in regard to the non-protected one, it was $p < 0.001$. Such a large backlog of the irradiated, non-protected group compared to the control group is a result of the summarized effect of the radiation and pituitary gland hypofunction on the growth of the upper jaw. In the protected group, only the effect of the radiation was manifested, which is why it is smaller than that in the control group, but also significantly longer than in the irradiated, non-protected group. Based on these observations, it can be said that the maxillary growth into the sagittal direction is more influenced by the hypofunction of the irradiated pituitary gland than by the radiation itself (Table 4).

Mandibular length

The control group of animals had longer mandibles than the other two groups. The level of difference in length compared to the control group in the irradiated, protected group was at the significance level $p < 0.05$ and in the non-protected group the level was $p < 0.001$. In the protected group, the length of the lower jaw was longer than in the non-protected one (the significance of the difference was at the $p < 0.001$ level). All this suggests that pituitary hypofunction, conjoined with the consequent growth hormone deficiency, caused a greater delay in the sagittal growth of the mandible than direct radiation exposure. A similar finding was ascertained in the analysis of sagittal parameters of the maxilla, so that it can be said that the irradiation of jaws causes a smaller growth delay of the sagittal dimension of the head and jaws compared to the lack of the growth hormone (Table 4).

Mandibular height and ramus length

Mandibular height in the protected group was significantly lower than in the control group, but also significantly higher compared to the irradiated, non-protected group. Based on the level of significance, it can be seen that there was a greater backlog of the non-pretected group compared to the protected one than of the protected group compared to the control group (Table 5).

Table 4

Length of maxilla and mandible in irradiated (IRR) rats

Group	Maxillary length (mm)		Mandibular length (mm)	
	mean ± SD	CV(%)	mean ± SD	CV(%)
Control (non-IRR)	21.73 ± 0.75	3.4712	20.68 ± 0.63	3.3965
IRR-protected	20.71 ± 0.42*	2.0159	19.95 ± 0.51*	2.5805
IRR	18.43 ± 0.62**†	3.3646	17.96 ± 0.61**†	3.3736

***p < 0.05, **p < 0.001 v.s. Control; †p < 0.001 v.s. IRR-protected (t-test).**
CV – coefficient of variation; SD – standard deviation.

Table 5

Height of mandible and ramus in irradiated (IRR) rats

Group	Mandibular height (mm)		Ramus height (mm)	
	mean ± SD	CV(%)	mean ± SD	CV(%)
Control (non-IRR)	6.18 ± 0.16	2.6197	10.02 ± 0.53	5.2555
IRR-protected	5.88 ± 0.18*	3.0850	9.48 ± 0.08*	0.8323
IRR	5.47 ± 0.24**†	4.3126	8.55 ± 0.22**†	2.5415

***p < 0.05, **p < 0.001 v.s. Control; †p < 0.001 v.s. IRR-protected (t-test).**
CV – coefficient of variation; SD – standard deviation.

Ramus length in the protected group was significantly lower than in the control group, but also significantly higher compared to the irradiated, non-protected group. Based on the level of significance, it can be noticed that there was a greater backlog of the non-protected group when compared to the protected one than of the protected group compared to the control group. This ratio was conditioned by the greater influence of the lack of the growth hormone induced by irradiation on the growth of the mandible in the vertical direction than the negative effect exerted by radiation itself. Since the same happens with the length of the mandible, it can be said that the total growth of the body of the mandible was more damaged by the irradiation-induced pituitary gland hypofunction than by radiation. The mandible growth center is in the condyle, so any adverse effects on the wrist have an impact on the entire jaw. The x-ray direction was such that they passed through the skull before the condyle, so they probably lost their strength and could not fully affect the joint (Table 5).

Compared to the control group, a significant delay in the ramus length of the 57th day of age [32] was found in the group of the rats whose heads were irradiated with 9.6 Gy on the 8th day of age [32], which is consistent with our findings.

The growth hormone deficiency had a somewhat greater effect on ramus length compared to the effect of x-rays, but it can be summarized that, compared to the control group, there was an equal delay in the growth of the vertical dimension of the mandible in both experimental groups.

Bicondylar distance

As with the other parameters of the mandible, the values of the bicondylar distance were the lowest in the irradiated, non-protected group. The protected group was lagging behind the control group. However, there was a greater backlog of the non-protected group compared to the protected one. Irradiation had less effect on the transverse growth of the mandible than the hypofunction of the irradiated pituitary gland (Table 6).

Table 6

Bicondylar distance in irradiated (IRR) rats

Group	Bicondylar distance (mm)	
	mean ± SD	CV(%)
Control (non-IRR)	19.68 ± 0.31	1.5845
IRR-protected	19.09 ± 0.41*	2.1592
IRR	15.40 ± 0.24**†	1.5903

***p* < 0.05, ****p* < 0.001 v.s. Control;**
†*p* < 0.001 v.s. IRR-protected (*t*-test).
CV – coefficient of variation; SD – standard deviation.

Discussion

In the available literature on this topic, we have not encountered any works that have separately examined the effects of irradiated pituitary gland and the corollaries of direct effect of x-rays on the growth of head bones. Therefore, some of the results obtained could not be compared or discussed with other authors. These results can be considered to be our original contribution to the study of the effects of pituitary hypofunction on the growth of rats' head bones. At the same time, it represents a baseline study in research of the effects of radiation-induced pituitary hypofunction on the growth of facial and jaw bones in patients irradiated in this region during the adolescence.

Length and weight of the tibia

Measuring tibial length as an indicator of systemic effects of head irradiation was performed by Demajo [32]. In the control group, the values agree with our finding, while the values of tibia length of the animals irradiated with 9.6 Gy were greater than the values of the irradiated animals from our study. Regardless of fractionation, the dose of 27.92 Gy is much higher than that used by Demajo [32], so the values are lower.

Frontal and hind skull height

The anterior skull heights were measured at the same points by Haralabakis and Dagalakis [18]. In the 60-day-old control group, the value is about 11 mm, which is slightly higher than in the animals in our control group.

Maxillary length

The animals from the same farm where we conducted the research were measured by Demajo [31, 32]. In the control group, the maxilla length was 21.82 ± 0.31 mm, which is entirely in accordance with our results in the control group. In the group where the heads of the rats aged eight days were irradiated with 9.6 Gy, the length of the maxilla on the 57th day was lower than the value recorded in our irradiated group. Although the rats in our study had received almost three times higher dose, their maxillae were less delayed in the sagittal growth. Total dose fractionation and the time between irradiation applications enabled the activation of reparative processes, which prevented more drastic growth retardation.

Mandibular length

In animals whose heads were irradiated with 9.6 Gy when they were eight days old, Sedlecki et al. [31] recorded a significantly shorter mandible length on day 57 compared to the control group. In the 57-day-old animals, Demajo [32] measured higher values than ours (26.03 ± 0.21 mm), and in the group where the heads were irradiated with 9.6 Gy when the animals were 8 days old, the values of the sagittal dimension of the mandible were also significantly higher than in our irradiated group (23.87 ± 0.38 mm).

Duterloo and Vilmann [17] measured a mandible length of 18.02 mm in the 30-day-old rats and found that the mandible had grown 2.17 mm from the 14th day. In our control group, the rats are twice older and the difference in length was slightly more than 2 mm. As the growth rate is twice faster in the first month than in the second month of life, our results are in line with the findings of these authors.

Mandibular height and ramus length

Sedlecki et al. [31] measured the height of the mandible of the first molars, unlike our measurement of the third molars; they also found that the irradiation of the head of 8-day-old rats caused a significant retardation in growth, recorded on day 57.

In the 57-day-old rats, Demajo [32] measured a mandible height that was lower than in animals from our control group. In animals whose head was irradiated with 9.6 Gy on day 8, the height of the mandible on day 57 was also lower than the value in our irradiated group.

Bicondylar distance

In the 30-day-old irradiated males rats [17], the measured bicondylar width was slightly lower than the values measured in the twice older animals in our irradiated group, so it can be said that the x-radiation with pituitary gland hypofunction caused the growth of the mandible in the transverse direction that is twice slower.

Conclusion

In the animals with locally irradiated heads, the measurements of the craniofacial system showed statistically significantly lower values compared to the measurements of the animals in the control group, as well as in the group where their pituitary glands were protected, which is a consequence of the combined effect of the x-ray radiation and irradiation-induced pituitary gland hypofunction.

The growth of the craniofacial system in rats in the sagittal direction was most sensitive to radiation and radiation-induced growth hormone deficiency.

The measurements of the growth of numerous parameters of the stomatognathic system in rats with the entire locally irradiated heads showed statistically significant delays compared to both non-irradiated control group and the group where the pituitary gland was protected.

In the group where the pituitary gland during the irradiation of the head was protected, there was a statistically significant delay in the growth of the stomatognathic system compared to the (non-irradiated) control group.

Damage to the stomatognathic system was greater due to irradiation-induced pituitary gland hypofunction than due to the direct exposure to x-rays itself.

Radiation-induced pituitary hypofunction and direct irradiation caused greater growth delay on the lower than on the upper jaw.

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The advantages and disadvantages of biodentine: satisfactory mechanical properties and radiopacity not meeting ISO standard

Prednosti i nedostaci biodentina: zadovoljavajuća mehanička svojstva i radiološka vidljivost koja ne zadovoljava ISO standard

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Abstract

Background/Aim. In dentistry, the concept of using inert materials for tissue repair has been replaced by the strategy to find bioactive materials which positively interact with human tissues. The aim of this study was to characterize the physicochemical properties of the commercially available calcium silicate and calcium carbonate-based dental cement, biodentine (Septodont, France). **Methods.** Material elucidation included the measurements of radiopacity, scanning electron microscopy and x-ray dispersive analyses, wettability, Fourier transform infrared spectroscopy, microindentation, micro- to nanoporosity, setting time, pH and calcium ion release. The cells (mouse bone marrow mesenchymal stem cells – BMSCs) were grown on biodentine surface in order to evaluate its behaviour under biological conditions. **Results.** The radiopacity of the cement (2.8 mmAl) was below ISO requirement for a root canal filling material. The cement was composed of fine powder with particles similar in size and shape, changing from oval to cubic after having been soaked in a simulated body fluid. Biodentine demonstrated good micromechanical properties and low porosity attributed to microporosity with the average pore size of 92 μm. Wettability (contact angle = 41°), calcium ion release (0.098 μg/cm²) and pH of storage solution (9.07) showed satisfactory characteristics. The BMSCa in intimate contact with cement particles remained viable, indicating biodentine good biocompatibility. **Conclusion.** Biodentine exhibits good mechanical and physicochemical characteristics, but possesses insufficient radiopacity.

Key words:
tricalcium silicate; dental cements; materials, testing; x-ray microtomography.

Apstrakt

Uvod/Cilj. U stomatologiji, koncept primene inertnih materijala u cilju reparacije tkiva zamenjen je strategijom pronalaženja bioaktivnih materijala koji pozitvno deluju na humana tkiva. Cilj studije bio je karakterizacija fizičko-hemijskih osobina komercijalno dostupnog dentalnog cementa na bazi kalcijum silikata i kalcijum karbonata, biodentina (Septodont, Francuska). **Metode.** Ispitivanje materijala obuhvatilo je merenja radiografskog kontrasta, skening elektronsku mikroskopiju i radiografsku disperzivnu analizu, kvašenje, Furier-ovu transformocionu infracrvenu spektroskopiju, čvrstinu, mikro- i nano-poroznost, vremena vezivanja, pH vrednosti i oslobađanja kalcijumovih jona. Ćelije (mezenhimalne stem ćelije kostne srži miša – BMSCs) su uzgajane na površini biodentina sa ciljem da se ispita njegovo ponašanje u biološkim uslovima. **Rezultati.** Radiološki kontrast cementa (2.8 mm Al) bio je niži od ISO standarda za materijale namenjene punjenju kanala korena. Cement se sastojao od finog praha sa česticama male veličine i oblika koji se iz ovalnog menjao u kockasti nakon potapanja u simuliranu telesnu tečnost. Biodentin je pokazao dobre mikromehaničke osobine i nisku poroznost sa prosečnom veličinom pora 92 μm. Kvašenje (Ca = 41°), oslobađanje jona kalcijuma (0,098 μg/cm²) i pH rastvora gde je čuvan (9.07), pokazali su zadovoljavajuće karakteristike. BMSCs u neposrednom kontaktu sa cementnim česticama zadržale su vijabilnost što je ukazalo na dobru biokompatibilnost biodentina. **Zaključak.** Biodentin je ispoljio dobre mehaničke i fizičko-hemijske karakteristike, ali bez zadovoljavajućeg radiografskog kontrasta.

Ključne reči:
trikalcijum silikat; dentalni cementi; materijali, testiranje; mikrokompjuterizovana tomografija.

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Introduction

Remarkable progress has been made over the last few decades in the field of materials science [1–3]. In dentistry, the concept of using inert materials for tissue repair has been replaced by the strategy to find bioactive materials which positively interact with human tissues [4, 5]. Numerous materials were manufactured with the goal to improve the treatment of pulp infection, which causes over five million interventions per year only in the United States [6, 7]. In this context, calcium silicate cements (CSC) were fabricated with the intention to replace mechanically weak calcium hydroxide materials used for healing of pulp lesions and orthograde/retrograde root canal filling. In order to meet the clinical requirements of CSC-based dental material, a radiopacifier has to be added to its composition to enable the visibility of cement on radiographs [2, 8, 9]. However, finding an ideal balance between the ratio of radiopacifier and cement powder remains a challenge of the dental and engineering community [3, 10, 11]. When choosing a proper radiopacifier for cement fabrication, several aspects should be taken into consideration. Namely, it should be biocompatible, which means that it does not damage the surrounding tissues, and it should be inert (meaning that it does not react with other cement compounds) [9, 12–16].

The first CSC-based dental material was introduced in 1993 by Torabinejad, as a mineral trioxide aggregate (MTA) [17] . This formulation consists of Portland cement and a radiopacifier bismuth oxide [Z(Bi) = 83] in 4 : 1 proportion. It was used effectively for root-end filling, pulp capping, apexification and root perforation repair [18, 19]. Nonetheless, MTA experiences some weaknesses including extended setting time (around two hours), washouts, staining of coronal dentine and impaired mechanical properties [2, 17, 20, 21]. Bismuth oxide is claimed as responsible for most of these downsides [22–24]. Therefore, a new formulation named biodentine (Septodont, Saint Maur, France) has been lunched recently [25–27]. It contains calcium silicate, calcium carbonate; zirconium oxide instead of bismuth oxide is added to improve material's radiopacity and calcium chloride is added in liquid component to reduce its setting time [13, 14]. Biodentine is considered superior to MTA due to higher biocompatibility of zirconium oxide in comparison to bismuth oxide and zirconium ability to increase the toughness of a material[16, 21, 28, 29]. Zirconium oxide is well-known in dentistry as a part of different forms of zirconia ceramics [17]. Unfortunately, in contrast to its superior biological and mechanical behavior, it has lower capacity than bismuth oxide to confer satisfactory radiopacity, due to the lower atomic number of zirconium (Z = 40) in comparison to bismuth (Z = 83) [30, 31].

Contradictory findings in the literature report biodentine to be as radiopaque as 2.8 mmAl [32], 4.1 mm Al [33], 1.5 mm Al [34], 2.06–2.52 mm Al [16] and 5.8 mm Al [35]. Therefore, there were some attempts to improve the radiopacity of biodentine by adding calcium tungstate ($BD+CaWO_4$) or zirconium oxide ($BD+ZrO_2$) [16].

This study was conducted to investigate physicochemical properties of biodentine, as well as its biocompatibility.

Methods

Material preparation

Biodentine specimens were prepared in accordance with the manufacturer's instructions. A capsule of biodentine was mixed with five drops of liquid component and placed in a mixing device Technomix (Lineatac, Italy) at the speed of 4000 rotations/min for 30 seconds.

Radiopacity determination

The radiopacity of the cements was calculated in accordance with the International Standard Organization (ISO) 6876 [36]. Five specimens for each testing procedure were made in accordance with the manufacturer's instructions and placed in teflon rings (1 mm × 8 mm). They were positioned on CCD radiovisiography (RVG)-based digital sensor (Trophy Radiology, Cedex, France) alongside an aluminum stepwedge varying in thickness from 1 mm to 10 mm, in 1 mm increments. An x-ray unit (Trophy Radiology) operating at 65 kV, 7 mA, a focus to target distance of 33.5 mm and exposure time of 0.074 s was used. Obtained digital images were analyzed using Trophy for Windows Software to make comparison between the grey scale values of the specimens and different thickness of the calibrated stepwedge.

Scanning electron microscopy (SEM) and energy dispersive x-ray analysis (EDX) of hydrated cements

Specimens of the hydrated cements were placed in a simulated body fluid (SBF) for two weeks, coated with gold and subjected to analysis using SEM (LEO 435 VP, LEO Electron Microscopy Ltd., Cambridge, England). In addition, scanning electron micrographs obtained in backscatter electron mode were used for x-ray dispersive analysis (EDX) to determine elemental constitution of the material. SBF was prepared from the following chemicals: 7.996 g NaCl, 0.350 g $NaHCO_3$, 0.224 g KCl, 0.228 g K2HPO4 x $3H_2O$, 0.305 g $MgCl_2$ x $6H_2O$, 0.278 g $CaCl_2$, 0.071 g Na_2SO_4 and 6.057 g $(CH_2OH)_3CNH_2$ and 1 l of ion exchanged water. The pH was set to 7.4 by stirring the solution with HCl.

Wettability measurements

Contact angle (CA) of glycerol (2 μL in volume) was measured by applying a sessile drop on six cement specimens. The profile of the liquid drops was recorded with contact angle analyzer ("Vinča" Institute, Belgrade, Serbia). Thereafter, CAs were calculated using Image J software by fitting the contour of the droplet placed on the surface (tangent method).

Fourier transform infrared (FTIR) spectroscopy

The hydrated specimens immersed in SBF for two weeks were desiccated and crushed into powder. Thereafter, the phase composition of the specimens was characterized by FTIR (Nicollet 380 FT-IR, Termo Electron Corporation) in attenuated total reflectance (ATR) mode. All peaks were assigned according to the established reference spectra.

Micromechanical measurements

Micromechanical characteristics of biodentine were evaluated by cyclic micro indentations using the Biodent 1000 Reference Point Indentation instrument (Active Life Scientific, Inc., Santa Barbara, CA) (RPI). Ten indentation cycles were performed on the cement surface at 2 Hz and the maximum force of 10 N. The following parameters were assessed: the 1st cycle indentation distance (ID 1st), indentation distance increase (IDI), total indentation distance (TID) and the average energy dissipated (Avg ED).

Micro- to nanoporosity analysis

For testing the microporosity, cements were kept for two months in SBF and scanned using micro-computed tomography (μCT) (Skyscan-Bruker 1172, Kontich, Belgium) operated at 100 kV, 100 μA, exposure time of 1150 ms, copper aluminum filter, rotating 180° in 0.4 steps, 5 μm isotropic resolution and 2,048 × 2,048 pixels per slice. Images were rebuilt using the NRecon v.1.6.9.8 software with beam hardening correction of 20%, ring artifact correction of 8%, post-alignment of -1 and smoothing of 1. For porosity assessment CTAn 1.14.4.1 software (Skyscan-Bruker) was used for all analyses. Threshold was set at the low mean value of 58 and the max of 255 to determine the following structural morphometric parameters: total porosity (%), closed porosity (%), open porosity (%) and pore size (μm).

For nanoporosity determination, specimens stored in SBF for two months were subjected to high-pressure mercury intrusion porosimetry (Carlo Erba Porosimeter 2000). It operated in the interval 0.1–200 MPa and Milestone 100 Software System was employed for the analysis.

Setting time determination

The initial setting time was determined at 60 s interval time using a 100 g indenter with a needle having a flat end of 2 mm in diameter. Material was considered set at the time when it was not possible to observe any mark on its surface.

Measurements of pH of storage solution and calcium ion release

For pH measurements, three cylindrical specimens (4 × 6 mm) were placed in hermetically sealed cylindrical polystyrene holders containing 25 mL of artificial saliva (Ca^{2+} concentration of saliva was 0.017 ± 0.005 kg/L at pH = 7.0) and maintained at 37 °C for two weeks. A pHmeter (WTW Ino Lab 1, Germany) with temperature-compensated electrode (WTW, Sen Tix 81, Germany) was used. For calcium ion release calculation, specimens were mixed with the artificial saliva (1 mL of saliva per 1 cm^2 specimen) and kept in an incubator at 37 °C for 14 days. Thereafter, the residue was dissolved with 10 mL of deionized water in a volumetric flask and subjected to the analysis by direct injection into an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (Nexion 300X, PerkinElmer, CA, USA). The double-spike composition was defined relative to calcium (Calcium ICP Standard 1,000 mg/L Ca CertiPUR) standard and calibrated.

Cell culture and testing

For testing the cell adherence to cement surface, three specimens were sterilized and cultured for five days with mouse bone marrow mesenchymal stem cells (BMSCs). Cells were cultured in a low-glucose Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (PS). The osteogenic medium for BMSCs consisted of the growth medium plus 100 nL dexamethasone, 10 mL β-glycerophosphate, 0.05 mL ascorbic acid and 10 nL 1α, 25-dihydroxyvitamin. Firstly, the presence of cells in media was confirmed using an optical microscope. Thereafter, the specimens with cells grown on their surfaces were fixed with 4% glutaraldehyde, dehydrated with different concentrations of ethanol (50 %, 60 %, 70 %, 80 %, 90 %, 95 % and 100%), dried at a critical point dryer for 30 min (critical point dryer bal tec CDP 030, Leica Microsysteme, Wetzlar, Germany), coated with gold and subjected to SEM observation.

Results

Figure 1 shows the results of radiopacity, EDX, wettability and FTIR measurements. Biodentine exhibited the radiopacity of 2.8 mm Al, while the glycerol droplet formed an average contact angle of 41°on its surface. EDX analysis revealed elemental peaks for the following elements: calcium, carbon, oxygen, sodium, aluminum, silicon, zirconium and chlorine. The FTIR spectrum indicated the presence of 3,414 cm^{-1} band corresponding to water molecules and Ca-OH groups in the structure. The band at 1,414 cm^{-1} can be assigned to the stretching vibrations of the carbonate group. The bands at 997 cm^{-1} and 983 cm^{-1} correspond to the Si-O stretching vibrations in Q2 site and lattice vibrations of tobermorite. The peak at 873 cm^{-1} can be assigned to out-of-plane vibrations of C-O bond, while the band at 712 cm^{-1} is typical of calcium carbonate.

Figure 2 depicts the SEM analysis of bioactivity experiment together with RPI outcomes. Having been soaked in SBF cement, crystals were transformed from round to cubic-like.

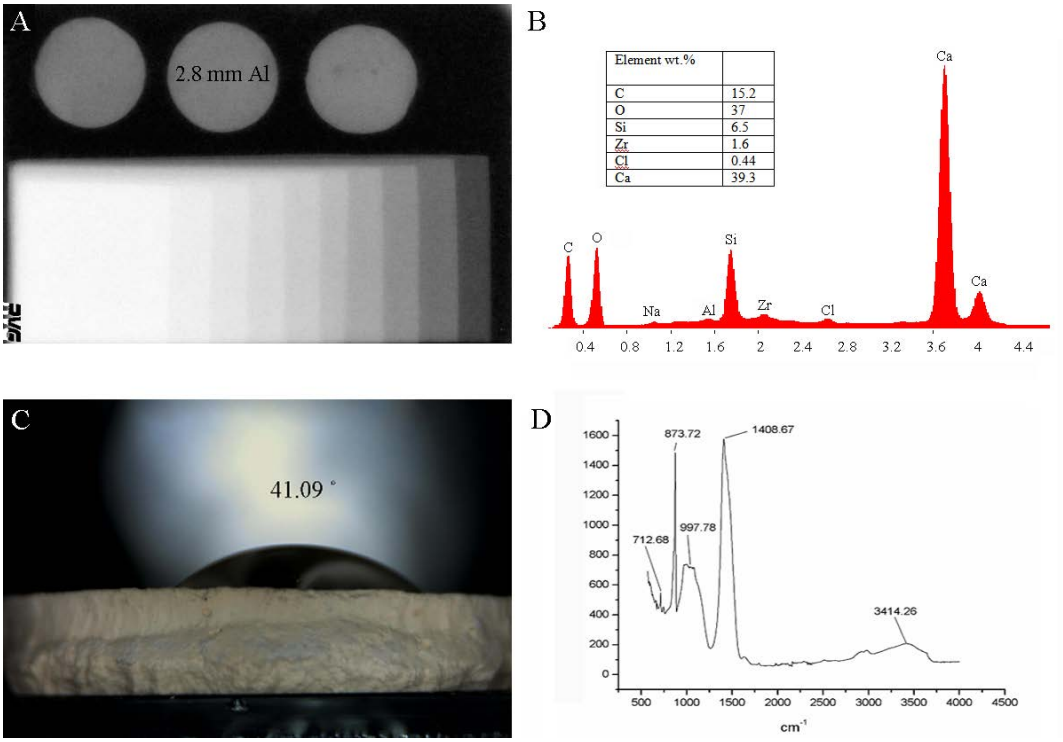


Fig. 1 – Physicochemical properties of biodentine: A) Radiopacity as expressed in equivalent thickness of aluminum showing cement specimens alongside an aluminum stepwedge; B) Energy dispersive x-ray analysis of hydrated cement specimen; C) Contact angle of glycerol droplet on cement surface; D) Fourier transform infrared spectroscopy of biodentine specimens after having been soaked in a simulated body solution for two weeks.

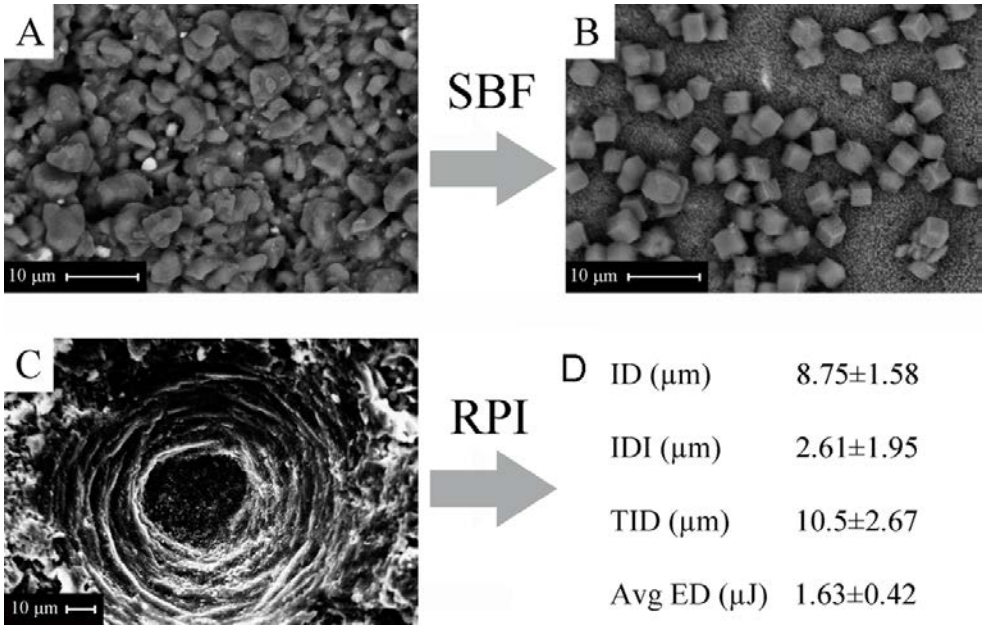


Fig. 2 – A, B) Morphology and surface precipitates of biodentine before and after having been soaked in a simulated body fluid; C, D) Scanning electron micrograph of indented cement specimen and reference point indentation outcomes of the measured parameters (results are shown as mean ± standard deviation).

Figure 3 reveals the findings of µCT measurements. Biodentine specimens exhibited low microporosity with the prevalence of closed pores. The analysis of pore size distribution demonstrated that 50% of the pores were in range between 10 and 49 µm. Figure 4 shows the results of nanoporosity analysis indicating almost total absence of nanopores in the cement structure after two months storage in SBF.

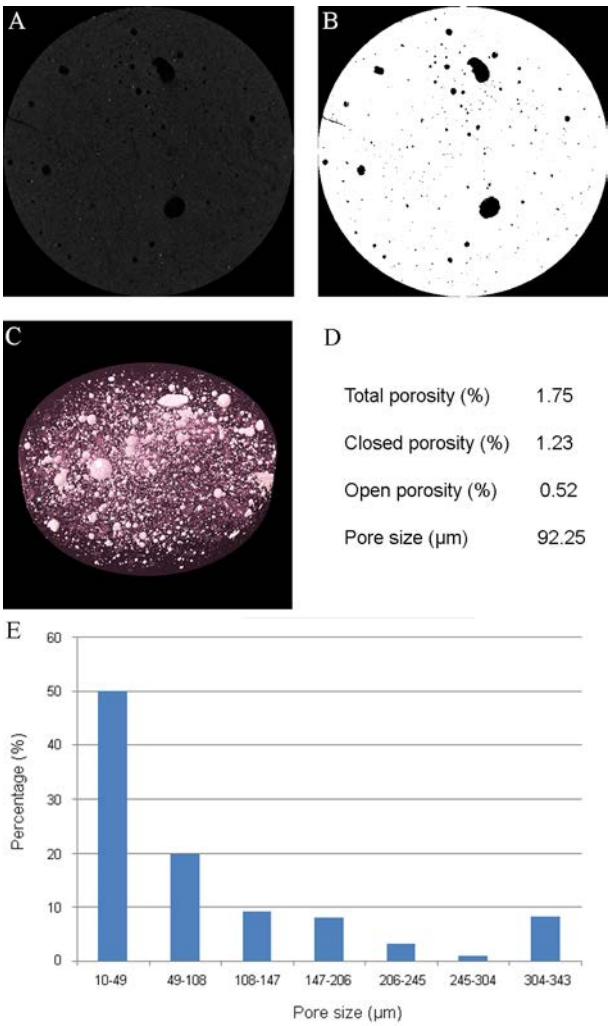
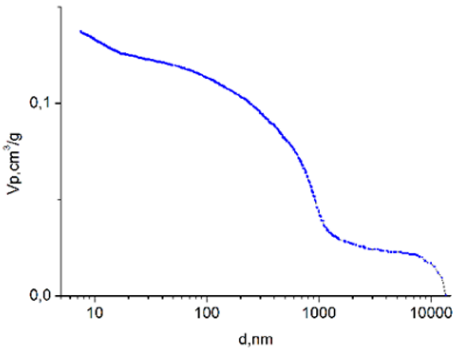


Fig. 3 – Micro-computed tomography measurements of biodentine porosity: A, B) Cross-sectional micro-computed tomographic slices; C) Three-dimensional volume rendering of biodentine; D) Results of microporosity measurements; E) Cement pore size distribution.



Nanoporosity	
Bulk density (g/cm^3)	2.37
Total sample porosity (%)	24.59
Pore radius average (nm)	432
Total cumulative vol.(mm^3/g)	137.4
Specific surface area (m^2/g)	6.32

Fig. 4 – Cumulative nanopore volumes obtained from mercury intrusion porosimetry measurements.

Table 1 reveals the results of initial setting time, pH of cement storage solution and calcium ion release.

Table 1

The results of initial setting time, pH value of specimen solution after the storage in a simulated body fluid for 14 days and the release of calcium ion after soaking specimens in artificial saliva for 14 days

Setting time (min, mean ± SD)	22 ± 2
pH	9.07
Calcium ion release (μg/cm²)	0.098

SD – standard deviation.

Figure 5 shows cells with numerous cytoplasmic processes attached to the cement surface after the 5-day incubation period. Cells infiltrated into pores with numerous cytoplasmic extensions.

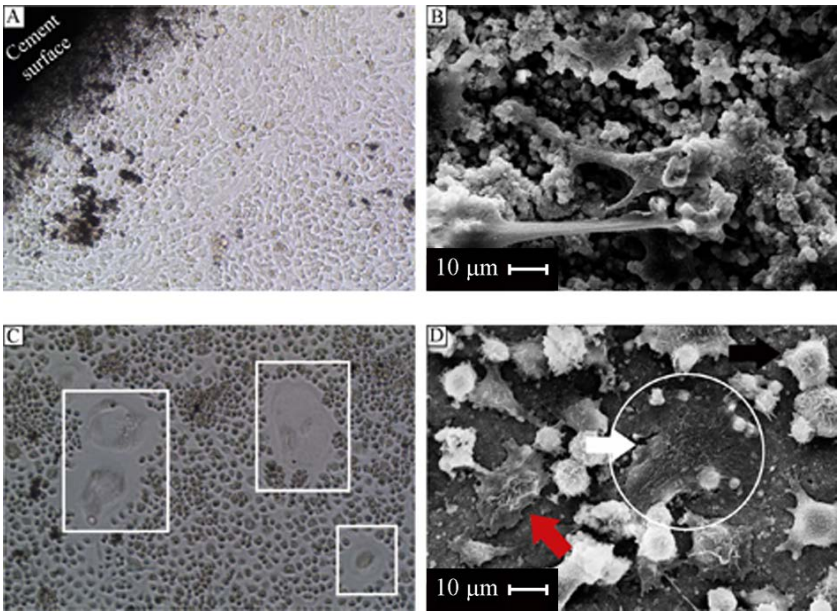


Fig. 5 – Cells attachment to biodentine surface: A) Optical microscopy of cell culture adjacent to cement surface, and B) cells adherence to cement crystals observed by scanning electron microscopy (SEM); C) Optical and D) SEM of cytoplasmic extensions on cement surface that indicate cell adhesion. Note the beginning of cement resorption caused by osteoclast-like cells (arrows).

Discussion

The long-standing goal in the field of CSC-based dental materials engineering is to find appropriate balance between the type and ratio of radiopacifier and cement powder [16]. This study encompassed biodentine's multilevel characterization, since this is the first formulation on the market incorporating zirconium oxide as a radiopacifying agent. All in all, although the results revealed quite satisfactory biological and microstructural properties of Bbodentine, its radiopacity did not meet the required criteria for adequate contrast between material and surrounding anatomical structures on radiographs.

The SEM analysis has shown that biodentine possesses the structure consisting of nano- to micro-scale particles of uniform shape. As it is known from materials science, structures composed of particles having similar size possess higher mechanical strength due to the enhanced potential for particles packing [30, 37] . In addition, microporosity of biodentine was 1.75ww%, which is lower than previously reported (6%) [24]. The analysis of μCT pores size distribution showed that 50% of micro pores were between 10 and 49 μm; on the other side mercury intrusion porosimetry did not show any presence of pores. Micro CT measurements enabled detecting pores higher than 5 μm, while mercury intrusion porosimetry allowed the determination of pores in the range of 7 nm to 15 μm. It can be additionally concluded that in the micropores ranging 10–49 μm most were greater than 15 μm. The lower porosity of the cement is a desirable quality, since it is related to higher mechanical performance of cement and because it leads to lower microleakage between root canal dentine and the material [22]. The lower cement porosity consequently leads to a lower micro gap between dentine and cement. Subsequently, a lower micro gap is associated with the avoidance of periradicular fluids to penetrate at tooth-cement interface and cause bacterial contamination [23].

Lower setting time of biodentine than previously reported for MTA comes as the consequence of finer cement particles and represents an important advantage for dental practitioners [23, 38]. The change in morphology from round shape to cubic-like crystals in SFB-soaked specimens confirmed that biodentine is modified during interaction with a biologically simulated system indicating its bioactivity – an ability of the material to provoke positive reaction from the host tissue [39]. Here, it is attributed to the potential of the cement to produce calcium carbonated crystals in the presence of SBF [10]. This was confirmed by FTIR spectra of the hy-

drated specimens having the calcium silicate phases transformed into tobermorite. In addition, calcium ion release is an indicator of biodentine's biointeractivity – an early interplay between cement surface and host tissue [40]. Finally, pH value of the solution was alkaline (9.07), which is also an important prerequisite for the successful healing of inflamed pulp tissue [6, 30]. All these favorable characteristics of biodentine were followed by cells adhering well to the cement surface. Also, biodentine induces reparative dentin synthesis and transforming growth factor beta 1 (TGF-β1) [7, 41]. To fully appreciate the significance of these and preceding findings, it may be useful to consider in more details whether biodentine may be used *in vivo* as a bone substitute material to fill bone defects after surgical procedures.

The average contact angle of reference liquid on biodentine was 41°. It has been reported during the recent years that osteoblasts preferentially adhere to the surfaces of biomaterials with the similar wettability to themselves [42]. Although glycerol was used instead of water and the literature data lack the knowledge about the wettability of CSC, this value is generally higher than reported for monolayer of bone-like proliferating cells (27°) and for nano-hydroxyapatite/chitosan bone cement (33.5°) [42]. A more recent research has shown the contact angle of distilled water on biodentine to be 25.3 ± 0.8°, indicating desirable hydrophobicity of the cement [35]. The factors affecting measured contact angles are material structural design and constitution, but divergences in the results may also arise from higher roughness of the materials, as shown in thorough and methodological study by Kisic et al. [39].

As measured by digital CCD-based radiography, the radiopacity of biodentine was equivalent to 2.8 mm Al, which is lower than minimum ISO requirement for radiopacity of an endodontic material (3 mm Al). This is in accordance with the results of Tanalp et al. [33], who reported that biodentine is as radiopaque as 2.8 mm Al, but in contrast with the findings of other authors where it exhibited the lower (1.5 mm Al [34] and 2.06– 2.52 mm Al [16]), and higher radiopacity value (4.1 mm Al [33] and 5.8. mm Al [35]) than ISO standard proposes. The differences could be due to various factors, such as various sensors for radiopacity determination [34]. The physical cause that explains different x-ray sensitivity of conventional film-based radiography and digital CCD-based sensor is the difference between the absorption of x-rays by silica and CCD sensor, silver and film emulsion, exposure time, tube voltage and source-to-object-distance [2, 31]. A material having similar composition to the detector absorbs most of the energies making the x-ray detector most sensitive. Thus, it appears more radiopaque in comparison to the material with different composition than the detector [43]. Having all the above-mentioned in mind, we concluded that more radiopacifier should be added to biodentine.

Conclusion

Despite satisfactory micromechanical characteristics of biodentine, it does not meet 3 mm Al ISO requirement for radiopacity, which is extremely significant for its clinical application. Therefore, the fabrication of CSC with adequate characteristics remains a challenge for the dental and engineering community.

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Conflict of Interest

All authors declare that there is no conflict of interest.

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Neuropathic pain as independent predictor of worse quality of life in patients with diabetic neuropathy

Neuropatski bol kao nezavisan prediktor lošijeg kvaliteta života kod bolesnika sa dijabetesnom neuropatijom

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Abstract

Background/Aim. The prevalence of diabetes mellitus in general population is constantly increasing. On the other hand, the number of diabetic patients with neuropathic pain is large. The aim of the study was to examine influence of neuropathic pain on quality of life (QoL) in patients with diabetic sensorimotor polyneuropathy (DSPN) who did not have any other diabetic complication or any other significant comorbidity. **Methods**. A total of 32 patients with DSPN and definitive neuropathic pain were compared with 32 patients with DSPN without neuropathic pain. The respondents were matched according to age, gender, and duration of illness. The following scales were used: the Pain Detect Questionnaire, Leeds Assessment of Neuropathic Symptoms and Signs, *Douleur Neuropathique* EN 4 *Questions*, Hamilton depression and anxiety rating scales, Neuropathy Impairment Score of the Lower Limb (NIS-LL), and the Short Form 36 Health Survey Questionnaire (SF-36). **Results.** Patients with neuropathic pain had significantly more severe DSPN measured with NIS-LL ($p < 0.01$). They were more likely to be engaged in physical work ($p < 0.05$), and had more symptoms of depression ($p < 0.05$) than patients without neuropathic pain. Patients with neuropathic pain had significantly lower QoL in both physical and mental domains ($p < 0.01$). Independent predictors of worse QoL in DSPN were presence of depression (beta=-0.58, $p < 0.01$) and presence of neuropathic pain (beta = -0.23, $p < 0.05$) - $R^2_{adjusted} = 0.48$. **Conclusion**. Independent predictors of QoL in patients with DSPN were presence of depression and neuropathic pain, which signifies importance of their early recognition and early treatment.

Key words:
anxiety; depression; diabetic neuropathies; neuralgia; quality of life; risk factors; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Prevalenca šećerne bolesti u opštoj populaciji raste. Sa druge strane, veliki je broj obolelih od dijabetesa sa neuropatskim bolom. Cilj studije je bio da se ispita uticaj neuropatskog bola na kvalitet života kod bolesnika sa dijabetesnom senzorimotornom polineuropatijom (DSPN), koji nisu imali ni jednu drugu dijabetesnu komplikaciju niti bilo kakve druge komorbiditete od značaja. **Metode.** Ukupno 32 bolesnika sa DPN i definitivnim prusustvom neuropatskog bola su upoređivani sa 32 bolesnika sa DSPN bez neuropatskog bola. Ispitanici su bili upareni po polu, starosti i trajanju bolesti. Korišćene su sledeće skale: *Pain Detect Questionnaire, Leeds Assessment of Neuropathic Symptoms and Signs, Douleur Neuropatathique* EN 4 *Question*, Hamiltonova skala depresivnosti i anksioznosti, *Neuropathy Impairment Score of the Lower Limb* (NIS-LL) i *Short Form* 36 *Health Survey Questionnaire* (SF-36). **Rezultati.** Bolesnici sa neuropatskim bolom su imali znatno težu DSPN mereno pomoću NIS-LL ($p < 0,01$). Bolesnici sa neuropatskim bolom su se češće bavili fizičkim poslovima ($p < 0,05$) i češće su imali simptome i depresije ($p < 0,05$) u odnosu na bolesnike bez neuropatskog bola. Bolesnici sa neuropatskim bolom su imali značajno lošiji kvalitet života, kako u fizičkim, tako i u mentalnim domenima ($p < 0,01$). Nezavisni prediktori lošijeg kvaliteta života kod bolesnika sa DSPN su bili: prisustvo depresije (beta = -0,58, $p < 0,01$) i prisustvo neuropatskog bola (beta = -0,23, $p < 0,05$) – pilagođeno R2 = 0,4. **Zaključak.** Nezavisni prediktori lošijeg kvaliteta života kod bolesnika sa DSPN bili su prisustvo depresije i neuropatskog bola, što ukazuje na značaj njihovog ranog prepoznavanja i ranog lečenja.

Ključne reči:
anksioznost; depresija; dijabetesne neuropatije; neuralgija; kvalitet života; faktori rizika; ankete i upitnici.

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Introduction

Neuropathic pain (NP) is, as defined by the International Association for the Study of Pain (IASP), a pain that arises as a direct consequence of the lesion or disease of the somatosensory system [1]. According to the site of the lesion, it can be peripheral (lesions of peripheral nerve, nerve plexus, dorsal ganglion, nerve root) and central (lesion of the spinal cord or the brain) [2]. Peripheral NP is significantly more common and better studied than central NP, and its most common cause is diabetic polyneuropathy (DPN). The most common form of DPN is chronic, symmetrical, length-dependent sensorimotor polyneuropathy (DSPN), observed in 3%–50% of patients with diabetes mellitus (DM) [3]. Taking into account that the prevalence of DM in general population is above 8% and that it is constantly increasing, number of diabetic patients with NP is large [4]. In the United States prevalence of diabetes in 2014 was even 9.3% [5].

It has been shown that DPN reduces quality of life (QoL) [6, 7]. In addition, even patients with subclinical forms of DPN may have reduced QoL [8]. Numerous studies on QoL in patients with DPN were conducted, but they usually included patients who had other significant complications of DM or other associated comorbidities that could also strongly affect QoL [7, 9, 10]. Also, in many of these studies patients who did not have diagnosis of definite DPN were included. Assessment of NP in these studies has not been consistent, and some patients even used NP medication and antidepressant drugs.

The aim of this study was to assess the impact of NP on QoL in patients with confirmed diagnosis of DSPN and definitive diagnosis of NP, along with excluding all other significant complications of diabetes and other illnesses that could affect QoL, as well as excluding all patients that use or have used NP medication anytime in their life.

Methods

This research was approved by the Ethics Committee of the University Clinical Center of the Republic of Srpska, Banja Luka, Bosnia and Herzegovina. Prior to the research, informed consent was obtained from all patients.

A total of 140 consecutive patients with suspected DPN as complication of DM type 2 were tested in the period from December 1, 2014 to December 1, 2015 at the Electromyography Laboratory, Neurology Clinic of the University Clinical Center of the Republic of Srpska. Among them, 58 patients had diagnosis of definitive NP according to criteria made by Haanpää et al. [11] and diagnosis of definitive DSPN in accordance to criteria proposed by Dyck et al [12]. Of these, 15 patients were excluded due to comorbidities, including history of stroke, and myocardial infarction, heart failure, renal failure, and limb amputation. Patients who abused alcohol, or use NP medication or psychiatric therapy were also excluded.

We used three questionnaires for the diagnosis of NP (the Pain Detect Questionnaire – PD-Q, the Leeds Assessment of Neuropathic Symptoms and Signs – LANSS and the *Douleur neuropathique* EN *4 Questions* – DN4). The questionnaires were filled in by patients in the presence of a neurologist who was available to them in case of difficulties in understanding certain questions. The PD-Q scale score ≥ 19 indicates a clear presence of NP [13]. Score ≥ 12 on the LANSS scale indicates the presence of NP [14], while score ≥ 4 indicates the presence of NP according to the DN4 [15]. The study focused on 32 patients who had a diagnosis of NP, according to all three questionnaires – definite NP.

The control group included 32 patients with diabetic neuropathy who did not have a clinical diagnosis of NP according to Haanpää et al.[11] and who had insignificant scores on all three applied diagnostic questionnaires for NP. Patients in the experimental and control groups were matched according to gender, age, and duration of the DPN.

In all 64 patients involved in the study, we excluded other causes of polyneuropathy (urea, creatinine, B12, thyroid hormones serum levels, immunological and virusological analyses, serum and urine protein electrophoresis with immunofixation, tumor markers etc.). General questionnaire was used to examine the demographic characteristics of patients, including sex, current age, level of education, occupation, employment and marital status. Clinical characteristics of the disease were also examined: age at the onset and duration of both DM and DPN, therapy for DM (oral, insulin or both), concomitant medication for any other disease, type of polyneuropathy according to the type of affected nerves (sensory, sensorimotor, motor), type of polyneuropathy according to the type of pathological impairment (axonal, axonal-demyelinating, demyelinating) and severity of polyneuropathy (Neuropathy Impairment Score of the Lower Limb – NIS-LL) [16]. The NIS-LL consists of three groups of tests: muscle strength, muscle reflexes and sensibility testing. Values range from 0, representing a normal finding, to 88 representing muscle paralysis, absence of muscle reflexes, and impairment of superficial and deep sensibility on the dorsal side of the thumb of the foot.

A nerve conduction study (NCS) was performed in all patients by the same examiner on the Oxford Synergy device. Temperature of the tested limbs was above 31ºC. NCS were conducted using superficial stimulation and registration electrodes. Motor conduction velocity (MCV) (median, ulnar, peroneal and tibial nerves) and sensory conduction velocity (SCV) (median, ulnar and sural nerves) were examined. Also, we tested the amplitudes of the compound muscle action potentials (CMAP) and minimum F-wave latency for previously mentioned motor nerves and amplitude of the sensory nerve action potentials (SNAP).

21-item Hamilton Depression Rating Scale (Ham-D) was used to assess depression, where score > 8 indicates the presence of depression [17]. Hamilton Anxiety Rating Scale (Ham-A) was used to estimate anxiety, where score > 18 indicates the presence of anxiety [18]. As a measure of health-related QoL, each patient filled in the Serbian version of the Short Form 36 Health Survey Questionnaire (SF-36) [19] which is a generic measure that combines eight general health concepts: physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT),

social functioning (SF), role emotional (RE), and mental health (MH). Beside the total SF-36 score, physical composite score (PCS) and mental composite score (MCS) are two main scores to summarize these eight scales. All scores were interpreted with a 0–100 scale, where higher numbers represent better QoL.

Statistical data processing was performed in SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA). All examined variables were first analyzed using the Kolmogorov-Smirnov test to determine whether they were distributed by normal distribution. For the comparison of nominal and ordinal variables, the χ^2 test or Fisher test were used. Difference between two continuous nonparametric variables was investigated using the Mann-Whitney *U*-test, while Student's *t*-test was used for continuous parametric variables. All variables that differed between patients with and without NP were included in the multiple linear regression analysis (stepwise method) as independent variables, while the SF-36 score was considered dependent variable. Level of statistical significance was 0.05 for statistically significant difference and 0.01 for statistically highly significant difference.

Results

Sociodemographic and clinical characteristics of our cohort of patients are shown in Table 1. Patients with NP had significantly more severe form of DSPN measured with the NIS-LL scores ($p < 0.01$), and were more likely to be engaged in physical work than patients without NP ($p < 0.05$). Other parameters did not significantly differ.

Results of psychological status assessment are shown in Table 2. Patients with NP had a significantly higher score on depression scale and a higher percentage of depression compared to the patients without NP.

Results of QoL assessment are shown in Table 3. All SF-36 domains were significantly worse in patients with NP, except for RE. Patients with NP also had significantly lower PCS, MCS, and total SF-36 scores.

In multiple linear regression analysis (stepwise method), we included all variables that differed between patients with and without NP (presence of NP, NIS-LL total score, occupation and results on Ham-D) (Table 4). Presence of NP and presence of depression appeared as independent predictors of poor QoL in DSPN.

Table 1

Sociodemographic and clinical characteristics of DSPN patients with and without neuropathic pain (NP)

Characteristics	Patients with NP (n = 32)	Patients without NP (n = 32)
Gender (% of men)	50.0	50.0
Age (years, mean ± SD)	60.0 ± 6.7	59.2 ± 8.6
Education (%)		
lower	25.0	6.2
medium	62.5	71.9
high	12.5	21.9
Occupation (%)*		
physical job	65.6	31.2
intellectual work	34.4	68.8
Employment (%)		
employed	43.8	53.1
unemployed	56.2	46.9
Marital status (%)		
lives with a partner	87.5	87.5
lives alone	12.5	12.5
Age at onset of DSPN (years, mean ± SD)	51.5 ± 7.9	51.3 ± 8.0
Disease duration (years, mean ± SD)	8.4 ± 2.6	7.9 ± 1.8
Diabetes therapy (%)		
oral	21.9	37.5
insulin	56.2	53.1
both	21.9	9.4
Type of polyneuropathy (%)		
sensory	43.8	43.8
sensorimotor	56.2	56.2
Type of polyneuropathy (%)		
axonal	56.2	50.0
axonal-demyelinating	43.8	50.0
NIS-LL motor score (mean ± SD)*	1.3 ± 1.8	0.2 ± 0.7
NIS-LL sensory score (mean ± SD)**	6.6 ± 1.2	2.8 ± 1.0
NIS-LL reflex score (mean ± SD)**	4.4 ± 2.1	1.8 ± 0.8
NIS-LL total score (mean ± SD)**	12.4 ± 3.5	4.7 ± 1.6

***$p < 0.05$; **$p < 0.01$.**

SD – standard deviation; DSPN – diabetic sensorimotor polyneuropathy; NIS-LL –Neuropathy Impairment Score of the Lower Limb.

Table 2

Anxiety and depression in DSPN patients with and without neuropathic pain (NP)

Score	Patients with NP (n = 32)	Patients without NP (n = 32)	Test (*p*)
HamD (mean ± SD)	8.5 ± 5.1	4.9 ± 3.4	*U* (0.01)
% of patients with depression	37.5	15.6	χ^2 (0.04)
HamA (mean ± SD)	11.4 ± 7.8	7.1 ± 5.8	*U* (0.08)
% of patients with anxiety	28.1	12.5	χ^2 (0.12)

DSPN –diabetc sensorimotor polyneuropathy; SD – standard deviation; HamD – Hamilton Depression Rating Scale; HamA – Hamilton Anxiety Rating Scale.

Table 3

Quality of life in DSPN patients with and without neuropathic pain (NP)

SF-36 domain	SF-36 score		
	patients with NP (n = 32)	patients without NP (n = 32)	Test (*p*)
PF	83.0 ± 12.3	91.9 ± 6.1	*t* (0.00)
RP	29.7 ± 37.8	70.3 ± 40.4	*U* (0.00)
BP	45.1 ± 13.2	64.6 ± 10.4	*t* (0.00)
GH	29.3 ± 9.1	39.8 ± 10.2	*t* (0.00)
VT	49.1 ± 18.8	68.0 ± 17.5	*U* (0.00)
SF	54.7 ± 6.7	71.9 ± 14.9	*t* (0.00)
RE	65.6 ± 48.3	71.8 ± 43.3	*U* (0.60)
MH	56.1 ± 21.4	76.0 ± 17.7	*U* (0.00)
PCS	47.2 ± 15.6	66.9 ± 14.3	*t* (0.00)
MCS	51.0 ± 18.8	65.5 ± 18.8	*U* (0.00)
Total SF36	51.6 ± 17.5	69.3 ± 17.3	*t* (0.00)

SF-36 – Short Form 36 Health Survey Questionnaire; **DSPN – diabetic sensorimotor polyneuropathy; PF – physical functioning; RP – role physical; BP – bodily pain; GH – general health; VT – vitality; SF – social functioning; RE – role emotional; MH – mental health; PCS – physical composite score; MCS – mental composite score.**

Table 4

Predictors of total SF-36 score in patients with DSPN (a multiple linear regression analysis – stepwise method)

Included variables	Beta	*p*
Neuropathic pain	-0.23	0.02
HamD	-0.58	0.00
	$R^2_{adjusted}$ = 0.48	

SF-36 – Short Form 36 Health Survey Questionnaire; HamD – Hamilton Depression Rating Scale (excluded variables: NIS-LL total score, occupation); DSPN – diabetic sensorimotor polyneuropathy.

Discussion

The results of our research showed reduced QoL in patients with DSPN, especially in those with NP. This means that treatment of NP might significantly improve QoL of these patients, particularly because there is no effective causative therapy for polyneuropathy. In our group of patients with NP compared to those without NP, much lower PCS, MCS and total SF-36 scores were observed. Furthermore, the study showed that all QoL domains (except RE) were significantly worse in patients with NP. We demonstrated that NP was an independent predictor of poorer QoL in patients with diabetic neuropathy.

Aslam et al. [9] found similar results regarding individual SF-36 domains, PCS and MCS while examining 25 patients with painful DSPN and 25 patients without NP. In another study, authors found lower PCS and MCS scores on 12-Item Short Form Survey (SF-12) in patients with NP compared to those without it [7]. One recent study conducted in Croatia has shown worse scores on all SF-36 domains in 80 patients with painful DSPN compared to 80 patients with DSPN and no pain [10]. However, these authors included patients with significant comorbidities and those who used NP drugs that altogether may have influence patients' QoL and results of the study.

Patients with DSPN from both groups (with and without NP) had the best scores in PF domain. Similar findings were obtained by Wasserman and Trifonova [20], as well as in our work from 2014 [21]. Such a good result for PF in our patients is due to the fact that DSPN is predominantly sensory

polyneuropathy in which motor fibers are long preserved and that patients with significant comorbidities were excluded. On the other hand, different results were obtained in other studies that found PF to be among the worst scores [9, 10, 22]. It is of note that patients with significant comorbidities were included in these studies which certainly may affect the results.

The worst results on the SF-36 were obtained for GH in both investigated groups of patients, which is a score where patients directly estimate and anticipate their health and diseases. These findings correspond to the results in most of the studies published so far, in which it was found that this item was among the most commonly and severely affected on the SF-36 scale [9, 10, 20, 21]. RP was a subdomain with very low scores in patients with NP, and very high in patients without NP. Similar results were found in other studies which suggest significant influence of NP on patients' working ability and other daily activities scale [9, 10, 20, 21]. It should also be mentioned that patients with NP in our study more frequently performed physical than intellectual job. So, it is possible that NP has impact on their working ability and *vice versa*.

Patients with NP in our study had more severe form of neuropathy since they probably have more nerve fibers affected. Similarly, multiple sclerosis patients who were more affected had a greater physical disruption of myelin and axons in their central nervous systems so they were more likely to suffer from NP [23]. Ruts et al. [24] reported similar findings in Guillain-Barré syndrome and noticed that the prevalence of pain was significantly higher in the severely affected patients.

Patients with DSPN and NP had a significantly higher score on the depression scale and a higher percentage of depressed patients compared to the patients without NP. Moreover, depression was an independent and the most significant predictor of QoL in our patients with DSPN. In the group of patients with NP, the percentage of depressed (38%) and anxious patients (28%) corresponds to the results in other studies [9, 20, 25, 26]. Aslam et al. [9] used the Hospital Anxiety and Depression Scale (HADS) in their study to assess the presence of depression and anxiety and obtained worse scores, as well as a higher percentage of depressed and anxious patients in the group of patients with painful DSPN compared to the patients without NP, although the results were statistically significant only for anxiety. Gore et al. [25] obtained a significant association between painful DSPN and depression/anxiety in the study on 255 patients with painful DSPN.

Linear regression analysis showed that the presence of depression and NP were predictors of worse QoL in patients with DSPN and that the obtained model explained almost 50% of the total SF-36 score variance. It is of note that there are another factors not included in our analysis that could explain remaining 50% of the variance. Previous studies showed that the most important predictors of QoL in diabetic patients with or without neuropathy are associated with disease itself rather than with sociodemographic factors [27, 28], while the presence of macrovascular complications is the most important predictor, as was noted in one review article [29]. In a prospective study of 53 patients with DSPN in DM type 2, Lyracos et al. [27] found that QoL was significantly reduced compared to the general population, and the most important predictors of QoL were: symptoms and signs of DPN measured by the Michigan Neuropathy Screening Instrument, HgbA1C level, reduction of activity, mental fatigue, depression, neuropathy treatment and the presence of cardiovascular diseases. Papadoupolos et al. [28] found that significant predictors of QoL were female sex, complications of DM, other associated diseases not related to DM and duration of DM

Main limitation of our study is a relatively small sample size. The strength is in the fact that the research involved patients who had a confirmed DSPN diagnosis and a definitive diagnosis of NP, and that control group of patients with DSPN without NP was also included. In addition, we included only the patients with no other significant complications of DM or comorbidites that could affect QoL.

Conclusion

We found significantly lower QoL in both physical and mental domains in patients with DSPN and NP compared to patients with DSPN without NP. Independent predictors of QoL in patients with DSPN were presence of depression and NP, which signifies importance of early recognition and early treatment of these symptoms.

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Scoliosis, life style and low back pain in adolescents

Skolioza, način života i bol u donjem delu leđa kod adolescenata

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Abstract

Background/Aim. Various internal and external factors could have an influence on the appearance of scoliosis and other postural disturbances in adolescents. The aim of this study was to investigate the correlation between scoliosis and other postural disturbances, physical activity, nutritional habits, as well as its association with comorbidities and age. **Methods.** This cross-sectional study involved 212 adolescents with the average age of 12.74 ± 1.34 years that were selected by a random selection of attended class of elementary school. A modified "The Physical Activity and Postural Disturbance Test" was used for the study. For statistical analysis Pearson's test of correlation and linear regression were used to estimate the association between scoliosis and postural disturbances, age, and physical activity, nutritional habits and comorbidities in adolescents. **Results.** It was shown that there was a statistically significant association between scoliosis and age (r = 0.228, $p < 0.05$), a significant negative correlation with lordosis (r = -0.207, $p < 0.05$) and a statistically significant positive correlation between scoliosis and flatfoot (r = 0.279, $p < 0.01$), *metatarsus varus* (r = 0, 2, $p < 0.05$) and low back pain (r = 0.304, $p < 0.05$). The results of linear regression with the scoliosis, as a dependent variable, and low back pain and other diseases as independent variables have shown that low back pain was significantly associated with scoliosis ($p < 0.05$). **Conclusion.** Scoliosis in adolescents is significantly associated with age and postural disturbances, especially with foot postural disturbances, as well as with low back pain.

Key words:
adolescents; back, pain; body, posture; risk factors; scoliosis; spine, curvatures.

Apstrakt

Uvod/Cilj. Različiti unutrašnji i spoljašnji faktori bi mogli uticati na pojavu skolioze i drugih posturalnih poremećaja kod adolescenata. Cilj studije bio je da se istraži korelacija između skolioze i drugih posturalnih poremećaja, fizičke aktivnosti, navika u ishrani, kao i njena udruženost sa godinama života i komorbiditetima. **Metode.** Studijom je bilo obuhvaćeno 212 adolescenata prosečne starosti 12,74 ± 1,34 godina, koji su selektovani metodom slučajnog izbora razreda koji su pohađali u osnovnoj školi. Za istraživanje je korišten modifikovani „Test fizičke aktivnosti i posturalnih poremećaja". Za utvrđivanje udruženosti skolioze i posturalnih poremećaja, godina starosti, fizičke aktivnosti, navika u ishrani i komorbiditeta kod adolescenata korišćeni su Pearson-ov test korelacije i linearna regresija. **Rezultati.** Utvrđena je statistički značajna pozitivna korelacija između skolioze i godina života (r = 0,228, $p < 0,05$), značajna negativna korelacija sa lordozom (r = -0,207, $p < 0,05$), pozitivna sa ravnim stopalom (r = 0,279, $p < 0,01$), deformitetom stopala *metatarsus varus* (r = 0,2, $p < 0,05$) i bolom u donjem delu leđa (r = 0,304, $p < 0,05$). Rezultati linearne regresije sa skoliozom kao zavisnom varijablom, a bolom u donjem delu leđa i drugim oboljenjima kao nezavisnim varijablama, pokazali su da je bol u donjem delu leđa značajno udružen sa skoliozom ($p < 0.05$). **Zaključak.** Skolioza kod adolescenata je značajno udružena sa godinama života i posturalnim poremećajima, posebno sa posturalnim poremećajima stopala, kao i sa bolom u donjem delu leđa.

Ključne reči:
adolescenti; leđa, bol; telo, držanje; faktori rizika; skolioza; kičma, krivine.

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Introduction

The term posture represents a complex of different domains (psychological, kinesiological, biomechanical and physiological). This is the reason of disagreements about the definition, diagnosis and means of treating various disorders. Optimal load on the skeletal system, balance between antagonistic muscle groups, optimal activity of musculoskeletal system and internal body systems are essential for the maintenance of normal posture. Various external and internal factors during growth and development can influence the occurrence of postural disturbances (reversible and irreversible) of the spine (scoliosis, kyphosis, lordosis) and lower extremities [1, 2].

Structural scoliosis is a three-dimensional deformity of spine with lateral curvature, changes in sagital profile end rotation in transversal plane. These lead to the appearance of truncal asymmetry with rib or lumbar prominence on the convex side of curvature. Functional scoliosis refers to a disorder in which there are no structural changes in the spine, so it has a better prognosis than structural scoliosis [3–5]. Scoliosis appears in 2–3% of population [6], but when a smaller angle of curvature (less than 10°) is taken for the definition of scoliosis [7], a significantly higher prevalence of scoliosis is registered (4.5%) [8]. Functional scoliosis is completely reversible in comparison to structural scoliosis that is mostly idiopathic scoliosis (around 90%). Scoliosis can cause serious health problems, as well as the back pain, make difficulties in breathing, disturbance of lung and heart function, psychological and social problems [7–9].

The angle of thoracic kyphosis is normally 20–40°, measured by Cobb method from Th2-Th12 [10]. Poor posture with kyphotic postural disturbance is characterized by complete correction.

Lumbal lordosis is the key postural component. Physiological lordosis is determined with the specific characteristics of every person [7].

Several studies suggested that the pediatric flatfoot as a postural disorder in the lower extremities is a frequent presentation in clinical practice [11, 12]. Potential pain and disability are the reasons to discuss the prevention and treatment of this problem.

Physical activity is necessary for normal growth and development of children. Fast growth in adolescence is associated with muscle disbalance and insufficiency. Increased sedentary activities could be the reason for poor physical condition of adolescents. Lack of physical activity is in direct relation with health problems in childhood years, as well as obesity, metabolic diseases and poor health status of musculoskeletal system [7, 13].

Results of studies confirm that regular physical activity is useful for health. Also, participation in sports activities of high level can influence the occurrence of postural disturbances and functional painful syndromes. Strenuous physical activity can cause structural changes: increased angle of thoracic kyphosis, lumbar lordosis associated with higher cumulative time of training and certain type of sport, as well as the presence of back pain [14].

Back pain is often present in children. The occurrence of the back pain is increased with age, especially in the early period of adolescence [15, 16].

The increase of sport activities and improvement of the eating behavior in childhood are identified as the target for future policy and health care planning [13]. Previous studies reported different results of researches concerning recommended guidelines from at least one hour, to moderate and intensive everyday physical activity [17–19]. The SPEEDY study (Sport, Physical activity and Eating behavior: Environmental Determinants in Young people) also studied the level of physical activity and eating behaviors in the large population of children aged 9–10, as well as individual and collective factors associated with these habits [20]. There are reports in literature about risk factors for the development of scoliosis and other postural disturbances [21, 22], about diagnostics and therapy of scoliosis [5, 23, 24], about the treatment of the other postural disturbances [1], physical activity and eating behavior in children with scoliosis [20], other postural disturbances [25], back pain in children and the causes of the pain [14, 16, 25, 26].

However, a little is known about the association between scoliosis and other postural disturbances, the association between scoliosis and physical activities, eating behavior, back pain and other diseases that could help in the identification of risky groups and promotion of changes in the domain of health behavior and habits. That is why the aim of this study was to examine the association between scoliosis and other postural disturbances, scoliosis and physical activity, scoliosis and eating behavior, as well as its association with comorbities (back pain and other diseases).

Methods

This cross-sectional study included 212 adolescents, average age of 12.74 ± 1.34 years (range of 10 to 14 years). The sample of children included elementary-school pupils from Banjaluka region, of a certain age, who were selected randomly by the attended class of elementary school (Table 1).

The exclusion criteria were the presence of congenital musculoskeletal deformities, neuromuscular diseases and injuries that resulted in deformities and/or changes of the function of the musculoskeletal system.

All the participants underwent a standardized physical examination of the spine and feet [5, 12] and a modified "The Physical Activity and Postural Disturbance Test" was used for each participant [9]. This test includes two parts: 'The Physical Activity Test' with the questions from the domain of physical activity, eating behaviors and comorbidities and 'The Test of Postural Disturbances' (screening on scoliosis, neck asymmetry, kyphosis, lordosis, flatfoot, *metatarsus varus*).

The domain of physical activity was related to the questions about the average time that children spent every day walking, participating in sports, learning and sitting in front of a computer. The domain of eating behaviors contains the questions about the variety of foods in nutrition and about appetite. The domain of comorbidities contains the information about the presence of the back pain and other diseases (a chronic

Table 1

Characteristics of study participants (n = 212)

Parameter	Adolescents	Total number of postural dysfunctions
Age (years), mean ± SD	12.74 ± 1.34	
Sex, n (%)		
girls	164 (77.3)	212
boys	48 (22.7)	
Sport, n (%)		
active participation	35 (16.51)	212
regular activity	177 (83.49)	
Postural disturbances, n (%)		
neck asymmetry	30 (14.15)	30
scoliosis		
functional	56 (87.5)	64
structural	8 (12.5)	
kyphosis		
functional	30 (76.9)	39
structural	9 (23.1)	
lordosis		
functional	42 (85.71)	49
structural	7 (14.29)	
flatfoot	90 (42.45)	90
metatarsus varus	8 (3.77)	8
Total		280

SD – standard deviation.

illness, as well as a chronic illness of respiratory organs, allergy, visual, hearing, heart problems).

The diagnosis of postural disturbances was based on visual screening of the spine and feet with the required Adams bending's test performed in scoliosis, test of reclination with contraction of the back extensor muscles in kyphosis and test with contraction of abdominal muscles in lordosis. Children were classified as having normal findings (estimated "0"), flexible postural disturbance or mild asymmetry (grade "1") or structural, clear, nonflexible deformity or asymmetry (grade "2"). The average time spent on a certain physical activity related to the average duration of activities every day (we did not consider the time spent in school): learning, sitting in front of a computer longer than 2 hours, the duration of walking less than 1 hour and the time spent in moderate to intensive physical activity less than 1 hour, we recorded as "1". With "0" we recorded the time spent on sedentary activities in duration less than 2 hours, walking one or more hours daily, and having moderate to intensive physical activity one or more hours every day.

We marked active participation in sport with "1"and regular activities of children of the same age with "0". If children declared that they consumed variety of foods in nutrition, this parameter was marked with "0" and if they did not, with "1".

If children did not have back pain and other diseases, this parameter was marked with "0", but if they did, it was marked with "1".

Informed consent was obtained from all subjects. All parameters that were collected and testing procedures are the part of the regular clinical and ethical procedures with children and medical practice. Study methods were approved by the institutional Ethics Committee.

For statistical analysis, we used software package SPSS 17. Statistical significance of differences was set on the level of $p < 0.05$.

Results

Characteristics of the study participants and postural disturbances, diagnosed in adolescents from the examined sample are presented in Table 1. We diagnosed the total of 280 postural disturbances, mostly flatfoot. The percentage of scoliosis was smaller in adolescents who participated in sports activities than in those who did not.

Correlation between the occurrence of scoliosis and other postural disturbances is presented in Table 2. The results of the study showed that there was a significant, but weak correlation between scoliosis and the age of

Table 2

Correlation between the presence of scoliosis and other postural disturbances (n = 212)

Tested correlations	r	*p*
Scoliosis - age	**0.228**	**< 0.05**
Scoliosis - neck asymmetry	-0.227	> 0.05
Scoliosis - kyphosis	0.145	> 0.05
Scoliosis - lordosis	**-0.207**	**< 0.05**
Scoliosis - flatfoot (*pes planus*)	**0.279**	**< 0.01**
Scoliosis - *pes metatarsus varus*	**0.200**	**< 0.05**

***Note*: Significant correlations are bolded.**
r – Pearson's test of correlation.

adolescents (r = 0.228, $p < 0.05$), scoliosis and flatfoot (r = 0.279, $p < 0.01$), scoliosis and *pes metatarsus varus* (r = 0.2, $p < 0.05$), and a negative correlation between scoliosis and lordosis (r = -0.207, $p < 0.05$).

Scoliosis and back pain statistically significantly correlated (r = 0.304, $p < 0.05$) (Table 3), but other examined parameters did not show a significant correlation.

The results of linear regression (Table 4) showed the association between scoliosis (as a dependent variable) and other postural disturbances (F = 5.467, $p < 0.001$). We found a significantly higher association between scoliosis and flatfoot ($t = 4.155$, $p < 0.001$), as well as *pes metatarsus varus* ($t = 2.090$, $p < 0.05$) in relation to the other studied postural disturbances ($p > 0.05$).

In our study, there was no significant association between scoliosis and the domain and parameters of physical activities (walking, sport, learning, sitting in front of a computer), nor with the nutritional habits. The results of linear regression with scoliosis (as a dependent variable), back pain and other diseases (as independent variables), showed that scoliosis was significantly associated with low back pain ($t = 2.095$, $p < 0.05$).

Discussion

Postural disturbances in adolescence, especially scoliosis, are the subject of many studies, as well as of the studies of other factors that could have the influence on their occurrence and development.

We studied the occurrence of postural disturbances, the association between scoliosis and postural disturbances, and the influence of certain factors of a lifestyle on the appearance of scoliosis in adolescents. The results of our research showed that in adolescents the flatfoot was most frequently diagnosed (42.4%). It was followed by scoliosis (30.1%), lordosis (23.1%) and kyphosis (18.3%). Neck asymmetry was present in 14.15% and *pes metatarsus varus* in 3.77% of adolescents. There was higher percentage of functional spinal postural disturbances in relation to structural.

Our results are in accordance with the findings of a study carried out in Brazil in order to establish the prevalence of trunk postural deviations by visual methods on the sample of 864 scholars, 8 to 15 year-olds from the city schools [27]. Authors reported that the prevalence of postural disturbances was 16.6% for dorsal kyphosis, 27.9% for lum-

Table 3

Correlation between the presence of scoliosis and physical activity, nutritional habits and comorbidities (n = 212)

Parameters	Number (%) of adolescents	Tested correlations	r	*p*
Walking (< 2 h *per* day)	95 (44.9)	Scoliosis - walking	-0.216	> 0.05
Participation in sport	34 (16.3)	Scoliosis - sport	0.016	> 0.05
Learning (> 2h per day)	203 (95.9)	Scoliosis - learning	0.098	> 0.05
Sitting in front of a computer (> 2 h *per* day)	99 (46.9)	Scoliosis - sitting in front of computer	-0.024	> 0.05
Variety of foods in nutrition	199 (93.9)	Scoliosis - variety of foods in nutrition	-0.121	> 0.05
Appetite (good)	182 (85.7)	Scoliosis - appetite	0.108	> 0.05
Back pain	8 (3.8)	Scoliosis - back pain	**0.304**	**< 0.05**
Other diseases	56 (26.5)	Scoliosis - other diseases	-0.166	> 0.05

***Note*: Significant correlation is bolded.**
r – Pearson's test of correlation.

Table 4

Association between scoliosis (as a dependent variable) and physical activity, nutritional habits and comorbidities – linear regression (n = 212)

Domain	F	*p*	*t*	*p*
Postural disturbances	**5.467**	**< 0.001**		
neck asymmetry			-0.227	0.114
kyphosis			1.064	0.293
lordosis			-0.806	0.425
flatfoot (*pes planus*)			**4.155**	**0.000**
pes metatarsus varus			**2.090**	**0.042**
Physical activity	0.685	> 0.05		
walking (< 2 h *per* day)			-1.442	0.156
sport			0.334	0.740
learning (> 2 h *per* day)			0.603	0.550
sitting in front of a computer (> 2 h *per* day)			-0.124	0.902
Nutritional habits	0.723	> 0.05		
variety of foods in nutrition			-0.947	0.349
appetite			0.865	0.391
Comorbidities	2.905	> 0.05		
back pain			**2.095**	**0.042**
other diseases			-1.006	0.320

***Note*: Significant associations are bolded.**

bar hyperlordosis and 33.2% for scoliosis. The ages of 8 to 12 were a risk factor for lumbar hyperlordosis. Scoliotic attitude did not show a significant association with the independent variables. The need for including preventive and therapeutic procedures was indicated in order to correct bad postural habits, which could cause irreversible damages in the future [27].

The results of the study aimed to identify the prevalence and the main risk factors of poor posture in school children in the Czech Republic have shown that poor posture was diagnosed in 38.3% of children, more frequently in boys [28]. The authors detected an increase in lumbar lordosis (32%), and round back (31%) in children. The prevalence of scoliosis was increased with age [28]. Our findings are in accordance with the results of these studies and have shown that there is a significant correlation between scoliosis and the age of children.

Many studies agree that rapid growth in puberty can be associated with asymmetrical growth of spine and the appearance of scoliosis [29–31]. We also explored the occurrence of association between scoliosis and other factors that could have the influence on the appearance of scoliosis or that could be associated with it, as well as physical activity, nutritional habits and comorbidities, which included the occurrence of the low back pain in adolescents aged about 13.

Flatfoot has been shown to cause abnormal stress on the foot and lower extremity. The results of our study have shown that there is a significant correlation between scoliosis and flatfoot, as well as between scoliosis and *metatarsus varus.*

We did not find studies that examined and found a significant correlation between scoliosis and flatfoot. However, a recently published cross-sectional study, which included 822 school children (mean age 12.2 ± 1.3 years), and was conducted with an aim to assess the prevalence of generalized joint hypermobility in school children in relation to scoliosis and to identify musculoskeletal problems, found no association between scoliosis and hypermobility [32]. All of the adolescents with scoliosis except one girl had mild scoliosis and among subjects having generalized joint hypermobility, the most common clinical finding was *pes planus* (34.3%).

These results are partly in accordance with our findings, although we have not assessed the presence of generalized joint hypermobility. The correlations between hypermobility and clinical symptoms are not clear. The authors concluded that generalized joint hypermobility should be considered in adolescents with scoliosis, which may be an important aspect of a treatment [32]. There is a report which showed that the occurrence of hypermobility was more frequent in patients with idiopathic scoliosis in comparison with healthy controls. Muscular weakness and ligament laxity are believed to contribute to the development of spinal deformity [33].

One study indicated that *pes planus* was one of the main features in children with generalized joint hypermobility [34]. However, there is a statement that this is not so only in these children, but in all children [32]. In the study conducted to estimate the prevalence of spine and feet deformities among children who are regularly involved in basketball trainings, the authors found that despite regular participation in basketball training, subjects in this study had high prevalence of deformities (spinal deformities were present in 53.13% and feet deformities in 64.06%) [35].

A recently published study that included 667 of the school children aged between 7 and 14 in Iran has shown the prevalence of flatfoot of 17.1% in the population studied [25]. The differences in the results of this study in relation to our results could be due to various characteristics of the sample and methodology of the study, but also to various life habits in different cultural environments.

The results of linear regression have shown the association between scoliosis (as a dependent variable) and other postural disturbances. It can be seen that there is a significant association between scoliosis and flatfoot, as well as *pes metatarsus varus* in relation to other examined postural disturbances. It is difficult to explain these findings etiologically, so for an explanation future studies are needed.

There are different reports about the results of the studies on adhering to the recommended guidelines for at least of 1 hour of moderate to intensive everyday physical activity [17–19]. A recently published study reported that there is a relationship between the physical activity of a child and the occurrence of postural defects [21]. The percentage of scoliosis in relation to participation in sports was smaller in the group of the adolescents who participated in sports activities (28.6% in regard to the adolescents who did not 30.5%), but in our research we did not obtain a significant association between scoliosis and the domain and parameters of physical activity (walking, sport, learning, sitting in front of a computer). The variation in the results of studies mostly depends on the characteristics of the participants included in the study and on the applied research method. However, in the majority of studies the results have shown that the level of physical activity decreases with age, especially in children at final elementary school age. This period is potentially important for the use and promotion of health protection procedures, as well as for doing sports activities. It is reported that, on average, children spent 4 hours weekly participating in sports activities and 14 hours weekly watching TV or playing computer games. In the group of children that did not do sport there was a significantly higher probability of the occurrence of poor posture than in children doing sport [28]. In our research, the percentage of scoliosis, kyphosis, lordosis and flatfoot was lower in adolescents that participated in sports activities than in their peers who did not.

However, one recently published article reported that performing competitive sports can be significantly associated with scoliosis [22]. The authors of cross-sectional study that examined the prevalence of spinal deformities and low back pain in adolescent competitive swimmers (n = 112) and normal controls (n = 217) of the same age (12.5 years), reported that competitive swimming was associated with the increased risk for trunk asymmetries, hyperkyphosis and with increased low back pain in females by 2.1-fold. Although

swimming was discussed as an option in the treatment of scoliosis, these results showed contrary [36].

Scoliosis is not significantly associated with the variety of foods in nutrition in our research. There are reports about the association between the nutritional status and scoliosis in children in other studies [37, 38]. Patients with idiopathic scoliosis had significantly lower weight compared with healthy controls [38].

Scoliosis and low back pain significantly correlate in our study. Parameters of physical activities (walking, sport, learning, sitting in front of a computer) do not show a significant correlation with scoliosis, which also holds for parameters from the domain of nutritional habits (variety of foods in nutrition, appetite) and from the domain of comorbidities (other diseases). These results are in accordance with other studies that reported that children with poor posture had reported pain in the lumbar spine more frequently [4, 14, 28, 39].

Scoliosis in children is considered to be painless. However, the true prevalence of back pain in scoliosis is essentially unknown. It is commonly accepted that every child who has scoliosis and reports back pain should be examined thoroughly, especially if the patient has a painful left thoracic curve. The association between scoliosis and back pain has been demonstrated in the study which found that 23% of patients with adolescent idiopathic scoliosis at the initial time of presentation had back pain, and that an additional 9% developed back pain during the period of observation [40].

The presence of scoliosis was found on radiographs in 18% (16/87) of patients in one study. Five out of 16 (31%) patients had no other underlying pathology to explain their symptoms, and therefore, scoliosis was considered to be the direct cause of their back pain. [41].

Scoliosis is significantly associated with the low back pain in our study. An increased number of adolescents with back pain, which is associated with static physical activity, as well as with standing, sitting or weightlifting during daily activities, was reported. The yearly incidence of back pain during growth increases from 12% at the age of 11 to 22% at the age of 15. Idiopathic scoliosis is not related to back pain, even in big curves, but significant thoracolumbar or lumbar scoliosis can be painful itself because of asymmetrical load of the muscles [14].

A recently published study included 116 adolescents with the average age of 13.6 years suffering from spinal pain and reported that there were 32 cases of non-specific low back pain, 31 of lumbar or thoracolumbar scoliosis and 23 of Scheuermann's disease [42]. The study in which the prevalence of the low back pain was examined on the sample that included 966 adolescents from Portugal, aged between 10 and 16, and association with risk factors, showed that 152 (15.7%) of adolescents had low back pain at the present time and 456 (47.2%) had experienced it in the last year [43]. The authors reported that low back pain is a common condition in children and adolescents, and the prevalence has been increasing over the years.

Students who sit with the spine incorrectly positioned have 2.49 times greater probability of experiencing low back pain. Those who adopt an incorrect standing posture have a 3.39 times greater chance of experiencing low back pain [43].

Conclusion

The results of our research have shown that scoliosis is significantly associated with postural disturbances and with low back pain. These results could be of significance for planning preventive, diagnostic and therapeutic procedures that would include changes in behavior and habits in adolescence, as well as creating the system of education (parents, teachers and children) in regard to postural disturbances, risks and factors that could be associated with them.

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Probiotics and fecal bacteriotherapy: the line between deception and treatment

Probiotici i fekalna bakterioterapija: linija između obmane i lečenja

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Introduction

Probiotics are a living microbial food supplement that favorably affects the host by improving the intestinal microflora, as well as live microorganisms, which by ingestion cause significant improvement of health when compared to a regular diet [1]. Initially, they were applied as an alternative therapy or simply healthy food. However, their reputation in medicine had problems due to the extravagant claims of the producers of the probiotics. In reality, the consumption of these various strains of bacteria (many of which have not shown any probiotic activity or survival ability) has shown to be inconclusive. The amount of clinical data supporting the use of proven probiotic organisms in the prevention or treatment of many disorders is lacking [2, 3].

Thus, in recent years another approach has gained considerable attention. Fecal bacteriotherapy (FBT) represents a method that consists of feces infusion from a healthy human donor to the gastrointestinal tract of a patient, with the goal of treatment of a disease that is related to gut microbiota alteration. Reports of FBT effect in Western literature started to appear in the previous 60 years, first as a treatment for antibiotic-associated diarrhea [4], although the first use of this treatment was recorded 1,700 years ago [5]. Today, the admirable effect of this approach is reported in various conditions. However, it is still classified as an investigational treatment, so it requires further standardization and developing.

Gastrointestinal flora

Coevolution led to a symbiotic bond between eukaryotes and prokaryotes with the development of a sophisticated two-way signaling system in mucous epithelium and the immune system, as well the integration of gut microflora with various signaling pathways in the central nervous system [6–8]. It has been clearly established that gastrointestinal flora is of utmost importance for the mucosal protection of the immune function. Laboratory animals without microbiota (germ-free animals) are sensitive, and with a reduced mucous immune function. The reintroduction of the flora to germ-free animals restores intestinal function, mucosal proliferation, immunity development, animal growth, and normal behavioral development [9–12].

This complex microbial world is different in composition throughout the length of the intestine with an increased inclination of the host microbe number and diversity from the stomach to the colon [13–17]. The gastrointestinal flora is described as the most adjuvant and renewable metabolic organ in the body whose composition and activity can affect both the intestines and the physiology of the individual [6, 7, 9, 18, 19]. Such an effect is not surprising since dietary byproducts, intestinal secretion of the epithelial cells within the lumen, form the basis for microbial transformations. Compared to other regions of the intestine, the colon contains the most complex microbial population showing a certain level of metabolic activity that cannot be compared to those in the liver [17, 20].

Gram positive species, above all *Lactobacillus*, are the most common isolates since they have tolerance to stomach acids. Below the ileocecal valve, the number of bacteria grows. Out of these, we can more easily study specific *Clostridium*, *Bifidobacteria*, *Bacteroides* and *Peptostreptococcus*. Despite a large number of differences between individuals in the intestinal flora, the composition of the main groups of

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bacteria within an individual appears to be relatively constant [16]. The importance of intestinal microflora is reflected in the creation of a barrier against any potentially transient pathogens. The examples of the proliferation of pathogens are: pseudomembranous colitis caused by the action of *Clostridium difficile* and *Enterococcus faecium* toxins, intra-abdominal abscesses for which *Bacteroides fragilis* can be responsible.

Depending on the genetic and other host-related factors, intestinal flora can contribute to pathogenic processes as indicated by growth and bacterial displacement in the establishment of an immune or microvascular compromise, mobility disorder, irritable bowel syndrome (IBS) or blind loop syndrome. In addition, the initiation and maintenance of intestinal disturbances, such as ulcerative colitis and Crohn's disease can occur in persons with genetic predisposition [21–23].

During and after childbirth, the fetus is exposed to microbial contamination. The level of contamination impact is related to the duration and type of the delivery process. For example, initial contacts with cesarean-born neonate microbes are related to air, medical staff and neonatal care [24]. After giving birth (either natural or by the cesarean section), infants are continuously exposed to food-derived microorganisms, both to those useful and to those which are not. Healthy breast milk contains a significant number of bacteria. These transient bacteria include *Streptococci*, *Lactobacilli*, *Micrococci*, propionic bacteria and special *Bifidobacteria* [25, 26].

For breastfed babies, *Bifidobacteria* are bacterial species that is dominated by microbial flora, and significantly less *Escherichia coli*, *Streptococci*, *Bacteroides* and *Clostridium* species. In contrast, newborns on artificial nutrition have a much more complex composition of microflora, and *Bifidobacteria* and potentially pathogenic anaerobes are predominant [25]. Recently, this has influenced the development of artificial baby foods based on formulas with bifidogenic properties similar to mother's milk in an attempt to reduce the development of enterocolitis. Twelve to 24 months after birth, independently of diet or probiotic intake, children's flora becomes much more complex and more similar to that of adults' [27].

Probiotic products – the world of "arranged chaos"

Lactobacilli and *Bifidobacteria* are most commonly associated with probiotic activities, although other organisms are used, such as the certain strains of *Escherichia* and any non-bacterial organisms, such as *Saccharomyces boulardii* [28]. This is primarily due to the understanding that they are the members of the intestinal microflora. Furthermore, these bacteria have traditionally been used in the production of fermented dairy products and have the status of "GRAS: generally recommended as safe" [29]. Most of these organisms are derived from feces of healthy people, safe for human use, and are available in large numbers. Due to the continuing skepticism of such products, the European Union has established research groups, including medical, scientific and industrial interests that have harmonized the criteria for the selection and application of probiotics. In order to meet the criteria, probiotic microorganisms should be of human origin, show nonpathogenic behavior, even in immunocompromised hosts, demonstrate resistance to technological processes, have proven resistance to acids of the stomach and bile, adhere to epithelial tissue, be able to shortly survive in the gastrointestinal tract, produce antimicrobial substances, modulate immune responses, and can have the ability to influence metabolic activities (such as, for example, cholesterol assimilation, lactase activity, and vitamin production) [1]. Nevertheless, a product can be classified as a probiotic if it contains another bacterium that is accepted as not harmful or commensal, and where no serious adverse effects are expected. As a consequence of its classification as a food supplement, the main challenge of probiotics arises – the lack of regulation and rigorousness in the process of manufacturing.

The effects of probiotics are known to be dependent on the strain and dose, as well as for their transitory effect. In addition, the commercial formulation of probiotic product can be a significant factor in bacteria delivering process [29, 30]. Considering the commercial success of probiotics in the previous years, many clinical trials were conducted and published, mostly praising their therapeutic effect. Francavilla et al. [31] reported that the 6-week probiotic supplementation with 5 combined strains of lactic acid bacteria and *Bifidobacteria* [(*Lactobacillus casei* 101/37 (LMG P-17504), *Lactobacillus plantarum* (CECT 4528), *Bifidobacterium animalis subsp. lactis* Bi1 (LMG P-17502), *Bifidobacterium breve* Bbr8 (LMG P-17501) and *Bifidobacterium breve* Bl10 (LMG P-17500)] reduced the severity of irritable bowel syndrome-related symptoms in patients suffering from celiac disease with IBS on strict gluten-free diet.

Oh et al. [32] conducted a randomly controlled trial where they examined the effect of probiotic supplementation on gut microflora during standard triple therapy for *Helicobacter pylori* eradication (clarithromycin, amoxicillin, and lansoprazole). As probiotic supplementation Medilac-S® was used. It consists of *Streptococcus faecium* and *Bacillus subtilis*. After two weeks of the treatment, proportions of the gut microbiota in the group that received triple therapy for *Helicobacter pylori* eradication were higher than those in the group that received the same therapy combined with probiotics. They also noticed an increase in the levels of antibiotic-resistant bacteria, where higher levels were present in the conventional treatment group than in the probiotic one. In addition, Haghdoost et al. [33] conducted a trial where they examined the effect of a triple therapy for the eradication of *Helicobacter pylori* combined with probiotic supplement in the form of capsules that contain strains *Lactobacillus* and *Bifidobacterium*. In this case, the supplementation continued up to 4 weeks after the triple therapy, while during this time control group received placebo. The authors found that the eradication rate of *Helicobacter pylori* infection was higher in probiotic group and the adverse events were less prevalent in patients that received probiotic supplementation. Thus, they found no significant difference in terms of the infection recurrence during a 6-month follow-up.

Ljungquist et al. [34] examined the effect of eight different living bacterial strains mixture administration in adult patients intestinally colonized for at least three months with extended spectrum β-lactamase-producing *Enterobacteriaceae*. Probiotic supplement contained eight living bacterial strains: *Bifidobacterium longum*, *Bibidobacterium infantis*, *Bifidobacterium breve* and *Streptococcus thermophiles*, *Lactobacillus plantarum*, *Lactobacillus paracasai*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii ssp. bulgaricus.* Administration lasted for two months in placebo-controlled, single-blind clinical trial. Finally, 12.5% of the patients in the probiotic group achieved successful eradication of extended spectrum β-lactamase-producing *Enterobacteriaceae*, while in the placebo group 5% of the patients achieved successful eradication. The authors of the study concluded that probiotic supplementation was not superior compared to placebo for intestinal decolonization in patients with chronic colonization of extended spectrum β-lactamase producing *Enterobacteriaceae*.

Despite various reports, there is a difficulty for consumers, as well as for physicians when one should choose a specific probiotic product [35]. The state of seemingly "organized chaos" within the probiotic industry market is a result of their non-standardized manufacturing, as well as intense and often false advertising for potential beneficial effects of their products. Moreover, in cases where a therapeutic effect of probiotics lacks, the highest price is paid by the patients themselves, depending on their socioeconomic position, as well as their health status [36–39].

Regarding future probiotic applications, there is no doubt that a treatment should be approached in an individualized manner that considers the patient's diet, hygiene habits, comorbidities, and current health status. No space should be left for biased decisions to be made. And indeed, the studies that included a personalized probiotic treatment showed an advantage over commercial products [40, 41].

Fecal bacteriotherapy

Fecal bacteriotherapy (FBT) or fecal microbiota transplantation/microbiota transfer therapy represents transplantation of the fecal bacterial flora from a healthy donor into the gastrointestinal tract of the recipient.

Repulsive for some, FBT has been reported as highly effective in the treatment of recurrent *Clostridium difficile* infection (CDI), slow-transit constipation, inflammatory bowel disease and IBS, where quality of life improvement lasted for up to 28 weeks [42–46]. The donor can be a healthy person that is a near or distant relative of the patient or a community member. The major advantage of this approach is the high probability of genetic compatibility between a donor and a recipient, as well as the related living habits and diets that have influence on gut microbiota composition. With the growing interest in FBT, novel indications that are not directly related to gastrointestinal diseases are emerging. Promising effects were shown in patients with metabolic syndrome [47], hepatic encephalopathy [48], hepatitis B infection [49], and neurobiological disorders [50, 51].

European consensus conference strongly recommends FBT for the treatment of CDI [43], although Food and Drug Administration (FDA) recommends it as an alternative therapy for the recurrent CDI after the pulsed application of vancomycin [52]. The most reported adverse effect related to FBT recipients is "abdominal discomfort", predominantly after the treatment that involved upper gastrointestinal routes of application (nasogastric tube, nasojejunal tube, gastroscopy) [53]. Kelly et al. [54] reported the death of one patient directly related to FBT treatment, where the aspiration of inoculum during a sedation phase occurred. Thus, that incident can be attributed to the complications related to the application rather than a hazard of FBT itself.

Addressing the unpleasant method of the application of fecal microbiota by colonoscopy or by upper gastrointestinal route infusion, several research groups reported that the effect of fecal microbiota delivered via oral capsules did not differ from classical delivery in adult patients with CDI [44, 55, 56]. Therefore, with the introduction of more conventional ways of microbiota administration, we could expect the elimination of most adverse effects related to FBT.

Arbel et al. [57] addressed the cost-effectiveness of FBT through the treatment of nosocomial CDIs, compared to other regiments, including probiotics. Since the appearance of the recurring hospital CDIs has turned into common and severe incidents, costs related to CDIs with current treatment regimens in the United States are exceeding $3.2 billion per year.

As mentioned previously, FDA approves of offering FBT to a patient only when a relapse of the recurrent CDI occurs after the treatment with vancomycin, with or without probiotics [52]. Regarding that, FBT showed admirable effects with the resolution rates up to 94% in the treatment of the recurrent CDIs. It is believed that FBT induces the repopulation of *Firmicutes* and *Bacteroides* spp., which are deficient in patients with the recurrent CDIs [57]. Moreover, other study groups reported that FBT showed better cost-effectiveness and outcomes when compared to vancomycin treatment [58].

During 2019, FDA released Safety Alert due to two serious adverse reactions in immunocompromised patients that resulted from the transplantation of fecal microbiota. The Safety Alert highlighted that donor material contained extended-spectrum beta-lactamase-producing *Escherichia coli*, which was the causative agent of lethal outcome in one of the two patients. FDA finally recommended thorough screening of donors for risk factors that can lead to possible infection with multi-drug resistant organisms [59].

Addressing the effects of FBT on extraintestinal diseases, there are several possible indications that deserve to be mentioned here. To our knowledge, Vrieze et al. [47] conducted the only human study related to FBT effect in patients with metabolic syndrome. The authors reported that six weeks after the infusion of microbiota via duodenal tube from donors, insulin sensitivity of recipients significantly increased, as well as the levels of butyrate-producing intestinal microbiota.

Hepatic encephalopathy represents a common complication of liver cirrhosis. Kao et al. [48] presented a case where

a patient suffering from liver cirrhosis and hepatitis C infection was treated with FBT. The patient received FBT treatments during seven weeks, after which the authors reported a "dramatic clinical improvement", and thus the beneficial effect of FBT faded after the discontinuation of treatments.

Another possible link between gut microbiota and the progression of liver diseases was addressed by Ren et al. [49] where 18 persistently HBeAg positive patients resistant to standard entecavir or tenofovir disoproxil fumarate-based therapy were enrolled in the research. Thus, from the total number, only five patients received FBT, while others served as a control. The authors reported that HBeAg titer declined gradually after each treatment of FBT given parallel with the standard therapy.

Several authors reported a possible link between autism spectrum disorder severity and the alteration of microbiota composition in children [50, 60].

Xu et al. [60] found lower percentages of several bacterial strains, including *Bacteroides*, *Bifidobacterium*, and *Parabacteroides* and a higher percentage of *Faecalibacterium* and higher abundance of *Lactobacillus* in the total detected microflora, compared to control specimens.

Kang et al. [50] conducted an open-label clinical trial, where children with autism spectrum disorder were treated with FBT for seven or eight weeks after two-week antibiotic treatment. The authors reported that behavioral symptoms in children improved significantly and remained improved eight weeks after the treatment ended. Moreover, increased bacterial diversity was registered and the abundance of *Bifidobacterium*, *Prevotella*, and *Desulfovibrio*, among others.

Unlike probiotics, the current situation with FBT is not a case of introducing another poorly regulated food supplement. Thus, it is reasonable to assume that there is still a long way for FBT to become routinely used for wide specter of indications. Furthermore, when compared to probiotic products, its classification as an emerging therapeutic treatment is one of the biggest advantages of FBT. One could expect that if FBT fulfills the given requirements and becomes classified as a therapeutic treatment, the much needed line between deception and actual treatment related to microbial therapy will be drawn.

Conclusion

Although probiotics are accepted as beneficial products, there is a great burden of production inconsistencies between manufacturers leading to the deception of patients, as well as physicians in cases of inadequate selection of a dose, strain or formulation. The future of probiotics should be oriented to a personalized probiotic treatment that considers patients' diets, hygiene habits, comorbidities and current health status. On the other hand, fecal bacteriotherapy is conducted by strict regulations and is currently under the process of evaluation as a genuine treatment option for many indications. Considering current data, fecal bacteriotherapy represents an emerging and promising low-cost solution to diseases with which antibiotic and probiotic products have been struggling for years.

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Conflict of interest

The authors declare no conflict of interest.

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Incidental misplacement of a percutaneous nephrostomy tube in the inferior vena cava

Incidentalni neadekvatni plasman perkutanog nefrostomskog katetera u venu kavu inferior

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Abstract

Introduction. An isolated renal pelvis rupture is a rare type of blunt renal trauma that can occur mostly in patients with pre-existing hydronephrosis due to different causes. We presented a patient with the misplacement of a percutaneous nephrostomy tube during the treatment of isolated renal pelvis rupture in a patient with pre-existing hydronephrosis caused by post-irradiation ureteral stricture. **Case report.** A 36-year-old woman was referred to our institution by her gynaecologist for the treatment of a retroperitoneal urinoma. She had completed the irradiation treatment with concurrent cisplatin chemotherapy for a uterine malignancy one year ago. A computed tomography scan showed an isolated rupture of the left renal pelvis with the pre-existing hydronephrosis. A nephrostomy catheter was misplaced in the inferior vena cava during the percutaneous urinary drainage attempt. The patient underwent a laparotomy, renal pelvis suture and ureteroneocystostomy with an indwelling double pigtail stent. The percutaneous nephrostomy was removed during the same surgical procedure. **Conclusion.** Inadvertent injury of vascular structures is a possible complication of percutaneous nephrostomy under ultrasound guidance. It may have been possible to avoid the reported complication if the dilation of the nephrostomy tract over the guidewire had been performed under contrast-enhanced X-ray fluoroscopy.

Key words:
hydronephrosis; intraoperative complications; nephrostomy, percutaneous; tomography, x-ray computed; urologic surgical procedures; vena cava, inferior.

Apstrakt

Uvod. Izolovana ruptura pijelokaliksnog sistema je redak tip tupe traume tog sistema koja se može pojaviti kod bolesnika sa preegzistentnom hidronefozom izazvanom različitim uzrocima. Prikazali smo bolesnicu sa preegzistentnom hidronefrozom izazvanom postiradijacionom stenozom uretera sa neadekvatnim plasmanom nefrostomskog katetera prilikom tretmana izolovane rupture pijelokaliksnog sistema. **Prikaz bolesnika.** Bolesnica, stara 36 godina, upućena je u našu instituciju od strane ginekologa radi lečenja retroperitonealnog urinoma. Godinu dana ranije završena je kombinovana zračna terapija i primena hemioterapije cisplatinom zbog ginekološkog maligniteta. Kompijuterizovanom tomografijom utvrđena je izolovana ruptura pijelokaliksnog sistema levog bubrega sa preegzistentnom hidronefrozom. Nefrostomski kateter je nehotično plasiran u venu kavu inferior prilikom pokušaja preliminarne perkutane drenaže. Bolesnici je urađena laparotomija, sutura pijelokaliksnog sistema i ureterocistoneostomija sa postavljanjem dvostrukog *pigtail* katetera. Nefrostomski kateter je uklonjen u toku iste procedure. **Zaključak.** Incidentalna povreda vaskularnih struktura je moguća kod ultrazučno vođenog plasmana nefrostomskog katetera. Navedena komplikacija bi možda bila izbegnuta da je dilatacija nefrostomskog trakta preko žice vodilje rađena pod kontrastnom radiološkom fluoroskopijom.

Ključne reči:
hidronefroza; intraoperativne komplikacije; nefrostoma, perkutana; tomografija, kompjuterizovana, rendgenska; hirurgija, urološka, procedure; v. cava inferior.

Introduction

Ureteral stricture is a common complication of irradiation treatment for uterine cervical malignancies. An overall incidence of ureteral stricture with consecutive hydronephrosis in patients following irradiation treatment varies between 1% and 2.5% [1]. An isolated renal pelvis rupture is a rare type of blunt renal trauma which occurs most frequently in patients with

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pre-existing hydronephrosis due to ureteral stones, tumours, retroperitoneal fibrosis, pelvic masses or congenital anomalies, such as stenosis of the ureteropelvic junction or vesicoureteral reflux [2]. In such cases, the formation of urinoma and consequent abscess formation can occur. Percutaneous nephrostomy (PCN) is a safe and efficient procedure for temporary urinary diversion and is rarely associated with serious complications [3].

We present a single case who represents a simultaneous appearance of a rare injury and an unusual complication of the treatment.

Case report

A thirty-six-year-old woman was referred to our institution by her gynaecologist to treat a retroperitoneal urinoma. It was found during a routine computed tomography (CT) scan (Figure 1a), scheduled as part of a check-up visit following the irradiation treatment for a uterine cervical malignancy (FIGO stage 2b). The previous year, the patient had completed combined irradiation treatment (at a total dose of 74 Gy) delivered by conformal external beam radiation treatment and brachytherapy with concurrent cisplatin chemotherapy. Preceding follow-up monitoring had revealed no sign of the recurrence of the disease. However, the patient's recent history reported moderate pain in the left flank following an accidental fall in the bathroom, although she did not seek medical attention at the time. On admission she reported urological complaints and haematuria. A physical examination revealed mild tenderness, located predominantly in the upper left region of the abdomen and flank. The laboratory findings were unremarkable. Intravenous urography (IVU) revealed contrast extravasation in the left retroperitoneum, mild hydronephrosis and stricture of the distal third of the left ureter (Figure 1b).

The initial treatment plan was to place a PCN catheter under ultrasound guidance. The patient was positioned in the supine position. A Chiba needle was inserted in the posterior lower calix under ultrasound guidance (Acuson X500, C6-2 transducer, Siemens, Erlangen, Germany). The intervention proceeded with the placement of the flexible tip guidewire and dilation of the nephrostomy tract over a guidewire. Following the placement of the nephrostomy catheter (8 French Bard, Becton, Dickinson and Company, United States), unusual blood drainage was noticed. The PCN was closed and the patient underwent a CT scan immediately, which revealed that the pigtail nephrostomy tube had passed through the left renal vein into the inferior cava vein (Figures 2a and b). Under intensive

Fig. 1 – a) Computed tomography after trauma – contrast extravasation around the psoas muscle. No injuries to the kidney; b) On urography retroperitoneal extravasation (black arrow indicating) and stenotic distal ureter (white arrow indicating) are visible.

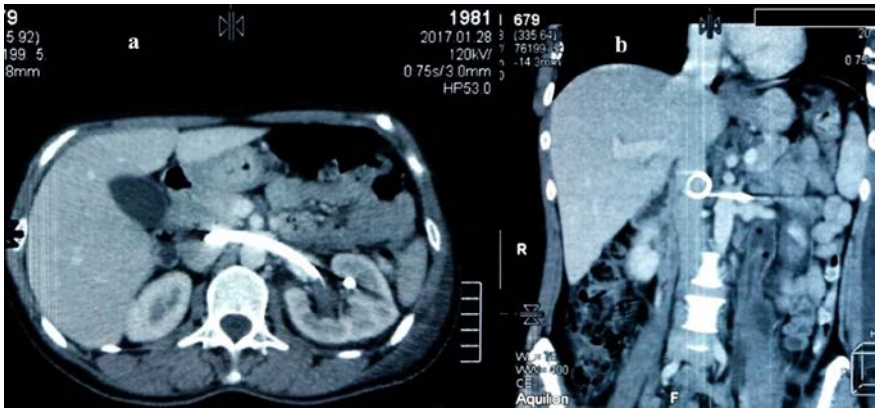

Fig. 2 – a) Percutaneous nephrostomy in the renal vein; b) Tip of the nephrostomy pigtail in the vena cava

care unit monitoring, the patient had stable haemodynamic parameters and showed no symptoms. The patient's haemoglobin blood levels were stable, excluding significant blood loss.

The patient underwent immediate surgery following the median laparotomy with a vascular surgeon present. Strict vascular control of the pigtail in the cava vein and unfolding conducted by the vascular surgeon allowed for the removal of the PCN by a urologist. There was no bleeding or haematoma formation after the removal of the PCN. A simultaneous renal pelvis suture and ureteroneocystostomy (a psoas hitch and a double pigtail stenting of the ureter) was performed. The procedure was completed, a drain inserted and the wound closed.

Thromboprophylaxis (nadroparin potassium, 0.3 mL) was introduced on the day of surgery, as well as third-generation cephalosporines (ceftriaxone 2 g/day), and continued during the seven days while the patient remained in hospital. The patient was discharged with a double pigtail catheter in the left renal unit and a urinary catheter. The drain was removed on postoperative day 2 and the urinary catheter on postoperative day 10. The double pigtail catheter was removed on postoperative day 14 during an outpatient follow-up appointment. The patient's postoperative recovery was uneventful.

A controlled IVU revealed the patient's left kidney functional, complete healing of the renal pelvis, patent ureteroneocystostomy and persistent hydronephrosis (Figure 3).

Fig. 3 – Postoperative control intravenous urography.

Discussion

Isolated ruptures of the renal collecting system are more common in cases with pre-existing hydronephrosis. To the best of our knowledge, there have been no previous reports of isolated renal pelvis rupture in patients with hydronephrosis as a result of postirradiation stricture of the distal part of the ureter.

Although the CT scan is considered a standard of care for renal trauma, IVU remains a useful method for the reliable diagnosis of urinary extravasation [4, 5]. Injuries of the renal collecting system remain a challenging issue in CT diagnostics. Extravasation of contrast will not occur during the early phases of CT scanning. Delayed CT scans are required to diagnose significant injury of renal pelvis or ureters. Contrast extravasation may be confirmed by additional IVU exposures at 30 minutes or later after intravenous contrast administration [6].

The majority of cases including an isolated injury of the renal collecting system require an active approach: a placement of a PCN or double pigtail stent, or even an open surgery [6]. Although spontaneous healing of the injury was reported, drainage should be advised for cases with persisting or increasing urinoma after five to seven days [7]. The surgical approach is indicated in cases with pre-existing ureteral obstruction.

Following a proper puncture of the pelvicalyceal system, flexible-tip guidewire problems can occur under ultrasound guidance. A standard set guidewire was placed in our patient. The protrusion of the Chiba needle deep into the collecting system of the kidney and inadvertent movements of the needle during the insertion of the guidewire may have resulted in direct cannulation of the vein. Therefore, Chiba needle tips should be inserted minimally and carefully controlled during the introduction of the guidewire. A lack of space needed for a flexible guidewire tip to wrap and secure the position for dilation may be another problem. In some reported cases the guidewire curled within the calyx itself, resulting in a vein puncture following the dilation of the tract [8]. The placement of the PCN in the renal vein and vena cava is an uncommon complication, with a total of 10 cases reported in the literature to date (Table 1) [8–15]. The majority of these 10 cases occurred in patients intended for percutaneous nephrolithotomy treatment; only two of the affected patients were scheduled for preliminary drainage. The majority of the misplacements involved the left renal vein. Large dilation tracts were reported in the majority of cases and all catheters were withdrawn without open surgery.

Possible PCN placement in the renal vein and vena cava can occur because of the existence of an anastomotic collar of veins around the calyceal infundibulum with significant antero-posterior connections and a close relationship to the renal vein. An accidental peri-infundibular vein puncture could occur in cases without permanent radiographic control and in patients without a clear distension of the calyceal infundibulum. A guidewire will follow the puncture route

Table 1

Demographic, clinical and operative data of previously published cases of misplacements of percutaneous nephrostomy (PCN) catheter within the inferior cava vein

Age/ gender	Medical history	Catheter size	Side	Location	Catheter withdrawal	Original operation	Definitive operation	Ref.
42/M	NA	14F	left	renal vein, IVC	2-step under CT	PCNL	late PCNL	[8]
38/F	right ureterolithotomy	14F	left	renal vein, IVC	2-step under fluoroscopy	PCNL	PCNL	[8]
48/M	right nephrectomy	14F	left	renal vein	1-step under ultrasound	PCNL	late ureterolithotomy	[8]
63/F	UCM, EBRT	12F	left	renal vein, IVC	1-step under fluoroscopy	PCN	PCN	[9]
54/M	left nephrectomy cystectomy	14F	right	renal vein	2-step under fluoroscopy	PCNL	laparotomy late PCNL	[10]
NA	NA	10F	NA	IVC	1-step under fluoroscopy	NA	NA	[11]
52/M	right nephrectomy	14F	left	renal vein	1-step removal	PCNL	NA	[12]
35/F	right nephrectomy	12F	left	renal vein, IVC	2-step under fluoroscopy	PCNL	NA	[13]
32/F	left lithotomy	14F	left	renal vein, IVC	2-step under ultrasound	PCNL	NA	[14]
50/M	left PCNL	8F	left	renal vein, IVC	1-step pyelotomy	PCN	pyelotomy	[15]

NA – not available; IVC – inferior vena cava; PCNL – percutaneous nephrolithotomy; PCN – percutaneous nephrostomy; UCM –uterine cervical malignancy; EBRT – external beam radiotherapy.

through the vein, and after dilation the PCN will be eventually placed through the renal vein into the lumen of the inferior vena cava [8, 16].

Chen et al. [8] suggested that another possible mechanism involved is an injury to the infundibular vein with the large dilatators of nephrostomy tract during percutaneous stone treatment. In this case, a calyceal fornix is strictly advised as a PCN puncture site [16].

Closure of the nephrostomy tube is the first-line manoeuvre after noticing blood flow draining through the PCN. Subsequent removal of the PCN can be performed in one or two stages, in the operating room or under CT or fluoroscopy control and with a surgical team on standby [8]. An intravenous balloon tamponade was recently reported as a successful treatment [17]. In the described case, ureteral implantation was necessary anyway, so the active approach was the primary choice, including ureteral reimplantation with the placement of a double pigtail stent after the removal of the PCN and renal pelvis suture in the operating room.

Thromboprophylaxis was introduced on the day of surgery during the hospital stay, as well as antibiotic support. Thromboembolic complications are rare and long-time prophylaxis is not obligatory in the absence of other reasons [8]. Communication of the urinary and vascular systems through a nephrostomy tube suggests obligatory antibiotic use in order to prevent systemic inflammatory complications, especially in cases of an infected kidney [8]. This complication seems to be preventable. The authors suggest regularly checking the position of the Chiba needle and guidewire during dilation of nephrostomy tract, using X-ray fluoroscopy with contrast medium.

Conclusion

Inadvertent injury of vascular structures is a possible complication of percutaneous nephrostomy under ultrasound guidance. It may have been possible to avoid the reported complication if the dilation of the nephrostomy tract over the guidewire had been performed under contrast-enhanced X-ray fluoroscopy.

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Conflict of interest

Authors have nothing to declare.

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The first case of benign familial neonatal epilepsy diagnosed in Serbia

Prvi slučaj benigne familijarne neonatalne epilepsije dijagnostikovan u Srbiji

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Abstract

Introduction. The exact prevalence of benign familial neonatal epilepsy (BFNE) is unknown due to the likelihood of overlooking the disease and not diagnosing the affected patients correctly. The rare autosomal dominant inherited disorder usually occurs within a few days after birth of an otherwise healthy newborn, and disappears after one to four months. Most patients develop no psychomotor deficiencies, nor any other forms of seizures. The disorder is most commonly linked to the KCNQ2 gene, with mutations located on the chromosome 20q13.33 which cause voltage-gated potassium channel changes. This clinically rare condition manifests itself in repeated tonic-clonic episodes of focal and generalized convulsions which are effectively treated with antiepileptic therapy. **Case report.** We presented a five-day old affected male infant, with genetically proven KCNQ2 gene mutation, in addition to a positive familial history of epilepsy. Seizures did not reoccur after several episodes in the fifth day of life and further psychomotor development of the child proved normal. **Conclusion.** Neonatal seizures have extensive differential diagnosis. However, BFNE should be suspected when the most common neonatal seizure causes have been excluded, and factors, such as the hereditary factor, in addition to the typical clinical course resembling BFNE, can be observed. Genetic identification of BFNE has resulted in easier and more specific diagnosis of this rare disorder and is therefore the gold standard in its diagnostics.

Key words:
diagnosis; epilepsy, benign neonatal; genetic testing; mutation.

Apstrakt

Uvod. Prevalencija benigne familijarne neonatalne epilepsije (BFNE) je nepoznata zato što mnogi bolesnici ostaju nedijagnostikovani, odnosno bolest se ne prepozna. Ovo retko, autozomno dominantno, nasledno oboljenje, ispoljava se kod novorođenčeta u prvih nekoliko dana posle porođaja, bez drugih tegoba i povlači se posle jedan do četiri meseca. Kod većine bolesnika kasnije se ne javljaju napadi ili drugi psihomotorni poremećaji. Oboljenje je najčešće povezano sa KCNQ2 genom i mutacijama lokalizovanim na hromozomu 20q13.33, što dovodi do voltažno-zavisnih promena kalijumovih kanala. Oboljenje se retko sreće u kliničkom radu i manifestuje se toničko-kloničkim epizodama fokalnih i generalizovanih napada koje se efikasno leče antiepileptičnom terapijom. **Prikaz bolesnika.** Prikazano je muško novorođenče, uzrasta pet dana, sa genetski potvrđenom KCNQ2 mutacijom i pozitivnom porodičnom anamnezom na epilepsiju. Posle nekoliko epizoda u petom danu života, napadi se više nisu ponavljali, a dalji psihomotorni razvoj je bio normalan. **Zaključak.** Epileptični napadi kod novorođenčeta podrazumevaju obimnu diferencijalnu dijagnozu. Na BFNE treba posumnjati kada se isključe česti uzroci ovih napada, a postoji nasledni faktor i klinički tok bolesti koji je sličan BFNE. Identifikacija gena za BFNE doprinela je lakšoj i preciznijoj dijagnostici tog retkog oboljenja i zbog toga danas predstavlja zlatni standard u njegovoj dijagnostici.

Ključne reči:
dijagnoza; epilepsija, benigna, neonatalna; genetičko testiranje; mutacija.

Introduction

Benign familial neonatal epilepsy (BFNE) is a rare autosomal dominant inherited disorder, which manifests itself in sudden and generalized seizures occurring for the first time during the first days of life, in an otherwise healthy newborn [1]. Usually no specific antenatal history is present in BFNE patients, with equal gender distribution. Generally, an Apgar score of minimum 7 is achieved within the first minutes of life [2]. The neonatal seizures are characterized by afebrile, repeated tonic-clonic episodes of focal and generalized seizures, accompanied by hypertonia. The seizures usually disappear within one to four months after the first onset, and thereafter most patients live a seizure-free

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life [3]. Due to the spontaneous resolution of BFNE, it is rather controversial if it should be treated. However, to prevent the damage due to seizure attacks, anti-epileptic therapies are advised to be administered for no longer than six months [4].

Most cases have shown no psychomotor development impairment after the seizures, although some studies may suggest otherwise. On the one hand, the risk of subsequent occurrence of febrile seizures is stated to be 5%, which corresponds to the average frequency in the general population; on the other hand, a significantly higher risk with 11% of subsequent epilepsy could be observed, which differs from the general population [5]. Reoccurring seizure disorders and developmental delay in BFNE-affected individuals have been described based on molecular analysis cases [6]. The familial analysis of multiple generations has shown expected, clinical heterogeneity in phenotypes expressing KCNQ2 mutations [6, 7]. Thus, it is in our opinion inconclusive whether this condition causes late effects of neurological development. This autosomal dominant inherited disorder is caused by mutations, which are most frequently inherited from the affected parents [8]. For instance, this type of mutations in the KCNQ2 gene, located on the chromosome 20q13.33, has shown to be significantly homogenous with a voltage-dependent delayed reaction of the rectifying potassium channel gene, KCNQ1 [1]. Another relevant gene which was found in BFNE-affected patients, KCNQ3, is mapped on the chromosome 8q24. Both mutations are a part of the KQT-like family and may cause voltage-gated potassium channel changes, which are not only the cause of BFNE, but also of several other epileptic disorders [9].

Studies have shown that recorded electroencephalograph (EEG) changes alone are not specific diagnostic measurements of the condition. Interictal EEG waves may be unchanged or only show mild changes with focal or multifocal abnormalities, whereas ictal EEG may begin with a brief flattening of EEG waves, followed by asymmetric spike and wave complexes [10]. Most individuals affected by BFNE present with normal EEG readings, and only a small percentage of individuals may show theta point alternant pattern [11].

Due to the well-established connection of potassium-gated channel dysfunctionality with BFNE, the neurological hyperexcitability is likely a consequence of the impaired repolarization of action potentials. Besides potassium channel, calcium channel and nicotinic acetylcholine receptor subunit defects, and some biochemical markers are known as specific diagnostic parameters of neonatal epilepsy conditions [12]. Besides routine testing for sepsis, serum electrolyte markers such as hyponatremia, hypocalcemia and hypoglycemia have been found to be another possible metabolic causes of these conditions [13, 14]. However, in BFNE, it is important to note that neither infectious, nor metabolic disturbances are the cause of the disease but rather the M-type potassium channel protein disinhibition due to genetic mutation [15].

We presented the first recorded patient in Serbia with clinically and genetically proved BFNE.

Case report

A five-day-old male infant was referred to the University Children's Hospital in Belgrade, Serbia, due to the recurrent episodes of afebrile seizures.

The patient was born to a 32-year-old mother by cesarean section due to placenta previa at 37.2 gestational weeks. At birth, his Apgar scores were 9 and 9, at 1 and 5 min, respectively. After the delivery, the newborn received vitamin K and hepatitis B vaccine. He was the second child of healthy parents without any complications during pregnancy up to the delivery. Historically, the 32-year-old healthy father confirmed a positive history of neonatal seizures for himself. He reported to have taken phenobarbital therapy up to 3 years of age. Also, the patient's elder female sibling was reported to have had seizures starting at three days of age, reoccurring at six weeks and three months of life. She was given an oral sodium-valproate therapy up to four years of age, with no reoccurring seizures after that (Figure 1).

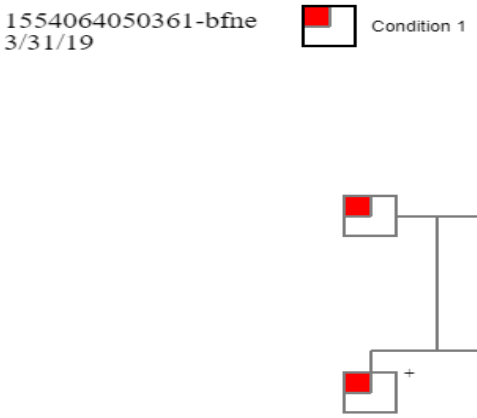

Fig. 1 – Autosomal dominant inheritance pattern of the affected patient and the closely related family members, such as parents and one female sibling in a short pedigree analysis.

The male neonate presented at our clinic with 3,200 g, the length of 57 cm and the head circumference of 36 cm (97 percentile). The infant presented in a conscious and overall healthy appearance, with normal tolerance of oral feedings and no other pathological signs.

The seizures appeared as tonic-clonic with the limb's involvement, which were reported by the mother to have first occurred on the left leg of the infant, expanding after the first episode with seizing to the right arm. The seizures lasted 5–10 sec, and would reoccur at 5–10 min. The episodes were not accompanied by apnea, and were successfully treated by pyridoxine amp i.v. and phenobarbital amp 10 mg/kg i.v. We want to emphasize that in this clinical situation phenobarbital is the drug of choice, and pyridoxine is given at the possibility of pyridoxine-dependent epilepsy [11]. Serum levels of bilirubin, sodium, potassium, calcium, C-reactive protein and glucose were 265.2 μmol/L, 146 mmol/L, 4.9 mmol/L, 2.1 mmol/L, 1.9 mg/L and 4.0 mmol/L, respectively. The blood type was B positive with negative Coombs test. No infec-

tions could be detected at this point, or at any point later. The complete blood count was within normal intervals. Sonography of the CNS and the abdomen showed no pathologic appearances, in addition to normal video EEG findings while awake and spontaneously asleep/sleeping. Henceforth, genetic analysis was needed for the diagnosis. DNA samples of the male infant, the elder female sibling and both parents were sent for genetic analysis to the genetic analysis laboratory of the hospital in Lyon, France.

SANGER sequencing of exon 13 of the KCNQ2 gene with a 3130XL sequencer (Seq Ref: NM_172107.2) was performed. The substitution c.1342C > T was found at the heterozygous state in the affected male infant patients, the elder female sister and their father´s sample. In contrast, the mother's sample was not affected. To our knowledge, the gene substitution leads to the creation of a stop codon (p.Glu130*), which was confirmed by the laboratory and proved therewith the diagnosis of BFNE.

Ten days after the observation, the male infant was discharged with a scheduled follow-up visits. In case of reoccurring seizures, the parents of the infant were instructed to bring the patient to the hospital, or administer appropriate doses of phenobarbital. Biochemical markers stabilized during the stay in the hospital. No significant situations, such as seizures, occurred any time after. Normal follow-up EEG readings were observed, in addition to normal psychomotor development.

Discussion

This case report represents a prototypical description of the BFNE disorder, fulfilling all of Miles and Holmes [4] proposed criteria of early infancy onset seizures, with otherwise normal neurological examinations, and normal neurodevelopmental progress with no other possible seizure etiologies, besides BFNE characteristic features, and a positive family history for infantile seizures. However, many more severe and more prevalent differential diagnosis, such as hypoxic ischemic encephalopathy (40%–60%), intracranial hemorrhages (7%–18%), cerebral infarctions or malformations (6%–17%, and 3%–17%, respectively), meningitis/septicemia (2%–14%), electrolyte disturbances (1%–4%), inborn errors of metabolism (1%–4%), maternal drug withdrawal, but also non-familial neonatal epilepsy have to be excluded before making a definite diagnosis of BFNE [16]. Similar to other etiologies, benign neonatal seizures need to be excluded. Genetic testing may be done to confirm the diagnosis.

As Zeng et. al. [17] have summarized KCNQ2 to be the most common causative gene for BFNE, we would like to highlight the importance of early genetic screening to exclude more harmful disease etiologies, such as those mentioned before. However, due to the heterozygous state of the genetic substitution, a frameshift or other forms of mutations of KCNQ2 could be expected, leading to possible pathologic states, such as neonatal seizure encephalopathy [17]. In our case, the gene sequence analysis showed a stop codon (p.Glu130*), which leads to potassium channel inhibitions. A small number of BFNE patients may suffer from psychomotor development delay, reoccurring epilepsy or even progress to neonatal seizure encephalopathy [18]. Therefore, in our opinion, it is also necessary to work on further investigations of potassium-channel-opening drugs, in addition to more antenatal routine genetic analysis in positive family history of BFNE.

Conclusion

BFNE is an autosomal dominant inherited disorder, affecting neonates up to six months of age. Due to the genetic heterozygous mutations of the KCNQ2 gene, M- ligated potassium channel disruption causes hypopolarization of action potentials leading to tonic-clonic seizures. The disorder is treated effectively with anti-epileptic agents, with phenobarbital as first choice.

In summary, genetic identification of BFNE has resulted in easier and more specific diagnosis of this rare disorder and is therefore the gold standard in its diagnostics.

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Conflicts of interest statement

The authors have no conflict of interest to declare.

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CASE REPORT

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The analysis of a sudden death caused by the unusual shape of the *foramen magnum* stenosis

Analiza iznenadne smrti izazvane neobičnim oblikom stenoze velikog potiljačnog otvora

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Abstract

Introduction. The anomalies of the size and shape of the *foramen magnum* (FM), followed by its stenosis, take a special place in clinical and forensic practice. The clinical picture of FM stenosis is variable, but it is not specific, which is why these anomalies are sometimes not recognized in time and are only detected by autopsy. **Case report.** This paper analyzes the case of a sudden death caused by the unusual shape of the FM stenosis. On autopsy, we found exostoses on the front and rear edges of the FM with their prominence into the FM. The exostoses reduced the sagittal diameter of the FM and practically divided it into two asymmetric semicircles, which is why we called this stenosis the "keyhole" stenosis of the FM. This form of the FM stenosis pushes the medulla oblongata aside, resulting in its incarceration into one of the FM semicircles. **Conclusion.** A stenosis of the FM can represent a threat to life, especially when combined with its unusual shape, as in our case. In this way, the critical stenosis of the FM becomes clinically clearly manifested, and there is a loss of consciousness that can also end with a sudden death, as in our case.

Key words:
constriction, pathologic; death, sudden; foramen magnum; forensic pathology.

Apstrakt

Uvod. Anomalije veličine i oblika velikog potiljačnog otvora (*foramen magnum* – FM) praćene njegovom stenozom zauzimaju važno mesto u kliničkoj i forenzičkoj praksi. Klinička slika stenoze FM je raznovrsna, ali nije specifična, zbog čega ovakve anomalije ponekad nisu na vreme prepoznate i otkrivaju se tek autopsijom. **Prikaz bolesnika.** U ovom radu analiziran je slučaj iznenadne smrti izazvane neobičnim oblikom stenoze FM. Autopsijom su nađene egzostoze na prednjoj i zadnjoj ivici FM sa prominencijom tuberkula egzostoza u sam otvor. Egzostoze su znatno redukovale sagitalni dijametar FM i praktično ga podelile u dva nejednaka polukruga, zbog čega smo ovakvu stenozu označili kao "ključaonica" stenozu. Ovakav oblik stenoze FM potiskuje produženu moždinu u stranu, sa njenim posledičnim uklještenjem u jedan od polukrugova FM. **Zaključak.** Stenoza FM može predstavljati pretnju po život, posebno kada je kombinovana sa njegovim neobičnim oblikom. Na ovaj način kritična stenoza FM postaje klinički jasno manifestna, nastaje gubitak svesti koji može da završi iznenadnom smrću kao u našem slučaju.

Ključne reči:
suženje, patološko; smrt, iznenadna; foramen magnum; patologija, sudska.

Introduction

The anomalies of the size and shape of the *foramen magnum* (FM), followed by its stenosis, take a special place in clinical and forensic practice. The clinical picture of the FM stenosis is variable, but it is not specific, which is why these anomalies are sometimes not recognized in time and are only detected by autopsy. The FM is characterized by the reduction of the diameter and the surface of the FM opening. The outcome of the stenosis is not only determined by the values of the diameter and the surface of the FM, but also by the shape of the FM. We found only a few cases in the literature in which a sudden death was described in association with the unusual shape of the FM stenosis [1, 2].

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We presented and analyzed the occurrence of a sudden death caused by the unusual form ("keyhole") of the FM stenosis.

Case report

A 15-year-old boy suddenly passed away during the game of rope jumping. In the medical chart, it was noted that he had had occipital headaches, dizziness, languor and occasional loss of consciousness. An autopsy confirmed the FM stenosis, which was induced by exostoses on the front and rear edges of the FM. The antero-posterior diameter of the FM was 13 mm. In addition to stenosis, we also found pronounced enlarged and asymmetric jugular tubercles of the occipital bone (Figure 1). The diameter of the *medulla oblongata* (MO) at the level of the FM was 12 mm.

The signs of compression of exostoses were visible as impressions and deformations of the shape and cross-section of the MO in the stenosis zone (Figure 2).

On microscopic examination, we found extensive edema, the collapse of the central canal in the compression zone, dilatation of the central canal above the compression level and partial gliosis (Figure 3). On the peripheral parts,

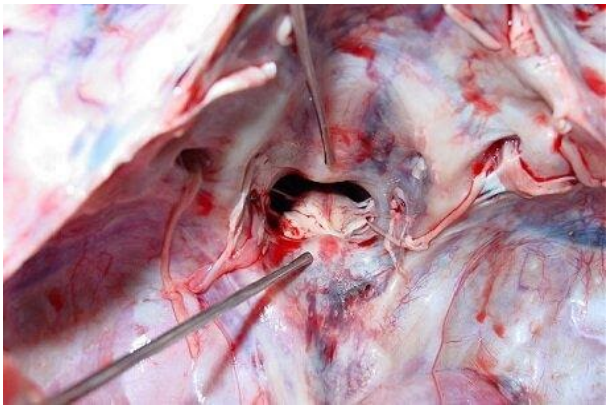

Fig. 1 – "Keyhole" stenosis of the *foramen magnum*.

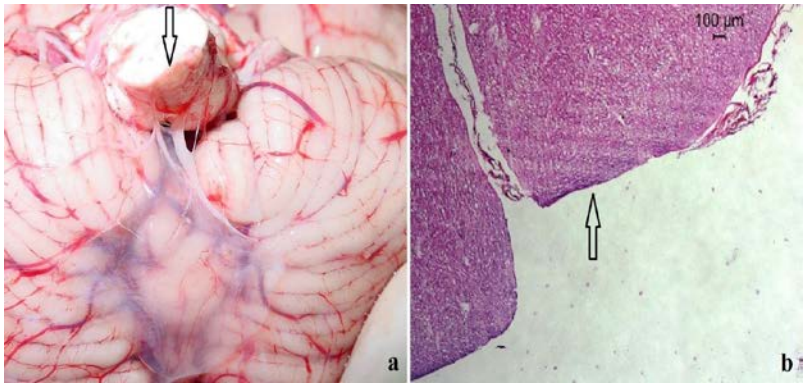

Fig. 2 – Deformations of the shape and cross-section of the *medulla oblongata* in the stenosis zone: a) macroscopic examination (arrow); b) microscopic examination (arrow), hematoxylin-eosin staining, ×100.

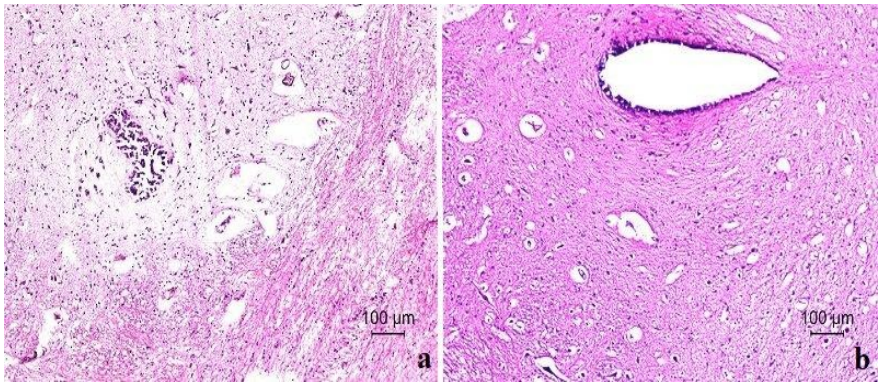

Fig. 3 – Microscopic examination of the brainstem (hematoxylin-eosin staining, ×100): a) collapse of the central canal at the level of compression; b) dilatation of the central canal above the level of compression.

we also found rare microtraces of iron in the tissue as a part of the decaying blood products (Figure 4).

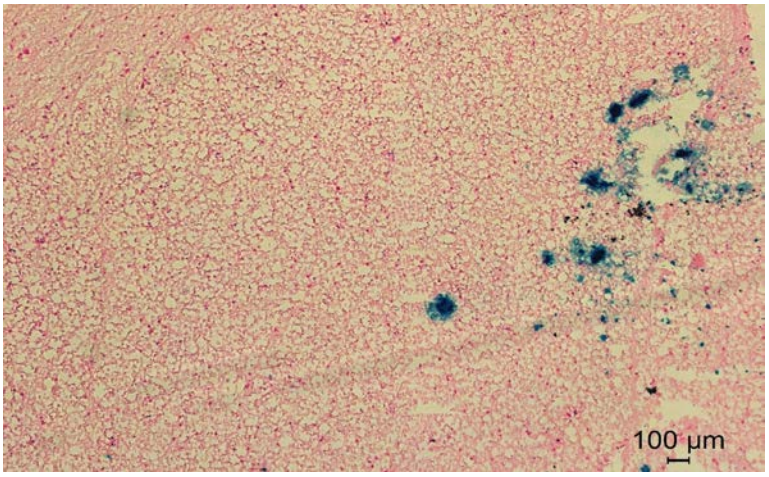

Fig. 4 – Perls' Prussian blue staining (×100) shows rare microtraces of iron in the tissue as a part of the decaying blood products.

Discussion

In the reviewed literature, most of the sudden deaths caused by the FM stenosis have been associated with achondroplasia [3], but only a few cases with bony formations at the rim of the FM and subsequent lethal stenosis have been described [1, 2].

The average length of the sagittal diameter of the FM determined by computerized tomography is 33.1 ± 3.5 mm, while the average length of the transversal diameter is 27.6 ± 3.1 mm. The FM surface area is 729.15 ± 124.87 mm^2 [4]. Vasović [5] states that the values of the FM diameters depend on the life age (Table 1).

Table 1

Mean values of sagittal and transversal diameter of the *foramen magnum* according to age [5]

Age	Sagittal diameter (mm)	Transversal diameter (mm)
1st day	20.8	15.3
6 months	28.7	22.7
2 years	29.5	25.8
3–6 years	31.5–32.5	25.8–27.7
8–10 years	33	28
15 years	34.4	28.4
> ww15 years	35	28.4

In our case, the diameter of the MO at the level of the FM was 12 mm, while the antero-posterior diameter of the FM in its narrowest part was 13 mm. In addition, the clinical data on the presence of occipital headaches, languor, dizziness, and intermittent loss of consciousness may have indicated a chronic compressive effect on that portion of the MO. Consequently, due to such narrowing and asymmetry of the jugular tubercles, we present a mechanism that explains the sudden death in this case.

The MO crosses over the most protruding points of the semicircular exostoses of the FM. Due to their size and the degree of the FM stenosis, the *medulla* made direct contact with them. The contact surfaces between them were semicircular and smooth, which is why the slipping of the MO into the left or right semicircle of the FM was facilitated. Head movements in the atlantooccipital joint in the form of flexion and/or rotation, as well as rotational brain acceleration in head trauma, contribute to the movement of the MO into one of the FM semicircles. Within the movements of the cervical spinal column, there are movements of the cervical spinal cord that is in direct anatomical relation to the MO. The movement of the MO aside pulls its rostral thicker part toward the FM. Its displacement to the side is confirmed by the deformation of the cross-sectional shape with the exostoses impressions on the posterior contour. In this way, in conjunction with the compressive effect on the ipsilateral vertebral artery, whose V4 segment passes through the FM, in our opinion, it had led to a sudden deterioration that resulted in the incarceration of the MO into one of the FM semicircles with the consequent reflex shutdown of vital centers in the brainstem, which eventually led to the fatal outcome. Because of this stenosis, the movement of the MO to the side had existed before, but not to the degree that would be termed as lethal incarceration.

All other possible causes of the sudden death, both by macroscopic and microscopic examination, were excluded. A toxicological analysis was not performed in this case because there were no indications that it was needed.

The vertebral arteries originate from the subclavian arteries and, as a result, there is a competition between the vertebrobasilar circulation and the circulation of the upper limbs. Some functional conditions of the organism that affect the circulatory flow rate through the vertebral arteries can contribute to final ischemic effects on the brainstem in the critical FM stenosis. Elevated body temperature redirects a portion of the circulatory volume to the upper extremities at the expense of the circulatory volume of vertebral arteries [6]. Pamphlett et al. [7] demonstrated the possibility of the compression of vertebral arteries in the stretching of the neck or rotation of the head in the experimental model of postmortem angiography in neonates. Saternus and Adam [8] state that the compression of the vertebral arteries is possible in

other positions of the neck and head. Excessive stretching of the neck and head rotation is especially possible in infants since they have instability of atlantooccipital joint [9]. The compression of the vertebral arteries is thought to be associated with sudden infant death syndrome [10, 11].

When it comes to critical FM stenosis, the question that arises is when the FM stenosis can be assessed as critical, or when it becomes a potential threat to the life of a patient. Vakili et al. [1] described the lethal ischemia of a brainstem in atlantooccipital fusion in a 24-year-old man, where the antero-posterior FM diameter was 16 mm, i.e. 45.7% of the normal value. In our case, the antero-posterior diameter of the FM in the boy aged 15 years was 13 mm, which is 37.8% of the normal diameter value for the age of 15 years. The values of the FM diameter and its shape, which can be obtained by radiological methods, can be parameters for assessing a patient's life-threatening condition.

Conclusion

A stenosis of FM can represent a threat to life, but when the stenosis is combined with an unusual shape of the FM, as a "keyhole" stenosis in our case, it can cause consequent incarceration of the *medulla oblongata*. In this way, the critical stenosis of FM becomes clinically clearly manifested, and there is a loss of consciousness that can also end with a sudden death, as in our case.

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c) Exact names and places of department(s) and institution(s) of affiliation where the studies were performed, city and the state for any authors, clearly marked by standard footnote signs;

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e) Data on the corresponding author.

2. ***Abstract and key words***

The second page should carry a structured abstract (250-300 words for original articles and meta-analyses) with the title of the article. In short, clear sentences the authors should write the **Background**/**Aim**, major procedures – **Methods** (choice of subjects or laboratory animals; methods for observation and analysis), the obtained findings – **Results** (concrete data and their statistical significance), and the **Conclusion**. It should emphasize new and important aspects of the study or observations. A structured abstract for case reports (up to 250 words) should contain subtitles **Introduction, Case report, Conclusion**). Below the abstract **Key words** should provide 3–10 key words or short phrases that indicate the topic of the article.

3. ***Text***

The text of the articles includes: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

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Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

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References

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Examples of references:

Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinska-Mladenovska O, Arsov T, Strezova A, et al. Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl 2003; 60(6): 657–612.

DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG*, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

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Primeri referenci:

Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

Balint B. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

Mladenović T, Kandolf L, Mijušković ŽP. Lasers in dermatology. In: *Karadaglić Đ*, editor. Dermatology. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG*, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

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