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ORIGINAL ARTICLES / ORIGINALNI RADOVI

International Human Rights Day, falling on 10 December, is marked every year as the anniversary of the day when the United Nations General Assembly adopted the Universal Declaration of Human Rights in 1948.
In 2018, Human Rights Day is the 70th anniversary of the Universal Declaration of Human Rights. The theme of the 2018 Day is "Stand Up For Human Rights" and implies a right to education, decent life, health care and a right to live free from any form of discrimination.

Međunarodni Dan ljudskih prava obeležava se svake godine 10. decembra kao dan kada je Generalna skupština Ujedinjenih nacija 1948. godine usvojila univerzalnu Deklaraciju o ljudskim pravima. Ove godine proslavlja se 70 godina od donošenja te Deklaracije.
Tema ovogodišnjeg Dana ljudskih prava je „Ustanimo za ljudska prava", što podrazumava pravo na obrazovanje, pristojan život, zdravstvenu zaštitu i pravo na slobodan život bez bilo kakvog oblika diskriminacije.

ORIGINAL ARTICLES

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Overall survival of patients with non-small cell lung cancer after surgery treatment

Ukupno preživljavanje bolesnika sa nesitnoćelijskim karcinomom pluća nakon hirurškog lečenja

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Abstract

Background/Aim. Lung cancer is one of the most common malignant tumors. About 80% of all lung cancers are non-small cell lung cancer (NSCLC). According to histopathological characteristics, the most common types of NSCLC are squamous cell carcinoma and adenocarcinoma. The aim of this study was to evaluate the overall survival rate in the NSCLC patients initially received surgery according to its histopathological type and T – primary tumor, N –regional lymph nodes, M – distant metastasis (TNM) stages which were treated with surgical treatment, and after that, according to the TNM stage, chemotherapy protocols and/or radiation therapy. **Methods.** This retrospective case series study included all patients with NSCLC admitted to the Military Medical Academy in Belgrade in the period 2010–2015. A total number of selected patients was 85 (27 females and 58 males). **Results.** Out of 41 patients with squamous cell carcinoma, 19.5% deceased. On the other hand, in the group of patients with adenocarcinoma, 43.2% out of 44 patients deceased. The average cumulative survival was statistically significantly lower in the adenocarcinoma patients in comparison to the patients with squamous cell carcinoma (1,605.2 vs.1,304.8 days; $p = 0.005$). On the other hand, the average cumulative survival was statistically significantly lower in our patients in the recurrence group with adenocarcinoma in comparison to the recurrence group with squamous cell carcinoma (1,212.8 vs. 1,835.5 days; $p = 0.032$). **Conclusion.** Adenocarcinoma is more aggressive cancer in comparing to squamous cell carcinoma with lower overall survival in comparing to squamous cell carcinoma. Additional studies are needed to identify risk factors for recurrence after surgery, and to additionally explain role of tumor markers and molecular biological techniques in the progression of this kind of cancer.

Key words:
carcinoma, non-small-cell lung; adenocarcinoma; squamous cell carcinoma; survival; recurrence.

Apstrakt

Uvod/Cilj. Karcinom pluća je jedan od najčešćih malignih tumora. Oko 80% karcinoma pluća jeste nesitnoćelijski karcinom pluća (NSCLC). Na osnovu patohistoloških karakteristika, najčešći tipovi NSCLC su skvamocelularni karcinom i adenokarcinom. Cilj studije bio je da se analizira preživljavanje bolesnika sa NSCLC na osnovu njihovog patohistološkog tipa i T – primarni tumor, N – regionalni limfni nodusi, M – udaljene metastaze (TNM) stadijuma koji su lečeni hiruški, a nakon toga prema TNM stadijumu hemioterapijskim protokolima i/ili radioterapijom. **Metode.** Izvršena je retrospektivna analiza preživljavanja bolesnika sa NSCLC lečenih u Vojnomedicinskoj akademiji u Beogradu u periodu 2010–2015. Ukupan broj bolesnika je bio 85 (27 žena i 58 muškaraca). **Rezultati.** Kod bolesnika sa skvamocelularnim karcinomom stopa smrtnosti bila je 19,5% kod ukupno 41 bolesnika, dok je kod bolesnika sa adenokarci-

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nomom stopa smrtnosti bila 43,2% kod ukupno 44 bolesnika. Prosečno ukupno preživljavanje bilo je statistički značajno kraće kod bolesnika sa adenokarcinomom u poređenju sa onima koji su imali skvamocelularni karcinom (1605,2 vs. 1304,8 dana; $p = 0.005$). S druge strane, prosečno ukupno preživljavanje je bilo statistički značajno kraće kod bolesnika sa adenokarcinomom kod kojih se javio recidiv bolesti u poređenju sa bolesnicima sa skvamocelularnim karcinomom kod kojih se takođe javio recidiv (1212,8 vs. 1835,5 dana; $p = 0.032$). **Zaključak.** Adenokarcinom je mnogo agresivniji karcinom u poređenju sa skvamocelularnim karcinomom sa kraćim ukupnim preživljavanjem. Potrebne su dodatne studije kako bi se identifikovali faktori rizika za pojavu recidiva bolesti nakon hiruškog lečenja i kako bi se dodatno objasnila uloga tumorskih markera i tehnika molekularne biologije u progresiji bolesti.

Ključne reči:
pluća, nesitnoćelijski karcinom; adenokarcinom; karcinom skvamoznih ćelija; preživljavanje; recidiv.

Introduction

Today, lung cancer is one of the most common malignant tumor [1–3]. It is a leading cause of cancer-related deaths [1, 3]. About 80% of all lung cancers are non-small cell lung cancer (NSCLC) [4]. In the time of diagnosis more than 65% of patients with NSCLC present with metastatic or locally advanced disease [4, 5]. According to the histopathological characteristics, the most common types of NSCLC are squamous cell carcinoma and adenocarcinoma [6].

Epidemiological data describe high aggressiveness of NSCLC. The overall five-year survival rate for all lung cancer in all stages is 16.8% [7]. This rate varies depending on the stage of lung cancer at the time of the diagnosis: up to 52.2% for localized disease, to 25% for regional metastatic disease, and to 4% for distant metastatic disease.

Non-small cell lung cancer has significant consequences in terms of survival, life quality and decreasing working ability [8]. Once the patient is diagnosed with clinically confirmed NSCLC, a comprehensive therapeutic approach depends on the stage of illness, histology, imaging diagnostics and tumor marker findings. Therapy in patients with NSCLC is a combination of surgical treatment, radiation therapy and/or one of the cytostatic drug treatment protocols [8].

A treatment of choice for patients with NSCLC from I to IIIA stages according to Tumor-Node-Metastasis (TNM) classification is surgery [9]. Patients with resected NSCLC from II to IIIA TNM stages, who have a high risk of relapse, in addition to surgery are treated with adjuvant chemotherapy (cisplatin or carboplatin with gemcitabine, paclitaxel, docetaxel, vinorelbin or pemetrexed) and/or radiation therapy [8, 10]. Patients with stage IIIB and IV NSCLC are usually treated with chemotherapy and radiation therapy. In the treatment of stage I and II NSCLC, radiation therapy alone is considered only when surgical resection is not possible because of limited pulmonary reserve or the presence of comorbidities [11]. Generally, radiation is a reasonable option for lung cancer treatment in patients who are not candidates for surgery [12]. Approximately 80% of patients with NSCLC are considered for chemotherapy at some point during the course of their illness. The current standard of systemic chemotherapy protocols for treatement of patients with NSCLC are platinum-based regimens and second-line chemotherapy [13–17]. Today, in these patients, new molecular-targeted therapies, such as an adjunct to conventional therapy, gefitinib, bevacizumab, erlotinib, pembrolizumab are used [2, 18].

After the treatment of the patients with NSCLC, the expected local and distant recurrence rates following complete resection by surgical stage are 10%, 12% and 15% for local relapse, for I, II and III TNM stages respectively [19]. The expected distant relapses are 15%, 30%, 40% and 60%, for IA, IB, II and III TNM stages, respectively [19].

The aim of this study was to evaluate the overall survival rate in the NSCLC patients according to its pathohistological type and the TNM stages which were treated by surgical treatment and, after that, according to the TNM staging, by chemotherapy protocols and/or radiation therapy.

Methods

This retrospective case series study is designed as a survival analysis according to the histopathological type and TNM stages in the patients with NSCLC. There were 85 selected patients with NSCLC who were treated at the Pulmonology Clinic and the Clinic for Chest Surgery, Military Medical Academy in Belgrade.

The clinical files from all patients with clinically confirmed lung cancer, admitted during 2010–2015 in the Military Medical Academy, were accessed in electronic and hard copies from the hospital registries. The following data were analyzed: demographic characteristics (age, gender), overall survival rate according to the pathohistology type and the TNM stages of NSCLC.

The patients with NSCLC who were treated in our hospital are classified according to the TNM stages [20]. Stage grouping of the TNM subsets was made to provide greater specificity for identifying patients with similar prognosis and options of treatment: T1N0M0 – stage IA; T2N0M0 – stage IB; T1N1M0 – stage IIA; T2N1M0 and T3N0M0 – stage IIB; and T3N1M0, T1N2M0, T2N2M0, T3N2M0 – stage IIIA. Stage IIIB is T4 any N M0 and any T N3M0. Stage IV is any T any N M1.

The patients with I TNM stage were only surgically treated. After surgery, the patients with IIA to IIIA TNM stage were treated with adjuvant chemotherapy which included etoposide and cisplatin (EP/PE protocol), and/or radiation therapy.

This chemotherapy protocol was applied in the following way: cisplatin 60 mg/m^2 intravenously on day 1 plus etoposide 120 mg/m^2 intravenously on days 1–3 every 21 days for 4 cycles, or cisplatin 80 mg/m^2 intravenously on day 1 plus etoposide 100 mg/m^2 intravenously on days 1–3 every 28 days for 4 cycles.

Radiotherapy was applied in the patients with positive resection surface for malignancy and with N2 TNM stage [8].

Continuous variables were presented as mean ± standard deviation with median values. Categorical variables were reported as frequencies unless otherwise stated. Differences between categorical variables were tested by χ^2-test, while a significance of differences between continuous variables were tested by non-parametric Mann-Whitney *U* test. Overall survival estimates were calculated using the Kaplan-Meier method, and Log-Rank (Mantel-Cox) test to assess differences between two histopathological types of NSCLC (adenocarcinoma vs. squamous cell carcinoma). The patients who stayed alive were censored at the cut-off date, that is, November 2016. A p value < 0.05 was considered statistically significant.

The underlying study was conducted in line with The Declaration of Helsinki and has been approved by the regional Ethics Committee of the Military Medical Academy, decision issued on June 9, 2015.

Results

Demographic patient characteristics are presented in Table 1. The males were significantly predominant in both histopathological groups (80.5% with squamous cell carcinoma, 56.8% with adenocarcinoma). The patients with squamous cell carcinoma were significantly older in comparison to those with adenocarcinoma (median age 63.56 in the group of patients with squamous cell carcinoma; median age 60.03 in the adenocarcinoma group).

In the group of patients with squamous cell carcinoma 19.5% of the patients died or 8 patients out of 41 (Table 2). On the other hand, in the group with adenocarcinoma 43.2% of patient deceased, or 19 patients out of 44. The mortality rate was significantly higher in the group of patients with adenocarcinoma (43.2%) in comparison to 19.5% in the group of patients with squamous cell carcinoma ($p = 0.035$).

Overall survival of the patients according to the histopathological type of NSCLC is presented in Table 2, while the cumulative survival curve (Kaplan-Meier analysis) is given in Figure 1. A statistically significant difference was observed [Log Rank (Mantel-Cox) test; $p = 0.005$] between groups. Cumulative survival was lower in the group with adenocarcinoma in comparison to the group with squamous cell carcinoma (approximately 550 days).

Overall survival of patients with squamous cell carcinoma according to recurrence as well as adenocarcinoma is presented in Table 3. A statistically significant difference between the groups was not observed [Log Rank (Mantel-Cox) test $p = 0.772$; $p = 0.295$, respectively]. On the other hand, the cumulative survival curves of the patients according to the histopathological type of NSCLC in the patients with recurrence (Kaplan-Meier analysis) are given on Figure 2. A statistically significant difference was observed [Log Rank (Mantel-Cox) test $p = 0.032$] between the groups. The cumulative survival was lower in the recurrence group with adenocarcinoma in comparison to the group with squamous cell carcinoma (approximately 620 days). This difference was not shown in the group without recurrence (Figure 3).

Overall survival was estimated and compared among patients according to the initial TNM stage in the patients with squamous cell carcinoma as well as adenocarcinoma. The baseline information is presented in Table 4. No statistical significance was observed among the patients with adenocarcinoma ($p = 0.665$ and the patients with squamous cell carcinoma ($p = 0.576$). No statistically significant survival difference was observed [Log Rank (Mantel-Cox) test] in the patients with adenocarcinoma and those with squamous cell carcinoma.

On the other hand, overall survival among the patients with squamous cell carcinoma and adenocarcinoma patients according to initially TNM stage was estimated. No statistical significance was observed between the patients with adenocarcinoma and squamous cell carcinoma in groups with IIA and IIB stage ($p = 0.278$) in contrasst to patients with IIIA stage ($p = 0.076$) (Figures 4 and 5). However, a statistical significance was observed between patients with adenocarcinoma and squamous cell carcinoma in the groups with IA and IB stage ($p = 0.038$) (Figure 6). Overall survival was lower in the group with adenocarcinoma in comparison to the group with squamous cell carcinoma in the patients with IA and IB stage (approximately 720 days).

Table 1

Demographic characteristics of the patient with non-small cell lung cancer (NSCLC) according to the histopathological type

Patients	Squamous cell carcinoma	Adenocarcinoma	*p* value
Total number, n (%)	41 (48.2)	44 (51.8)	
female	8 (19.5%)	19 (43.2)	0.035*
male	33 (80.5)	25 (56.8)	
Age total (years); mean ± SD (median)	62.07 ± 8.33 (63.56)	58.23 ± 8.34 (60.03)	0.034**
male	61.11 ± 8.31 (61.99)	59.06 ± 8.49 (60.85)	0.375**
female	66.05 ± 7.62 (69.03)	57.14 ± 8.25 (59.01)	0.013**
p value	0.374**	0.112**	

SD – standard deviation; *Pearson χ^2-tests; **Mann-Whitney *U* test.

Table 2

Overall survival of the patients according to a histopathological type of non-small cell lung cancer (NSCLC)

NSCLC	Total number	Deceased n (%)	Censored[1] n (%)	*p**	Survival (days) – estimated mean (95% CI)	*p***
Squamous cell carcinoma	41	8 (19.5)	33 (80.5)	0.035	1,858.3 (1,657.8–2,058.7)	0.005
Adenocarcinoma	44	19 (43.2)	25 (56.8)		1,304.8 (1,044.5–1,565.1)	
Overall	85	27 (31.8)	58 (68.2)		1,605.2 (1,427.2–1,783.2)	

[1]Alive at the end of the follow-up period.
***χ^2-tests; **Log Rank (Mantel-Cox) test; CI – confidence interval.**

Table 3

Distribution of overall survival of the patients with non-small cell lung cancer (NSCLC) according to recurrence

NSCLC	Recurrence	Total number	Deceased n (%)	Censored[1] n (%)	Survival (days) – estimated mean (95%CI)	*p**
Squamous cell carcinoma	Yes	17	4 (23.5)	13 (76.5)	1,835.5 (1,533.8–2,137.3)	0.772
	No	24	4 (16.7)	20 (83.3)	1,857.1 (1,597.6–2,116.5)	
Adenocarcinoma	Yes	30	15 (50)	15 (50)	1,212.8 (903.3–1,522.3)	0.295
	No	14	4 (28.6)	10 (71.4)	1,450.7 (1,032.0–1,869.5)	

[1]Alive at the end of the follow-up period.
***Log Rank (Mantel-Cox) test; CI – confidence interval.**

Table 4

Distribution of overall survival in the patients with non-small cell lung cancer (NSCLC) according to the clinically initial Tumor-Node-Metastasis (TNM) stage

NSCLC	TNM stage	Total number	Deceased n (%)	Censored[1] n (%)	Survival (days) – estimated mean (95%CI)	*p**
Squamous cell carcinoma	IA, IB	10	1 (10)	9 (90)	2008.9 (1750.3–2267.5)	0.576
	IIA, IIB	20	5 (25)	15 (75)	1624.3 (1345.2–1903.4)	
	IIIA	11	2 (18.2)	9 (81.8)	1845.3 (1428.3–2262.3)	
Adenocarcinoma	IA, IB	13	6 (46.1)	7 (53.9)	1290.5 (923.4–1657.6)	0.665
	IIA, IIB	19	7 (36.8)	12 (63.2)	1357.7 (980.7–1734.7)	
	IIIA	12	6 (50)	6 (50)	1116.9 (593.5–1640.3)	

[1]Alive at the end of the follow-up period.
***Log Rank (Mantel-Cox) test; CI – confidence interval.**

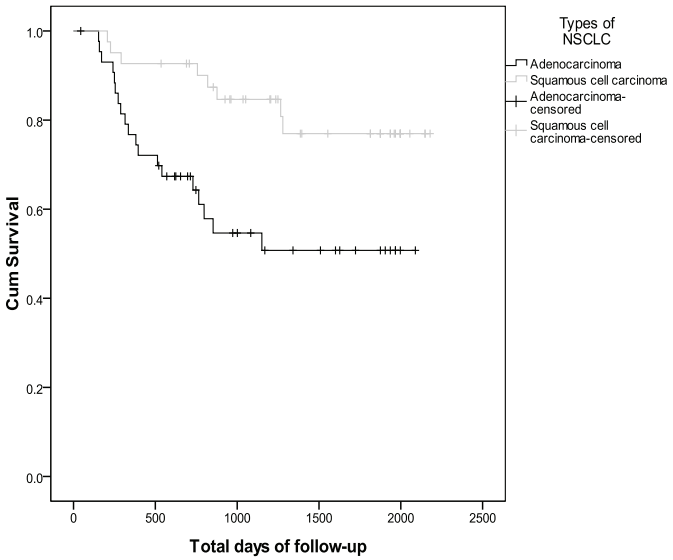

Fig. 1 – Kaplan-Meier analysis – survival curves of the patients according to the histopathology type of non-small cell lung cancer (NSCLC) (censored – alive at the end of the follow-up period).

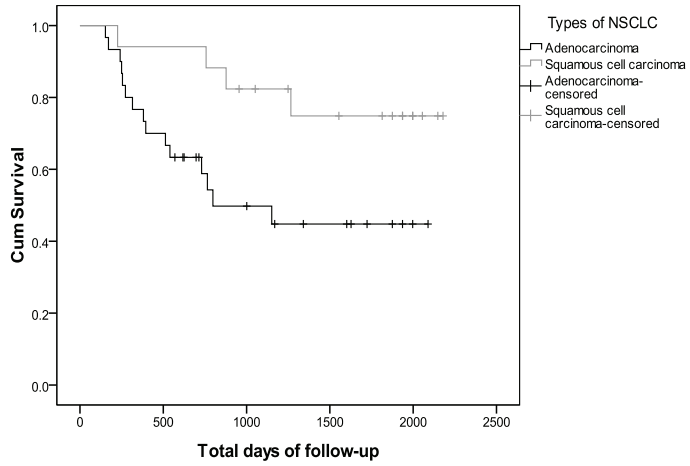

Fig. 2 – Kaplan-Meier analysis – survival curves in the patients with recurrence according to histopathology type of non-small cell lung cancer (NSCLC) (censored – alive at the end of the follow-up period). Log Rank (Mantel-Cox) test (p = 0.032).

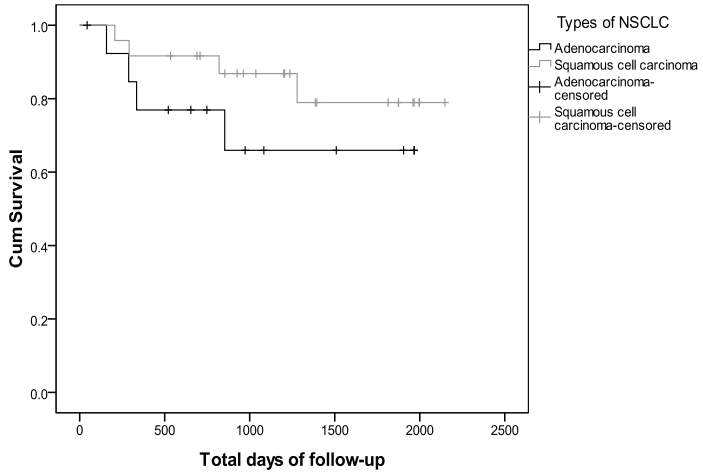

Fig. 3 – Kaplan-Meier analysis – survival curves in the patients without recurrence according to histopathology type of non-small cell lung cancer (NSCLC) (censored – alive at the end of the follow-up period). Log Rank (Mantel-Cox) test (p = 0.252).

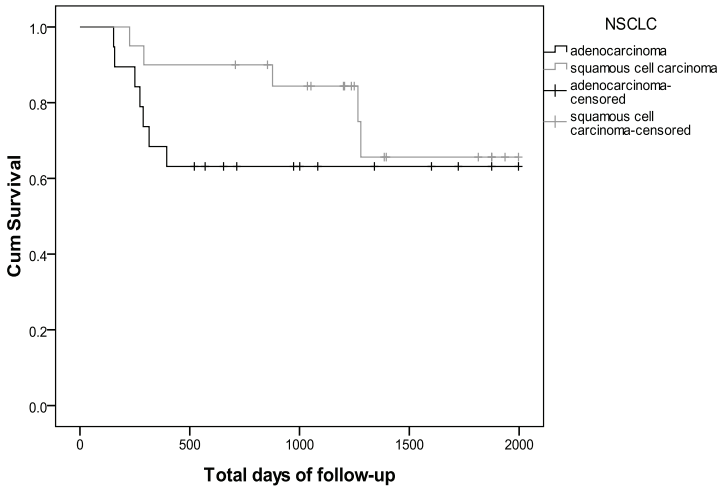

Fig. 4 – Kaplan-Meier analysis – survival curves in the patients with non-small cell lung cancer (NSCLC) in IIA and IIB Tumor-Node-Metastasis (TNM) stage according to histopathology type (censored – alive at the end of the follow-up period). Log Rank (Mantel-Cox) test (p = 0.278).

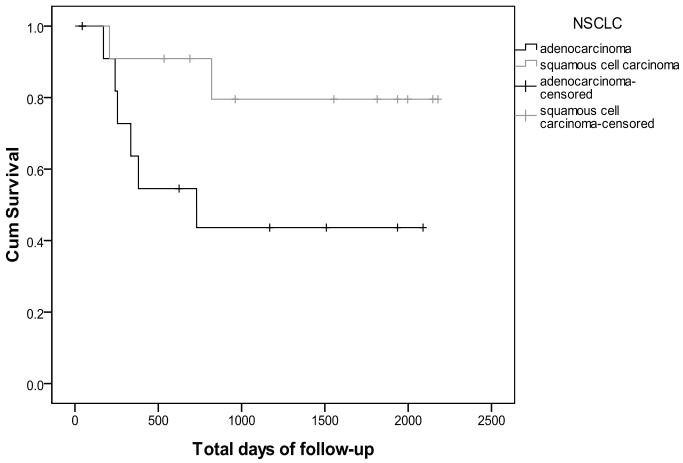

Fig. 5 – Kaplan-Meier analysis – survival curves in the patients with non-small cell lung cancer (NSCLC) in IIIA Tumor-Node-Metastasis (TNM) stage according to histopathology type (censored – alive at the end of the follow-up period). Log Rank (Mantel-Cox) test (p = 0.076).

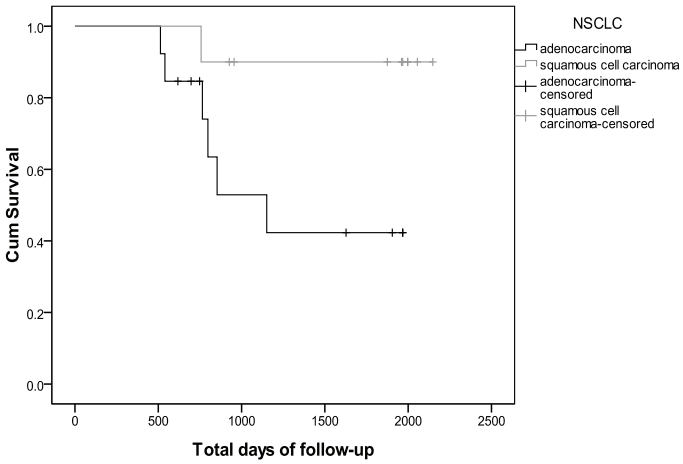

Fig. 6 – Kaplan-Meier analysis – survival curves in the patients with non-small cell lung cancer (NSCLC) in IA and IB Tumor-Node-Metastasis (TNM) stage according to pathohistology type (censored – alive at the end of the follow-up period). Log Rank (Mantel-Cox) test (p = 0.038).

Discussion

On the base of the Global Burden of Disease methodology, investigators estimated that there were 17,481 million cancer cases and 8,713 million deaths in 2015. Between 2005 and 2015, incident cancer cases increased by 33% [3]. Incidence of tracheal, bronchus and lung cancer was estimated to be 2,019 milion cases, and it is located on the second place after breast cancer (2.422 million cases). Non-small cell lung cancer continues to be one of the major causes of cancer-related deaths [2]. Therefore, our study was aimed to assess overall survival in the patients with NSCLC according to the TNM stages and pathohistological type of NSCLC.

After surgical resection of the tumor, adjuvant chemotherapy was considered a standard modality of treatment for NSCLC in the last 15 years [14–18, 21]. On the other hand, the molecularly targeted therapy significantly improved the outcomes of the treated patients with metastatic form NSCLC [2, 18]. However, for the majority of the patients, platinum-based chemotherapy remains the gold standard treatment and has to significantly improve median survival outcomes to about 10–11 months survival [22].

In our study, the males were more often in both the squamous cell carcinoma and adenocarcinoma groups. The men were more likely to develop tracheal, bronchus and lung cancer comparing to women, with 1 in 18 men and 1 in 45 women developing this cancer group between the birth and the age 79 years [3]. Similarly, in the United States, lung cancer ranks on the second place in both genders, with the estimated 115,060 new cases in men and 106,070 in women [23]. The estimated numbers of lung cancer cases worldwide has increased by 51% since 1985 (a 44% increase in men and a 76% increase in women). The higher increasing rates in women has been attributed to the fact that cigarette smoking in female gender peaked two decades later than in male [23].

Our patients with squamous cell carcinoma were significantly older in comparison to the adenocarcinoma patients. This ratio is explained by the fact that squamous cell carcinoma is connected with many risk factors, smoking, diet and food supplements, alcohol, air pollution, etc [9], while adeno-

carcinoma, although most cases are seen in smokers, develops more frequently than squamous cell carcinoma in individuals who have never smoked [6]. Due to this, adenocarcinoma earlier is diagnosed in comparison to squamous cell carcinoma.

The patients with adenocarcinoma were known to result in poorer prognosis comparing to squamous cell carcinoma patients [24]. Similarly, in our study, the mortality rate was significantly higher in the group with adenocarcinoma (43.2%) in comparison to 19.5% in the group with squamous cell carcinoma. In relevant literature, generally, it is reported that and the five-year survival rate of the patients with stage IA, IB, IIA and IIB NSCLC is about 49%, 45%, 30 and 31%, respectively [25]. For stage IIIA and IIIB NSCLC, this rate is about 14%, and 5%, respectively.

Overall survival of the patients according to recurrence is very important. Recurrence rates reported following surgical cancer resection range from 30% to 75% [26]. The majority of recurrent tumors are distant and more than 80% of recurrences occur within the first 2 years after resection. Cumulative survival was lower in the recurrence group of patients with adenocarcinoma in comparison to the group with squamous cell carcinoma, that is, about 620 days. This information support the fact that adenocarcinoma is more aggressive cancer in comparing to squamous cell carcinoma.

The complete resection of early stage NSCLC is the best treatment option. However, the post-resection recurrence rates remain high [27]. Right from the start of the therapy, in the patients with NSCLC, complete removal needs to be ensured both macroscopically and microscopically, because there are often occult micro-metastatic cancer cells undetected by standard staging methods, already present systemically at the time of the surgery, suggesting that there is an underestimation of the true tumor stage. Second, dissemination of cancer cells might occur during the handling of the tumor in the course of the surgery [27].

Statistically significant difference in overall survival according to the TNM stages was not observed between the patients with adenocarcinoma and those with squamous cell carcinoma. However, statistically significant was observed between our patients with adenocarcinoma and squamous cell carcinoma in the groups with IA and IB TNM stage, but this difference was not shown between the other groups (IIA, IIB and IIIA). Overall survival rate was lower for about 720 days in the group with adenocarcinoma of IA and IB stage in comparison to the group with squamous cell carcinoma of the same stage. This also supports the fact that adenocarcinoma is more aggressive cancer in comparing to squamous cell carcinoma.

After curative resection, the patients with lung cancer at the same TNM stage show wide variations in their incidence of recurrence [27]. The current TNM staging system, which is based on clinical and pathological findingshas the limit of its usefulness. Predicting the cases exactly in which the disease is likely to relapse can help guide the administration of adjuvant therapies. There are two methods for identifying factors related to recurrence following surgery: tumor markers and molecular biological techniques. Excellent prognostic markers for predicting the postoperative recurrence of cancer are KRAS, Ki-67, p16, epidermal growth factor receptor (EGFR), etc. An extensive pathological investigation is also important, because the histological differentiation, vessel invasion, lymphatic permeation and pleural invasion reported poor prognostic factors for the disease-free survival [28, 29].

Conclusion

Adenocarcinoma is more aggressive cancer in comparing to squamous cell carcinoma with lower overall survival. Cumulative survival was lower about 550 days in adenocarcinoma patients in comparison to patients with squamous cell carcinoma. On the other hand, cumulative survival was lower in the recurrence group of patient with adenocarcinoma in comparison to the recurrence group with squamous cell carcinoma, about 620 days.

Additional studies are needed to identify risk factors for recurrence after surgery as well as those which could additionally explain role of tumor markers and the molecular biological techniques in the progression of this kind of a cancer.

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Gender differences in suicide in Serbia within the period 2011–2015

Polne razlike kod suicida u Srbiji u periodu 2011–2015. godine

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Abstract

Background/Aim. The World Health Organisation (WHO) estrimates that approximately 1,000,000 people die by suicide every year. The aim of this study was to examine the gender differences in cases of committed suicides, including suicide rates, socio-demographic factors and methods of suicide in Serbia within the period 2011–2015. This investigation is continuing the previous investigation from the period 2006–2010. **Methods.** Data were obtained from the Statistical Office of the Republic of Serbia. Their classification related to the suicide method was carried out on the basis of International Classification of Diseases-Tenth Revions-Clinical Modification (ICD-X-CM) (WHO 1992). Statistical analysis was done by using the crude number of committed suicide. **Results.** Within the period 2011–2015, the total number of suicides in Serbia was 5,897, of which 74.56% were males and 25.44% females (male to female suicide ratio was 2.93). Annual suicide rate (per 100,000) showed constantly decreased from 2011 to 2015, and in 2015 it was 15. Male/female suicide ratio was the highest among adolescents and decreased with age. The suicide was the most often committed by married males (47.6%) and widowed females (38.86%) with completed high school, retired, Serbs. About a quarter (23.38%) suicide committers were older than 75 years, and 39.39% were older than 65 years. The most common suicide method males (64.63%) and females (59.00%) used was hanging, strangulation and suffocation. The second most common method males used was by firearm (18.96%) and females by poisoning (16.73%). **Conclusions.** Suicide Prevention Programme in Serbia should be primarily oriented towards two age groups at highest risk to commit suicide, towards the adolescents whose suicide was on the rise and towards the elderly male population, less ready to refer to the doctors for help because of problems related to their mental health. With the aim to suicide prevention, doctors should become familiar with community, state and national resources that are concerned with youth and elderly populations, including mental health institutions, family and crisis intervention centers.

Key words:
suicide; gender identity; risk factors; serbia.

Apstrakt

Uvod/Cilj. Prema procenama Svetske zdravstvene organizacije (WHO) oko 1 000 000 ljudi godišnje umire usled samoubistva. Cilj istraživanja bio je utvrđivanje polnih razlika kod izvršenog suicida, prema stopi suicida kao i prema socio-odemografskim karaktersitikama osoba koje su izvršile suicid i metodama suicida izvršenog u Srbiji u periodu od 2011. do 2015. godine. Rad je nastavak istraživanja suicida u Srbiji za period od 2006–2010. godine. **Metode.** Podaci za istraživanje su dobijeni od Republičkog zavoda za statistiku Srbije. Klasifikacija podataka koji se odnose na metod suicida su utvrđeni na osnovu međunarodne klasifikacije bolesti revizija – klinička modifikacija (ICD-X-CM) (WHO 1992.). Statistička analiza je rađena koristeći sirove podatke broja izvršenih suicida. **Rezultati.** U periodu od 2011. do 2015. godine u Srbiji je izvršeno ukupno 5 897 suicida, od toga se 74,56% odnosi na muškarce, a 25,44% na žene (muškarci su 2,93 puta češće izvršili suicid od žena). Stopa suicida (na 100 000) pokazuje konstantno sniženje od 2011. do 2015. godine, i za 2015. godinu iznosi 15. Polne razlike u stopi suicida su najveće kod adolescenata i opadaju sa godinama života. Suicid najčešće izvršavaju oženjeni muškarci (47,6%) i žene udovice (38,86%), sa srednjim obrazovanjem, penzioneri, srpske nacionalnosti (80,49%). Oko četvrtinu (23,38%) suicida su izvršile osobe starije od 75 godina, a 39,39% osobe starije od 65 godina života. Najčešći metod suicida kod muškaraca (64,63%) i žena (59%) su vešanje i davljenje. Kod muškaraca je na drugom mestu vatreno oružje (18,96%), a kod žena trovanje čvrstim i tečnim supstancama (16,73%). **Zaključak.** Program prevencije suicida u Srbiji bi trebalo da bude usmeren na dve dobne populacije sa najvećim rizikom da izvrše suicid, na adolescente kod kojih je suicid u porastu i na populaciju starih muškaraca, koji su manje spremni da se obrate lekaru kada imaju probleme u vezi sa mentalnim zdravljem. U cilju prevencije suicida, lekari bi trebalo da

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sarađuju sa državnim i nacionalnim institucijama koje vode brigu o mladima i populaciji starih, uključujući i institucije mentalnog zdravlja, porodicu i centre za intervencije u krizi.

Ključne reči:
samoubistvo; pol, faktor; faktori rizika; Srbija.

Introduction

The World Health Organization (WHO) estimates that approximately 1,000,000 people die by suicide every year. This roughly corresponds that about 3,000 people commit suicide every day throughout the world, or to one death every 40 seconds. At the same time, it is estimated that up to 25 times as many again attempt suicide. According to the WHO in all European countries, suicide is more common among men, while suicide attempts are more frequent among women [1, 2].

In 2012, suicide in the world was the fifth leading cause of death among people between 30 and 49 years of age, and the second one in people between 15 and 29 years of age [1]. Traditionally, suicide rate is the highest among older men, but suicide among young people is on the rise and makes the group with the highest risk in many countries [3]. The average suicide rate (number of suicides per 100,000 inhabitants) in the world was 16. Three countries with the highest suicides rate in the world were: Guyana (44.2), South Korea (28.1) and Sri Lanka (28.8). The lowest suicide rates in the world were in Saudi Arabia, Syria, Kuwait and Lebanon, where the suicide rate is less than 1 per 100,000 inhabitants. Two countries with the highest suicide rate in Europe are Lithuania (28.2) and Kazakhstan (23.8), followed by 10 countries of the former Soviet Republics [4].

Suicide rate in the world for the last 50 years increased by 60%. Since 1953, a growing trend of suicide rate has also been observed in Serbia. The lowest suicide rate was registered at the beginning of the 50s of the 20th century; it was about 12 to 100,000. Downward trend in suicide mortality occurred in Serbia in last two decades (1991–2014) [5]. The highest suicide rate was 20.9 per 100,000 in 1992 and 1997. The suicide rate in Serbia has been permanently decreasing since 2000 [6–8]. Investigations confirmed that particularly in last two decades the increase in mortality in older men, especially due to firearm suicides, air rifles, and explosives is worrying [5].

According to data obtained from the Statistical Office of the Republic of Serbia (Department for Demography), within the period 2006–2010, the total number of suicides in Serbia was 6,673, of which 71.9% were males and 28.1% females (male to female suicide ratio was 2.56). In this five-year period, their average rate was 18.15 per 100,000 persons, namely, 26.85 per 100,000 for males and 9.92 per 100,000 for females. The suicide was the most often committed by the married males and females with completed high school, retired, the Serbs. The suicide rate in Serbia has been increasing in parallel with the age of the suicide committers and it is the highest in subjects of both genders aged over 75 years. The most common suicide method in males (62.78%) and in females (58.38%) was hanging and strangling. The second most common method in males was by firearm (18.65%) and in females by poisoning (19.26%) [9].

The aim of this study was to examine the gender differences in cases of committed suicide, including suicide rates and/or trends obtained for population as a whole and to consider socio-demographic factors (age groups, education, employment, marital status, nationality) and methods associated with it in Serbia within the period 2011–2015.

This investigation is continuation of previous investigation of gender differences in suicide in Serbia 2006–2010.

Methods

The data for this study were obtained from the Statistical Office of the Republic of Serbia (Department for Demography). All completed suicides recorded in the foregoing population in Serbia (Central Serbia and Vojvodina) during the period from 2011 to 2015 were included in the study. Statistical analysis was done by using the crude number of committed suicides.

The male/female ratio of suicide was calculated for the total number of suicides, for number of deaths caused by suicide within the total mortality and for annual suicide rates, within the period from 2011 to 2015. We calculated male/female ratio for socio-demographic characteristics (education, employment and marital status, nationality and age) and for the methods of suicide within the observed five-year period. A classification of the data related to the suicide methods were defined on the basis of the International Statistical Classification of Diseases and Related Health Problems 10th revision, World Health Organization (ICD-X Code) [10].

Annual suicide rates *per* 100,000 population were calculated using the population data for total population, and for female and male population separately.

Data processing was carried out in the statistical package program SPSS (Statistical Package for the Social Sciences), software version 20.0.

Results

Gender differences in the numbers of suicides, number of deaths caused by suicide within the total mortality and annual suicide rates in Serbia within the period 2011–2015 are shown in Table 1.

Total of 5,897 suicides were committed in Serbia (Central Serbia and Vojvodina) within the period from 2011 to 2015. In observed period 4,397 (74.56%) males and 1,500 (25.44%) females committed suicide, namely, on average, males did it 2.93 times more often than females.

Male/female ratio in suicide number in the total mortality in Serbia, including Central Serbia and Vojvodina within the period 2011–2015 was 2.43 in 2011 to 3.00 in 2012, 2013 and 2015.

Table 1

Gender differences in number of suicides, number of deaths caused by suicide within the total mortality and annual suicide rates in Serbia within the period 2011–2015

Variable	Year of suicide	Total (n)	Males (n)	Females (n)	M/F ratio
Number of suicides					
	2011	1256	909	347	2.62
	2012	1245	934	311	3.00
	2013	1198	902	296	3.04
	2014	1134	857	277	3.09
	2015	1064	795	269	2.95
	Total	5897	4397	1500	2.93
Number of deaths caused by suicide within the total mortality					
	2011	1.2	1.7	0.7	2.43
	2012	1.2	1.8	0.6	3.00
	2013	1.2	1.8	0.6	3.00
	2014	1.1	1.68	0.55	2.96
	2015	1.0	1.5	0.5	3.00
Annual suicide rates (per 100,000)					
	2011	17.4	25.8	9.3	2.77
	2012	17.3	26.6	8.4	3.17
	2013	16.7	25.9	8.1	3.20
	2014	15.9	24.7	7.6	3.26
	2015	15.0	23.0	7.4	3.11

M – males; F – females.

Male/female ratio in suicide rate in Serbia continually increased from 2.77 in 2011. to 3.26 in 2014 and slowly decreased in 2015.

Annual suicide rate (per 100,000) within the period 2011–2015 showed constant decrease from 2011 to 2015 and in 2015, suicide rate was 15.0 (Figure 1).

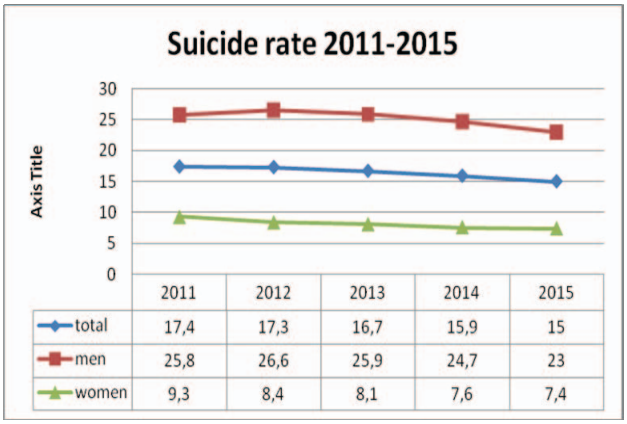

Fig. 1 – Annual suicide rates (per 100,000) in males and females in Serbia within the period 2011–2015.

Gender differences in socio-demographic data (marital status, educational level, employment, nationality and age range) of committed suicide in Serbia within the period 2011–2015 are shown in Table 2.

About half of the men who committed suicide (47.6%) were married, with secondary education (45%). About a third of women who have committed suicide were widowed (38.86%) or married (36.4%) with secondary education (31.33%). About a half of men (49.80%) and women (51.6%) who had committed suicide were retired.

According to the nationality, Serbs committed suicide the most frequently within observed period, then followed Hungarians and Croats ethnic minority. Male/female ratio in suicide was 2.92 in Serbs, 3.23 in Hungarians and 2.23 in Croats.

Male/female ratio in age differences varied from 2.5 in the youngest and the oldest group (younger than 15 years, older than 65 years) to 3.5 in the group of middle age (25 to 54). The highest ratio was in the group from 15–24 years of age.

The most common method of suicide both in males and females were hanging, strangulation and suffocation. Males 3.21 times more often committed suicide by hanging, strangulation and suffocation than females. The second most common method in males was by firearm and explosive material and by self-poisoning in females. Males 14.2 times more often committed suicide by firearm and explosive material than females, but females 1.17 times more often committed suicide by self-poisoning and by drowning and submersion than males (Table 3).

Table 2

Gender differences in marital status, educational level, employment, nationality and age range of committed suicide in Serbia within the period 2011–2015

Socio-demographic data	2011 M;F	2012 M;F	2013 M;F	2014 M;F	2015 M;F	Total M;F	M/F ratio
Marital status							
single	194;56	230;33	210;41	202;37	217;47	1053;214	4.92
married	468;130	453;115	427;119	413;104	348;78	2109;546	3.86
widowed	149;124	164;128	156;104	139;112	147;115	755;583	1.29
divorced	86;32	79;31	102;30	98;19	76;29	441;141	3.12
unknown	12;5	8;4	7;2	5;5	7;0	39;16	2.43
Educational level							
no school	21;39	11;33	21;20	19;27	63;56	135;175	0.77
uncompleted primary school	129;77	125;69	100;49	101;53	92;28	547;276	1.98
primary school	265;96	285;83	271;91	246;73	221;81	1288;424	3.03
secondary school	414;94	447;108	407;105	381;90	330;73	1979;470	4.21
high school	27;15	20;7	38;14	25;9	27;7	137;52	2.63
university	32;12	29;7	37;10	37;15	45;17	180;61	2.95
no data	21;14	17;4	28;7	48;10	17;7	131;42	3.12
Employment status							
employed	414;83	435;76	441;81	383;69	305;60	1978;369	5.36
unemployed	219;52	267;41	252;56	234;47	142;34	1114;230	4.84
retired	469;169	470;156	429;152	443;148	379;149	2190;774	2.82
dependents	25;95	29;79	32;63	31;60	31;60	148;357	0.41
Nationality							
Serbs	732;280	742;251	714;241	680;220	668;219	3536;1211	2.92
Hungarians	63;21	67;24	81;17	72;21	43;21	326;104	3.13
Croats	13;7	17;6	11;4	8;4	9;5	58;26	2.23
Age range (years)							
< 15	1;1	1;0	1;0	0;0	2;1	5;2	2.5
15–24	28;11	33;9	29;2	32;4	27;7	149;33	4.5
25–34	77;21	85;17	73;24	73;21	70;21	378;104	3.6
35–44	104;29	119;25	113;32	89;31	99;32	524;149	3.5
45–54	172;48	153;47	162;50	130;32	120;33	737;210	3.5
55–64	171;71	210;71	204;78	191;61	175;46	951;327	2.9
65–74	145;71	132;57	134;48	152;42	111;52	674;270	2.5
> 75	211;95	199;85	185;62	190;86	189;77	974;405	2.4

M – males; F – females.

Table 3

Gender differences in methods of committing suicide in Serbia within the period 2011–2015

Method of suicide	2011 M; F	2012 M; F	2013 M; F	2014 M; F	2015 M; F	Total M; F	M/F ratio
Self-poisoning by drugs and by exposure to liquid substances (X 60-65, X 68-69)	40;56	38;53	50;50	49;46	38;46	215;251	0.85
Hanging, strangulation and suffocation (X70)	603;215	618;191	562;172	558;162	501;145	2842;885	3.21
Drowning and submersion (X71)	19;23	14;23	20;24	24;23	20;20	97;113	0.85
Firearm and explosive material (X72-X75)	174;17	173;8	173;10	162;15	152;7	834;57	14.63

M – males; F – females.

Discussion

According to the data obtained from the Statistical Office of the Republic of Serbia (Department for Demography) within the period 2011–2015, about 1,200 people committed suicide on the average per year. In the observed five-year period (2011–2015), 776 suicides were committed less than in the previous five -year period (2006–2010) and recorded a reduction in the total number of suicides in Serbia by 11.62% [9]. A decrease of the total number of committed suicide is a result of yearly trend of permanent decreasing the total number of inhabitans in Serbia in the observed five-year

period. According to the data obtained from the Statistical Office of the Republic of Serbia (Department for Demography), the total number of inhabitans decreased in the period from 2011 to 2015 for 138,716 (1.97%) inhabitants, namely, from 7,234,099 in 2011 to 7,095,383 in 2015.

The trend of increasing number of suicides among male population comparing to female one, which began in 2006 continued in the following five-year period (2011–2015). The increase from 71.9% to 74.56% share of the male population in the total number of suicides in the observed five-year period was recorded. In that way, the male/female ratio increased from 2.56 on average (2006–2010) [9] to 2.93 in the observed period (2011–2015).

The male/female ratio in suicide number in the total mortality in Serbia, including Central Serbia and Vojvodina, continually increased from 2.52 times in previous period (2006–2010) [9] to 2.88 in the observed period (2011–2015).

In the observed period, the suicide rate decreased constantly from 17.4 in 2011 to 15.0 in 2015 and continued the tendency of suicide rate decrease in the last decade permanently from 19.43 (2006) [9] to 15.0 (2015).

As for socio-demographic data, differences were not observed in the socio-demographic characteristics of suicide committers according to the previous five-year period (2006–2010) [9]. The trend that suicide was most often committed by married males and females with completed high school, retired, the Serbs, which began in 2006 continued in the following five-year period.

Serbs most often committed suicide in Serbia, and it is expected, because the Serbian nationality is the majority nationality (83.3%) in Serbia. In accordance with the total Serbian population, the Serbs, both males (80.47%) and females (80.71 %), equally committed suicide, but regarding gender, males committed suicide about three times more often than females.

On the other side, in ethnic minority some differences with regard to gender are noticed. There are discrepancies in suicide rates between national minority, primarily in Hungarian national minority which makes 5.5% of committed suicide, according to 3.5% of Hungarian inhabitants in Serbia. Inversely, Croatian national minority makes 0.98% of committed suicide according to 0.8% of Croatian inhabitants in Serbia. Those differences are results of cultural factors that mediate suicide rates in minority ethnic groups. Suicide rates were higher in areas where ethnic minority groups were in lower concentration [11]. Comparing with the other regions of Serbia, Hungarian minority ethnic group could be considered the highest risk group, because of their constantly the highest suicide rate over last period [5]. Hungarian and Croats males more often committed suicide than females in the five-year observed period.

As for the age, there were two age groups at highest risk to commit suicide. Firstly, there were the adolescents, the people younger than 24 and secondly there were the elderly older than 65. It is well known that suicide is the third leading cause of death for 15 to 24-year olds, and that suicide among young people is on the rise and makes the group with the highest risk in many countries in the world [1–3]. Our investigation is in accordance with those investigations, and confirmed that male/female suicide ratio was the highest among adolescents and decreased with age. In Serbia, male adolescents more often committed suicide than female adolescents in the observed five-year period (2011–2015).

About a quarter of suicides in Serbia was pcommitted by individuals older than 75 years. Our investigation is in accordance with some investigations in other countries and confirmed that traditionally suicide rate is the highest among the elderly [1, 2].

On the other side, our results are not in accordance with the data in many investigations where individual suicide risk factors are varied, but in many countries suicide rates are highest in men, those who are divorced or separated, the unemployed and who are socially isolated [12–14].

It is shown that marital status was not a protective factor for suicide in Serbian population. Although loneliness is an important suicide risk factor it is not confirmed in our investigation [2]. Serbia in late 20th century and early 21st century faced a significant number of widowed persons and their participation in total population. The population of Serbia (excluding Kosovo) is extremely old. In Serbia, there are 1,250,316 people who are older than 65 years and among them, there are more women. Every second person is older than 70 years, and at the same time there are more widows (57%) than widowers (43%) [14].

It is well-known that loneliness, often manifested by intense feelings of emptiness and abandonment, can lead to depression and suicide. Social isolation is often associated with major life events, such as a death of a partner, divorce, unemployment or disability. Such events are usually accompanied by a loss of social ties. The relationship with the family and relatives changed, contacts are mostly focused on acquiring help. Seniors are more often exposed to such losses than young people. Reaching out to those who have become disconnected from others and offering them support and friendship may be a life-saving act [15, 16].

According to data obtained from the Statistical Office of the Republic of Serbia (Department for Demography), elderly people make 17% population in Serbia. More and more lonely pensioners are living without any help from their family and social institutions [14]. During the difficult economic situation, the large percentage of unemployed in the category of the working age population and economic stagnation, the elderly are faced with insufficient resources that could enable them to secure economic life and institutional care of them. These findings show a consistent trend at the individual level indicating that poverty, particularly in the form of worse economic status, diminished wealth, and retirement is associated with suicidal ideations and behaviors [17–19].

The most common cause for elderly suicide is untreated depression accompanied with health problems [17–19]. Social losses such as a death of a spouse, a loss of work roles and work sites provoke a desire to die caused by a lost sense of social belonging and the perception that life is not worth living [18]. Existence of chronic illness, physical impairment, unrelieved pain, sensory deficit and cognitive impoverishment

accompanied with depression, could be a trigger for suicide thoughts [17]. This implies that depression and factors causing depression might be more important suicide risk factors than social isolation.

The most common method of suicide both in males (64.63%) and females (59.00%) was hanging, strangulation and suffocation. The second most common method in males was by firearm and explosive material (18.96%) and by self-poisoning in females (16.73%). Differences in suicidal behavior could be explained by cultural factors. Males choose more efficient and lethal methods of suicide than women. On the other hand, females unlike males, more often commit suicide by poisoning either with solid or liquid substances, or drowning and submersion. Our results coincided with the results from the literature [2].

Based on the results of the present study, some initial ideas for potential intervention strategies can be proposed.

In our investigation, it is confirmed that there are two risk groups of suicide committers. First, there are adolescents, whose suicide was, like in many other countries, on the rise and the second, there are elderly male population, who the most often committed suicide in the observed five-year period (2011–2015) in Serbia.

In suicide prevention, all persons in suicidal risk should seek a professional help from a physician or a qualified mental health professionals. Doctors should recognize the medical and psychiatric needs of the suicidal persons and work closely with their families and health care professionals involved in the management and follow-up of those who are at a risk to commit suicide. Multidisciplinary treatment teams of mental health professionals for depression and anxiety treatment, including psychiatrist and other professionals such as psychologist, social worker and occupational therapist are recommended [2].

Some investigations confirm that, unfortunately, males, especially elderly, are less ready to refer to a doctor for help because of problems related to their mental health. With the aim to easier detect and treat them for psychiatric disorders and to reduce suicide risks, health education should have as a goal an improvement of motivation, particularly in elderly male population, to be ready to ask for doctor's help [20, 21]. Continuous education and training of general practitioners (GPs) and other health care staff are needed in order to gradually and steadily improve depressive treatment as effective in the prevention of suicide, too. With support from their family and appropriate treatment, suicidal persons can heal and return to a healthier path of development.

On the other side, it is necesery to establish some new forms of social protection of the elderly. This situation entails the need to undertake a series of social measures of care for elderly people. There are, among other things, gerontological and geriatric centers, opening counseling and services to help the elderly, the construction of special housing and homes for the elderly. It is necessery to think about their physical needs, including its psychological aspects. The elderly should be allowed to have guarantees that life in the institutions of social protection have adequate support and respect, and to participate in decisions concerning living conditions in the institution ensuring high quality of life. Financial support by the goverment is necessary. Intergenerational solidarity and the development of prevention measures and procedures to mitigate adverse environmental impacts of variety non-institutional forms of social protection and inclusion of elderly persons in society are also necessary [19, 20]. The elderly should be allowed to remain active members of society, to lead a decent life and play an active part in public, social and cultural life, to choose freely their lifestyle and to lead independent lives in their familiar environment.

The main limitation of the present study is that in our investigation we used only data from Statistical Office of the Republic of Serbia (Department for Demography). On the other hand, psychological autopsy could give more precise information, using hetero anamnesis data from a suicide's relatives, family members and friends, and medical data primarily in the field of mental health and somatic diseases of a suicide. Investigating a suicide, for example, by psychological autopsy is considered to be an effective method for clarifying the characteristics of suicide. So, psychological autopsy could give explanation of the different suicide risk factors, especially differences in sociodemographic characteristics (marital status, unemployment/retired status etc).

Add to these, there is a gap for explanation of a suicide risk factors in some ethnic minority groups and the skewed age distribution of ethnic minorities towards different age groups.

The present findings enrich the discussion of the spectrum of reasons for gender differences in lethality of suicidal behaviour and development of gender specific strategies of suicide prevention, consistent with our previous research of the same topic.

The present study provides initial data for researchers in the field of suicidology and should be further investigated.

Conclusion

Suicide Prevention Programme in Serbia should be primarily oriented towards two age groups at the highest risk to commit suicide, first, towards the adolescents whose suicide was on the rise and second, towards the elderly population, less ready to refer to the doctor for help because of problems related to their mental health.

Doctors should become familiar with community, state and national resources that are concerned with adolescents and elderly suicides, mental health institutions, family and crisis intervention centers, including social and financial support.

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Insulinoma – how to localize the tumor?

Insulinom – kako lokalizovati tumor?

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Abstract

Background/Aim. Arterial stimulation with calcium and venous sampling (ASVS) enables us to reach the goal of avoiding that any patient with insulinoma undergoes a blind surgical exploration. Since ASVS is both a functional and morphological localization procedure, its sensitivity is not influenced by factors that are causing the insensitivity of usual anatomical and morphological procedures. Based on our own experience in preoperative localization of insulinoma, we indented to show why we believe that ASVS should be performed to all patients regardless of data collected from other preoperative localization methods. **Methods.** We have analyzed the accuracy of preoperative localization methods retrospectively. First anatomical and morphological procedures like transabdominal ultrasound (US), endoscopic ultrasound (EUS), computed tomography (CT) and magnetic resonance imaging (MRI) were done. Then we analyzed the data collected during a functional procedure which, at the same time, allows regionalization (ASVS). To estimate the accuracy, the results of every single method were correlated with the operative findings in all sixteen cases. **Results.** Prior to ASVS, fourteen patients underwent US, fifteen had CT, MRI was performed in eight patients and EUS in thirteen. Using only one of these methods enabled identification of tumors in five patients, using two methods in six patients while three and four in one patient each. For three patients, none of these methods was successful. ASVS revealed that all seen tumors were functional except three of the six visualized with two methods (US and EUS). In two of these three cases, US and EUS localized the tumors in pancreatic tail/body while ASVS accurately identified the tumors in pancreatic head. For these patients US and EUS showed false positive results. In the third of these patients EUS showed the tumor localized in pancreatic head, while US and ASVS accurately pointed to tail. This, too, was a false positive result of EUS. ASVS successfully provided regionalization data in three patients where other visualization methods failed. Operative and later histological findings confirmed the accuracy of ASVS in all sixteen patients including two patients that previously underwent distal pancreatectomy based on false positive EUS findings. **Conclusion**. Two patients, with accurate insulinoma regionalization in pancreatic head, obtained with ASVS, previously underwent unsuccessful distal pancreatectomy based on the false positive EUS findings. The same goes to three other patients with the false positive results obtained with other anatomical and morphological findings, as well as those three patients that had no preoperative visualization with other methods prior to ASVS. Therefore we suggest ASVS performing in each suspected insulinoma patient before the surgery, regardless of the data collected using other methods. This would enable us to test functional characteristics of visualized findings and to regionalize part of pancreas with uncontrolled insulin secretion when no suspicious changes were found.

Key words:
insulinoma; **diagnosis; calcium gluconate; injections, intra-arterial; sensitivity and specificity.**

Apstrakt

Uvod/Cilj. Arterijskom stimulacijom kalcijumom sa venskim semplovanjem (ASVS) se moze ostvariti cilj da nijedan bolesnik sa insulinomom ne ode na slepu eksploraciju pankreasa. Pošto je ASVS funkcionalno morfološka lokalizaciona procedura na njenu senzitivnost ne utiču faktori koji ograničavaju senzitivnost anatomsko-morfoloskih preoperativnih lokalizacionih pregleda. Cilj rada bio je da se na osnovu sopstvenog iskustva stečenog u preoperativnoj lokalizaciji insulinoma pokaže zašto smatramo da kod svih bolesnika bez obzira na rezultate drugih preoperativnih lokalizacionih pregleda treba uraditi ASVS. **Metode.** Tačnost anatomsko-morfoloskih pregleda [transabdominalnog ultrazvuka (UZ), kompjutereizovane tomografije (CT), nuklearne magnetne rezonance (NMR), endoskopskog ultrazvuk (EUZ) i funk-

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cionalno-regionalizacionog pregleda (ASVS)] za potrebe preoperativne lokalizacije insulinoma je retrospektivno analizirana. Tačnost nalaza svakog pojedinog pregleda je proverena upoređivanjem sa operativnim nalazom kod 16 uspešno operisanih bolesnika. **Rezultati.** Pre ASVS sa ciljem da se lokalizuju insulinomi kod 14 bolesnika je urađen UZ, kod 15 CT, MR kod osam bolesnika i kod 13 bolesnika EUZ. Samo jednim od navedenih pregleda, promena u pankreasu je identifikovana kod pet bolesnika, sa dva kod šest, a sa tri i četiri kod po jednog bolesnika. Kod tri bolesnice ni jedan od primenjenih pregleda nije identifikovao promene u pankreasu. ASVS je pokazala da su identifikovane promene funkcionalne kod svih bolesnika izuzimajući tri bolesnice od šest bolesnika kod kojih su sa po dva pregleda identifikovane promene u pankreasu. Kod dva od ove tri bolesnice UZ i EUZ su identifikovane promene u telu/repu dok je ASVS tačno regionalizovala tumore u glavi pankreasa– nalazi US i EUZ su bili lazno pozitivni. Kod tri. bolesnice EUZ je identifikovana promena u glavi dok su UZ i kasnije ASVS tačno lokalizovali insulinom u repu; EUZ nalaz je bio lazno pozitivan. ASVS je tačno regionalizovala insulinome kod tri bolesnice kod kojih drugim pregledima tumor nije lokalizovan. Operativnim i kasnijim histološkim nalazom je potvrđena tačnost ASVS kod svih 16 bolesnika uključujući i dvoje koji su ranije neuspešno operisani (distalna pankreatektomija) samo na osnovu, ispostavilo se, lažno pozitivnog nalaza EUZ. **Zaključak.** Dvoje bolesnika kod kojih je samo na osnovu lažno pozitivnog nalaza EUZ urađena neuspešna distalna pankreatektomija, moguća, da nije urađena ASVS, slična sudbina još tri bolesnice sa lažno pozitivnim nalazima anatomsko-morfoloških pregleda i neizvestan ishod operacije bez ASVS kod tri bolesnice kod kojih drugi preoperativni lokalizacioni pregledi nisu lokalizovali insulinome, opravdavaju stav da kod svih bolesnika sa insulinomom, bez obzira na rezultate anatomsko-morfoloških pregleda pre operacije, treba uraditi i ASVS sa ciljem da se provere funkcionalne karakteristike identifikovanih promena ili regionalizacije mesta nekontrolisanog lučenja insulina kada promene nisu identifikovane.

Ključne reči:
insulinom; dijagnoza; glukonati; injekcije, intraarterijske; senzitivnost i specifičnost.

Introduction

In adults that are not treated for diabetes mellitus, who, apart from hypoglycemic episodes, seem to be healthy persons, the usual cause for endogenic hyperinsulinemic hypoglycemias is insulinoma. Insulinomas are extremely rare with incidence of 4/1000000 [1], very small (90% < 2 cm) in 90% benign and in 90% solitary. Practically, all are tumors of the beta cells in pancreatic Langerhans islets. Insulinomas are causing hypoglycemias by uncontrolled secretion of insulin. This diagnosis comes to mind when Whipple's triad is present and when it can be provoked by prolonged fasting, of course in the presence of necessary biochemical criteria for an adequate diagnosis [2]. The only adequate treatment option is surgery. Preoperative tumor localization is a very important step between the diagnosis and surgery. An accurate tumor localization is essential for successful surgical outcome. The importance of the preoperative tumor localization is best represented by the commonly adopted position by relevant professionals that none of these patients should undergo a blind pancreatic exploration [3].

Insulinomas could be localized by anatomical and morphological procedures like ultrasound (US), endoscopic ultrasound (EUS), computed tomography (CT), magnetic resonance imaging (MRI) and angiography. Furthermore we could use arterial stimulation with calcium and venous sampling (ASVS) both as functional and morphological exam.

Sensitivity data differ significantly among various morphological procedures and between diagnostic centers. For US, the sensitivity is 16%–64% [3, 4], for CT 33%–64% [5], for MRI 40%–90%, for EUS 65%–92% [5, 6] and for angiography 29%–50% [7]. Indirectly, the small size and rare occurrence can contribute to the inconsistency of the results. Different diagnostic centers use different diagnostic tools. These tools differ in power of resolution as well. Therefore, technical reasons may in part influence such difference, because insulinomas of the same size might be identified in one center and missed in the other center with inferior diagnostic appliance. On the other hand, to achieve maximum results with owned technology, a certain level of skill, experience and devotion is much needed. Given the same technology, a diagnostic center with significantly more patients will have more chance to reach desired skill and experience. A good example of the importance of skill and experience is EUS with a sensitivity span from 65% to 92% [3, 4]. Another reason for different success rates between diagnostic centers might be the fact that each center develops and masters some of the localization procedures more than some others. This comes regardless of the overall similar relevance and renown of centers in insulinoma management. In one of such famous institutions for insulinoma management the US exams provide accurate localization in 65% [3] and in another with comparable experience only in 16% [4]. To sum up, results of anatomical and morphological diagnostic exams differ significantly, particularly if skill and experience requiring methods are used.

Nevertheless, despite sophisticated equipment and admirable skills and experience, some tumors remain unseen – false negative results, while some tumors that are found will not be functional, will not be insulinomas – false positive results.

Because it is both a morphological and functional method, ASVS substantially differs from the previously mentioned procedures. The sensitivity of the ASVS results is not influenced by the factors that are causing insensitivity or limitations of usual anatomical and morphological exams. Regardless of the center that reported the ASVS data, the sensitivity of results is quite uniform and reproducible. This diagnostic procedure can be performed with the same accuracy anywhere. When done after other visualization methods, ASVS enables an insight into the functionality of the change found and, if none was found, it provides sufficient regionalization of pancreatic area that contains insulinoma.

Methods

Data gathered during preoperative insulinoma localization in sixteen patients was analyzed retrospectively. Our group includes eleven women and five men. The age range was between 23 and 77 years. All patients underwent surgery from 2002 to 2013. Two patients in this group were unsuccessfully operated in other institutions. ASVS was done for each patient after performing the usual anatomical and morphological exams. The accuracy of each localization method was compared to operative and histological findings.

The first two ASVS exams (1996 and 2002) were done in accordance with the original protocol [8]. Later, from 2006 we have modified the procedure in a way that all venous sampling was undertaken from the right hepatic vein [9], arterial stimulation with calcium was applied both in proximal and distal part of the lineal artery [10] and the dose of calcium was fixed to 1.35 mEq – 3 mL of 10% calcium gluconate [11].

The procedure was: A catheter used for venous sampling was inserted through femoral vein and fixed to position in the right hepatic vein. The catheter used for angiography of celiac plexus was inserted through the femoral artery. Then, selective catheterization was performed for each of the following arteries: *a. gastroduodenalis*, *a. mesenterica superior*, proximal and distal part of *a. lienalis* and *a. hepatica*. After each selective catheterization angiography and stimulation with 3 mL of 10% of calcium gluconate were performed. Blood samples from the right hepatic vein were taken 30 seconds prior to immediately prior to and 30, 60, 90 and 120 seconds after the stimulation with calcium. A double or higher rise in insulin concentration compared to the starting value was considered diagnostically significant. Thus, when a diagnostic rise in insulin concentration after calcium stimulation was noted in *a. gastroduodenalis* and a. *mesenterica superior*, we considered that insulinoma was regionalized in pancreatic head or *procesus uncinatus*. When the above was found after the stimulation of both ends of *a. lienalis insulinoma* was regionalized in pancreatic tail. On the other hand, when it was seen after the stimulation of only proximal part of *a. lienalis*, the tumor was in the body of pancreas. A double or higher rise in insulin concentration after the stimulation of *a. hepatica* suggests metastatic disease in liver. If angiographic findings (angiography during procedure) are positive and correlate with functional and regionalization data, then ASVS adds localization value to the test. It becomes more than a functional and regionalization test.

Prior to ASVS in our group, thirteen patients underwent US, fifteen CT, eight MR and thirteen EUS.

Results

All sixteen patients had ASVS done in a previously described way. In every case, after the stimulation with calcium, a diagnostic rise of insulin was noted and that enabled an accurate localization or regionalization of the tumors. This was confirmed with the operative and histological findings. Prior to ASVS, 7 suspected tumors were seen with US, but for two, ASVS showed no functionality – false positive findings. CT and MRI identified 5 and 2 changes, respectively, and all of them were confirmed functional by ASVS. When EUS was used there were 10 noted as suspected tumors, but only 7 were confirmed functional by ASVS and other 3 were not functional – false positive results (Table 1).

Table 1

Imaging tehniques performed to the patients

Imaging tehnique	Performed (n)	Localized (n)	False positive (n)	Negative (n)
US	14	5	2	7
CT	15	5	/	10
MR	8	2	/	6
EUS	13	7	3	3
Angiography	16	3	3	10
ASVS	16	16	/	/

US – ultrasonography; CT – computed tomography; MR – magnetic resonance imaging; EUS – endoscopic ultrasound; ASVS – arterial stimulation with calcium and venous sampling; n – number of patients.

Basic demographic characteristics, preoperative diagnostic results and operative and histological findings for patients are given in Table 2.

For three patients (N^o 4, 10 and 14) without preoperative localization with anatomical and morphological exams ASVS provided accurate regionalization of insulinomas.

By using only one anatomical and morphological method suspected tumors were identified in five patients (N^o 2, 5, 6, 7, and 9). In those cases, ASVS confirmed functionality for each one. The findings obtained by two diagnostic methods identified suspected change in six cases (N^o 1, 3, 8, 12, 13 and 16). For three of them (N^o 1, 3 and 12), ASVS showed functionality, but in other three cases it did not. US and EUS indicated that the tumor was in pancreatic body, while ASVS accurately showed that insulinomas were present in pancreatic head. For both of them, US and EUS results were false positive. In the third patient of this group a suspected change was identified by EUS on pancreatic head while the US result suggested the body/tail segment. ASVS showed that the US finding was accurate and that EUS gave a false positive result. In our group, two patients had accurate localization achieved by three or four morphologic exams and their functionality was also confirmed with ASVS.

The patients N^o 14 and 15 had unsuccessful distal pancreactomy in other institutions 1.5 and 5 years earlier, respectively. The diagnoses were based on the false positive EUS results.

Angiography was performed in all patients. For three patients it was accurate and corresponded to the functional findings. In three cases, it was false positive – it did not correspond to the functionality findings. Eventually, for ten patients angiography was negative although the tumor was found later on false negative results.

All patients were monitored after the surgery for at least 1 year and had no hypoglycemic episodes.

Table 2

Basic demographic characteristics, preoperative diagnostic results, and operative findings

Patient	Age	Gender	US	CT	MR	EUS	Postive arterial territory	Gradient	Surgery	Size (cm)
1	46	Male	N	P tail	ND	P body/tail	Splenic	10	Distal pancreatectomy - tail	0.8
2	59	Female	N	N	N	P tail	Splenic	7	Distal pancreatectomy - tail	0.9
3	23	Male	N	P head	ND	P	GDA	20	Enucleation - head	1.0
4	62	Female	N	N	ND	N	Splenic	14	Distal pancreatectomy - tail	1.5
5	62	Male	P tail	N	ND	N	Splenic prox. Splenic dist.	14 21	Distal pancreatectomy and splenectomy - hilum of the spleen	2.0
6	74	Female	ND	ND	ND	P head	GDA SMA	8 6	Enucleation - head	1.2
7	23	Female	ND	N	N	P tail	Splenic prox. Splenic dist.	8,5 2,7	Distal pancreatectomy - tail	1.1
8	68	Female	P body/tail	N	ND	FP head	Splenic prox. Splenic dist.	6 8	Distal pancreatectomy - tail	1.1
9	77	Male	N	P tail	ND	ND	Splenic prox. Splenic dist.	4 6	Distal pancreatectomy - tail	1.0
10	40	Female	N	N	N	N	Splenic prox. Splenic dist.	8 10	Distal pancreatectomy - tail	1.0
11	43	Female	P tail	P tail	N	P tail	Splenic prox. Splenic dist.	10 12	Distal pancreatectomy - tail	1.4
12	35	Male	P head	N	P head	ND	SMA	3	Enucleation - head	1.5
13	56	Female	FP body	N	ND	FP body	GDA	6	Enucleation - head	1.2
14	41	Female	N	N	N	ND	SMA	8	Enucleatio - head	1.3
15	61	Male	P head	P head	P head	P head	SMA GDA	25 3,5	Enucleation - head	4.0
16	41	Female	FP body	N	N	FP body	GDA	20	Enucleation - head	1.0

P – positive; N – negative; ND – not done; FP – false positive; US – ultrasound; CT – computed tomography; MR – magnetic resonance; EUS – endoscopic ultrasound; GDA – gastroduodenal; SMA – superior mesenteric arteries.

Discussion

The diagnosis of endogenous hyperinsulinemic hypoglycemias, commonly caused by insulinomas, is based on the established criteria, so that its detection is not that difficult [2]. On the other hand preoperative localization of insulinomas inside pancreatic tissue is difficult. Exact localization is necessary since surgery is the only valuable option for a definitive treatment. Having accurate tumor localization enhances chances for the successful tumor resection, shortens the time of operation, and therefore reduces the number of unsuccessful operations and a need for reoperations to the minimum. Reoperations correlate with higher morbidity. Therefore it is necessary to do everything to avoid that a single patient undergoes a blind pancreatic exploration [3].

For preoperative localization, we can use several anatomical and morphological exams (US, EUS, CT, MRI, angiography) and ASVS that is both a functional and regionalization test.

Based on previously given limitations of anatomical and morphological exams, it is not possible to localize all insulinoma cases using only these procedures, causing some of insulinoma cases to remain unseen – false negative results. It is not rare that all morphological methods fail to localize the tumor (occult insulinomas). Sending such patients to surgery would be in fact an undesirable blind pancreatic exploration. Another problems are false positive results. Some changes that are seen are not functional, therefore are not insulinomas. A patient sent to surgery based on false positive results will not be successfully operated. Difficulties arising from false positive or false negative results can be overcome if after anatomical and morphological exams ASVS is performed. Unlike these methods, ASVS is a functional exam that can point to a specific region of pancreas as a possible tumor site – regionalization. If there is a concurrence of angiographic and functional part of the test in a suspected region (characteristic angiographic finding in the area with diagnostic rise in insulin levels) ASVS becomes a functionally-anatomical localization exam. Since ASVS is a functional test, limitations of other anatomical and morphological methods do not influence its results. Consequently, the reports about ASVS sensitivity are reproducible and uniform regardless of the institution where it was done. In all institutions that are performing ASVS almost all insulinomas will be regionalized or localized.

ASVS enables localization or regionalization of insulinomas that were unseen with other methods and if a suspected change was identified, it provides additional information about the functionality of the explored lesion – it determents if it is actually insulinoma. So, ASVS, on one hand prevents patients from undergoing a blind surgical exploration of pancreas, and, on the other hand, it prevents surgery based on false positive results.

By performing ASVS after other anatomical and morphological methods we used all its benefits. We had false negative findings in three patients, but after ASVS, the accurate regionalization was made and that prevented a blind pancreatic exploration and led to a successful surgical outcome.

False positive results may lead to unsuccessful surgery. An example of this is the outcome of our two patients that were diagnosed with insulinomas in pancreatic body based on false positive EUS results. Other anatomical and morphological exams were negative. Distal pancreactetomies were unsuccessful in both cases. One and a half and five years after that these patients were admitted to our institution and after performing ASVS, we have determined the presence of insulinomas in pancreatic head, which was followed by a successful surgical enucleation. If ASVS had been done prior to the surgery, both patients could have been accurately diagnosed (EUS findings would have been seen as false positive) and properly operated during their first hospitalization.

Being operated based on false positive findings could have been an outcome of three more patients if ASVS had not been done to them. Fortunately, ASVS was used to check the functionality of suspected pancreatic changes identified by anatomical and morphological exams and such scenario was avoided. Two of them had suspected tumors based on the US and EUS findings in pancreatic body, but ASVS accurately regionalized insulinomas in pancreatic head. Both patients had successful surgical enucleation of insulinomas from pancreatic head. Chances for these patients to be misguidedly operated based on false positive results were much higher since not just one, but two methods gave wrong localization of the tumor. As for the third patient, if ASVS had not been done she would have been in danger of undergoing a complex Whipple pancreatic operation. In this case the EUS result indicated that the tumor was in pancreatic head and US pointed to body and tail. EUS is considered to be the most sensitive tool for localization of insulinomas in pancreatic head, superior to US, so it is not such a dilemma which result would have been chosen as true. If surgery was in order, it could turn into Whipple section, since a tumor in pancreatic head can easily be missed by palpation even when the surgeon is experienced. Never the less ASVS showed the EUS result to be false positive and the US finding to be true. Distal pancreatctomy was performed and the unnecessary Whipple operation avoided.

Five patients had pancreatic changes identified only by one method. Unlike the unsuccessfully operated patients, these five patients had ASVS done before surgery, and, although it could go the other way, all tumors were functional. There were no false positive results and patients were successfully operated.

The common ground for all medical workers that are treating insulinoma patients is a desire to achieve an exact preoperative localization of the tumor. Since such a goal cannot be reached universally with the same equipment and methods, every institution in this line of work need to develop their own methodology. The only measure of success is a concurrence of operative and histological findings with preoperative localization procedures. All approaches are equally good if their results are the same, and ideal if all insulinomas are accurately localized [11–13].

Conclusion

Our approach to perform ASVS in each patient after other anatomical and morphological procedures, regardless of the results, is based on two facts. Firstly, by doing ASVS, we were able to localize insulinomas in patients with false negative results; secondly, in this way we could check functionality of identified changes and avoid to be misled by false positive results. We will continue with such practice since we consider it to be the only way to send patients to surgery without a fear of failure. ASVS will be performed until it is proven that it can be replaced with noninvasive localization methods such as scintigraphy of glucagon like peptid-1 (GLP-1) receptors.

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Chronic diseases among university students: prevalence, patterns and impact on health-related quality of life

Hronične bolesti među studentima: prevalenca, obrazac i uticaj na kvalitet života u vezi sa zdravljem

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Abstract

Background/Aim. Around 30% of university students have chronic diseases and/or special care needs. As future taskforce in various job sectors will be drawn from current university student population, it is essential that their health-related problems are recognized and properly managed. The aims of this study were to estimate the prevalence and patterns of chronic diseases in the university student population and to assess their health-related quality of life (HRQoL). **Methods.** A total of 1,624 Belgrade University students were recruited from April to June 2009 at the Student Public Health Center. The students filled in sociodemographic and behavioral questionnaire, the Beck Depression Inventory (BDI) and the SF-36 questionnaire. Data on chronic diseases were self-reported and thereafter validated in medical records. The impact of chronic diseases on HRQoL was evaluated through series of linear regression models. **Results.** The prevalence of chronic diseases was 16.5%. The most common chronic diseases were asthma and chronic bronchitis (4.2% and 3.1%, respectively). All SF-36 domains, both composite and total scores were lower compared to healthy students ($p < 0.001$). Females with chronic diseases reported all eight HRQoL domains as worse, whilst males with chronic diseases reported some HRQoL domains as worse. After adjustment, having chronic diseases remained significantly associated with worse HRQoL [beta (β) -5.69; 95% confidence interval (CI) -8.09, -3.28]. **Conclusion.** To meet the needs of university students, the health care service should provide support in prevention and treatment of chronic diseases.

Key words:
chronic disease; prevalence; students; quality of life; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Oko 30% studenata ima hronične bolesti i/ili potrebu za posebnom negom. Imajući u vidu da će ova populacija činiti značajni deo radnog sektora, neophodno je da zdravstveni problemi studenata budu prepoznati i adekvatno tretirani. Ciljevi ovog istraživanja bili su procena prevalencije i distribucije hroničnih bolesti u populaciji studenata, kao i procena njihovog kvaliteta života povezanog sa zdravljem (KŽPZ). **Metode.** Ukupno 1,624 studenta Beogradskog Univerziteta je bilo uključeno u studiju u Studentskoj poliklinici. Studenti su popunjavali sociodemografski upitnik i upitnik o navikama, kao i Bekovu skalu depresije (BSD) i upitnik SF-36 za procenu KŽPZ. Podaci o hroničnim bolestima dobijeni su od ispitanika, a zatim su potvrđeni u istoriji bolesti. Uticaj hroničnih bolesti na KŽPZ procenjen je kroz seriju linearnih regresionih modela. **Rezultati.** Učestalost hroničnih bolesti u populaciji studenata bila je 16,5%. Najčešće hronične bolesti su bile astma i hronični bronhitis (4,2% i 3,1%). Svi SF-36 domeni, oba kompozitna i ukupan skor bili su niži kod studenata sa hroničnim bolestima u odnosu sa zdrave studente ($p < 0.001$). Studentkinje sa hroničnim bolestima imale su lošiji KŽPZ u svih osam domena, dok su studenti sa hroničnim bolestima naveli neke domene KŽPZ kao lošije u odnosu na zdrave studente. Nakon uključivanja više varijabli u konačan regresioni model, prisustvo hroničnih bolesti ostalo je značajan prediktor lošijeg KŽPZ [Beta (β -5,69; 95%) interval pouzdanosti (CI): -8,09, -3,28]. **Zaključak.** Da bi zdravstvene potrebe studenata bile zadovoljene, potrebno je da služba zdravstvene zaštite pruži podršku u prevenciji i lečenju hroničnih bolesti.

Ključne reči:
hronična bolest; prevalenca; studenti; kvaltet života; ankete i upitnici.

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Introduction

The university student population has been generally thought to be in good health. However, the estimates suggest that around 30% of students have chronic diseases and/or special care needs [1]. In the process of transition from high school to university, aside from undertaking responsibility for their own education, students are also expected to take care of their own health. Because of global ageing and an increase in life expectancy worldwide, efforts were made to address the importance and value of years spent in good health [2]. Since future leaders and taskforce in various job sectors will be drawn from current university student population, it is essential that their health-related problems are recognized and properly managed.

Overall, university students reported lower health-related quality of life (HRQoL) compared with community-based adult population of the same age [3]. Furthermore, female students seem to report more health problems, sustain greater psychological burden [4] and use more health care services due to both physical and psychological problems compared with males [5]. Beside challenges in the academic setting, there is evidence to suggest that presence of chronic diseases has influence on lower school achievements, regardless of ethnicity or socioeconomic status [6]. Despite some evidence in the available body of literature, chronic diseases among university students and their impact on overall well-being remain understudied. To gain deeper insight, measuring well-being, health and disease by means of HRQoL instruments, could offer valuable information to health-care providers and policy makers as to how current services could be revisited, redesigned or adjusted.

The aims of this paper were to estimate the prevalence and patterns of chronic diseases in a university student population, and to assess the HRQoL and comparatively analyze it in relation to their healthy peers.

Methods

Participants and setting

The study participants were undergraduate students registered at the University of Belgrade, Serbia. Belgrade is the capital of the Republic of Serbia with population of 1.6 million inhabitants. The University of Belgrade is the biggest and the oldest public institution offering higher education in the Republic of Serbia, with around 89,500 students. It consists of 31 faculties divided in four branches: social sciences and humanities, medical sciences, nature sciences and mathematics, and technology and engineering sciences. The participants were recruited between April and June 2009 at the only Student Public Health Center in Belgrade. As regular annual health check-ups are mandatory for all students at the university, this primary-health care facility was suitable for selection of the study sample. The sampling was based on convenience. Taking into consideration the expected prevalence of chronic diseases among university students of 30%, size of the Belgrade university population (roughly 89,000), confidence level of 95% and confidence interval (CI) of 2, the calculated sample size was 1,645 persons [7]. A total of 1,669 consecutive students who attended regular check-ups were invited to participate in the study, whilst 1,624 consented (response rate 97.3%). This sample represented approximately 1.8% of all Belgrade University students.

In the Republic of Serbia, the health care system is mainly financed by mandatory contributions to a social health insurance scheme. Delivery of health care is set according to three levels: primary, secondary and tertiary. The Student Public Health Center is the principal primary health care institution for the Belgrade University students, comprising outpatient and inpatient departments. Moreover, specialist consultations in all clinical fields are provided.

Ethical approval for the study was obtained from the Ethics Committee of the Faculty of Medicine University of Belgrade on April 24, 2009 (file No. 440/IV-1). Signed informed consent was received from all study participants.

Instruments

Data were collected by questionnaires. The general questionnaire was related to demographic data: age, gender, place of birth (rural/urban), type of faculty (social sciences and humanities/medical sciences/nature sciences and mathematics/technology and engineering sciences), type of current residence (with parents student dormitory rented apartment other), household monthly income; behavior and habits: alcohol use (yes/no), cigarette smoking (yes/no); ever illicit drug use (yes/no); physical activity (yes/no) – defined as moderate activities for at least 10 min at a time, such as brisk walking, cycling, swimming, or any other activity that causes some increase in breathing or heart rate. Data on chronic diseases were self-reported and thereafter validated in the Student Public Health Center's medical records by two independent physicians.

The Beck Depression Inventory (BDI) [8] was used to explore feelings and attitudes related to general depressive status. It is a one-dimensional scale consisting of 21 items. Answers were graded on a four-point scale from 0 to 3. The total BDI score was obtained as the sum of ratings for each item. The total BDI score ranged from 0 to 63, with higher values denoting presence of more severe depression symptoms. The BDI was approved for use in Serbian language by the publisher (Pearson, San Antonio) [9]. The Serbian version of BDI showed sufficient internal consistency (Cronbach alpha coefficient 0.87; test-retest reliability 0.63) [9].

The HRQoL was assessed by using the generic 36-item Short Form Healthy Survey (SF-36) questionnaire [10, 11]. This questionnaire is consisted of 36 questions divided in eight domains/dimensions: Physical Functioning, Role Physical, Bodily Pain, General Health, Vitality, Social Functioning, Role Emotional and Mental Health. Based on these eight domains two summary scores are made: the Physical Composite Score (comprising the former four domains) and Mental Composite Score (comprising the latter four domains). The total quality of life score represented the mean value of the two composite scores. The score ranged from 0 to 100, with higher values denoting better HRQoL [10, 11]. The SF-36 questionnaire has been approved

for use in Serbian language [12]. Serbian version of the SF-36 questionnaire showed good internal consistency as measured by the Cronbach's alpha coefficient of 0.73 [12].

Data analysis

Prevalence of chronic diseases in the study sample was expressed as percentage (in the total sample as well as according to gender). After testing for normality of distribution by means of Kolmogorov-Smirnov test, we determined that the age of students was normally distributed, while household monthly income and the BDI score were not. Difference in normally distributed variable was assessed by using *t*-test for two independent samples. Difference in not-normally distributed variables was assessed by using the Mann-Whitney test for two independent samples. The Chi-Square test was used to assess differences in categorical variables. To evaluate difference in the SF-36 scores, we applied ANOVA. We also tested the interaction terms between presence of chronic diseases and the BDI score. Because probability level was not statistically significant, we did not stratify according to the BDI scores. The Spearman's correlation coefficient (ρ) was used to examine a correlation between selected variables.

To examine the impact of having chronic diseases on HRQoL we performed a series of linear regression models, based on potential confounding effects of the other observed variables. In all models the dependent variable was the total HRQoL score as measured by the SF-36. The independent variable in crude model was a presence of chronic diseases only. In the "Basic model" age and gender were added as covariates. Next, in the "Socio-demographic model", we added the corresponding socio-demographic variables: place of birth, type of current residence, household monthly income, type of faculty and grade point average. In the "Behavior model" following habits were taken into consideration: smoking, alcohol use, ever drug use and physical activity. In the final "Full model", we additionally included the BDI score. The effect estimates were presented as beta coefficients, with corresponding 95% CI. Probability level of $p \leq 0.05$ was considered statistically significant. The SPSS 17.0 statistical software package (SPSS Inc, Chicago, IL, U.S.A.) was used to perform the statistical analysis.

Results

Basic demographic characteristics according to health status are presented in Table 1.

Table 1

Characteristics of the Belgrade University students according to health status

Variable	Students with chronic diseases (n = 201)	Healthy students (n = 1,423)	*p*-value
Age, mean ± SD	20.6 ± 1.6	20.8 ± 1.9	0.129
Gender			
male	72 (35.8)	633 (46.2)	0.849
female	129 (64.2)	737 (53.8)	
Place of birth			
urban	160 (79.6)	1,194 (83.8)	0.271
rural	41 (20.4)	229 (16.2)	
Type of current residence			
home (with parents)	96 (47.8)	665 (46.6)	0.580
students' dormitory	17 (8.5)	163 (11.5)	
alone (in rented apartment)	75 (37.3)	493 (34.7)	
other	13 (6.5)	102 (7.2)	
Family monthly income (in Euros)	600 (460)	600 (400)	0.870
Type of faculty			
social science and humanities	122 (60.7)	848 (59.6)	0.114
medical sciences	18 (9.0)	177 (12.4)	
natural sciences and mathematics	16 (8.0)	65 (4.6)	
technology and engineering science	45 (22.4)	333 (23.4)	
Grade point average*, mean ± SD	8.2 ± 0.8	8.1 ± 0.8	0.088
Smoking			
yes	50 (24.9)	288 (20.3)	0.440
no	151 (75.1)	1,131 (79.7)	
Alcohol use			
yes	163 (83.2)	1,151 (82.4)	0.836
no	33 (16.8)	246 (17.6)	
Ever drug use			
yes	43 (21.4)	200 (14.1)	0.008
no	158 (78.6)	1,223 (85.9)	
Physical activity			
yes	170 (85.0)	1,194 (84.3)	0.836
no	30 (15.0)	223 (15.7)	
BDI score	7.0 (10.0)	5.0 (8.0)	0.001

BDI – Beck Depression Inventory; *p* – probability level; values are presented as medians (interquartile ranges) unless otherwise marked; numbers in brackets for categorical variables denote percentages; SD – standard deviation; *grading system from 6 as minimum (lowest passing grade) to 10 as maximum (highest passing grade).

Table 2

Prevalence of chronic diseases among the Belgrade University students (n = 1,624)

Chronic diseases	n (%)
Asthma	66 (4.2)
Chronic bronchitis	50 (3.1)
Heart failure	39 (2.4)
Hypertension	33 (2.2)
Diseases of the digestive system*	14 (0.9)
Diabetes mellitus	10 (0.6)
Mental and behavioral disorders†	10 (0.6)
Diseases of the nervous system‡	8 (0.5)
Diseases of the urinary system§	8 (0.5)
Diseases of the skin**	4 (0.2)
Diseases of the circulatory system including diseases of the blood¶	5 (0.2)
Neoplasms***	5 (0.2)
Other††	16 (1.0)
Total	268 (16.5)

***peptic ulcers, gastritis, hiatus hernia, ulcerative colitis; †anorexia, insomnia, depression; ‡migraine, spinal disc herniation; §renal calculi, nephritic syndrome, nephritis; **psoriasis, eczema; ¶anemia, thrombocytopenia, haemorrhoids, venous varices; ***thyroid cancer, fibroadenomas; ††allergies.**

The students with chronic diseases more frequently reported ever drug use and higher BDI score. We observed the presence of 268 chronic diseases in 254 students. Of 254 students, 14 students (5.5%) had comorbidities. Prevalence of chronic diseases in the total sample was 16.5%. In addition, 15.6% of the students were diagnosed with one or more chronic diseases. Prevalence of chronic diseases is shown in Table 2.

The most frequent chronic diseases were asthma (4.2%) and chronic bronchitis (3.1%). Table 3 displays prevalence of chronic diseases according to gender. The pattern of chronic disease occurrence was, for the most part, similar in both genders. We did not find a correlation between having chronic diseases and grade point average during studies ($\rho = 0.059$, $p = 0.054$) nor with repeated years at the University ($\rho = -0.031$, $p = 0.627$).

The HRQoL scores according to the health status are given in Table 4. The scores in all domains, both composite and total scores were significantly worse when compared with the healthy students. The females with chronic diseases reported all eight domains, both composite score and the total score as significantly worse when compared with females without chronic diseases. Among the males, however, most scores were significantly worse in comparison with their healthy counterparts, except for the Physical Functioning, Vitality, Role Emotional and Mental Health (data not shown).

Table 5 summarizes linear regression models. In all models, the presence of chronic diseases was significantly associated with worse HRQoL. After adjustment for multiple confounding factors (Full model), the presence of chronic diseases among the University students remained associated with worse HRQoL (beta [β] -5.69; 95% CI: -8.09, -3.28; $p < 0.01$).

Table 3

Prevalence of chronic diseases among Belgrade University students according to gender

Chronic diseases	Males (752)	Females (872)
	n (%)	n (%)
Asthma	33 (4.4)	33 (3.8)
Chronic bronchitis	27 (3.6)	23 (2.6)
Heart failure	17 (2.2)	22 (2.5)
Hypertension	14 (2.0)	19 (2.2)
Mental and behavioral disorders*	6 (0.8)	4 (0.5)
Diabetes mellitus	5 (0.7)	5 (0.6)
Diseases of the digestive system†	4 (0.5)	10 (1.1)
Diseases of the circulatory system including diseases of the blood¶	4 (0.5)	1 (0.1)
Diseases of the nervous system‡	3 (0.4)	5 (0.6)
Diseases of the urinary system§	3 (0.4)	5 (0.6)
Diseases of the skin**	2 (0.2)	2 (0.2)
Neoplasms***	2 (0.2)	3 (0.3)
Other††	5 (0.7)	11 (1.2)
Total	125 (16.6)	143 (16.4)

***anorexia, insomnia, depression; †peptic ulcers, gastritis, hiatus hernia, ulcerative colitis; ‡migraine, spinal *disc herniation*; §renal calculi, nephritic syndrome, nephritis; **psoriasis, eczema; ¶anemia, thrombocytopenia, haemorrhoids, venous varices; ***thyroid cancer, fibroadenomas; ††allergies.**

Table 4

Mean T scores of the SF-36 scales among the healthy and students with chronic diseases (n = 1,624)

Scales of SF-36	Students		F value	*p*-value
	With chronic diseases mean ± SD	Healthy mean ± SD		
Physical functioning	89.7 ± 16.6	94.3 ± 11.8	23.9	0.001
Role physical	76.4 ± 31.8	84.4 ± 26.7	15.4	0.001
Pain	76.5 ± 21.8	84.3 ± 18.6	29.3	0.001
General Health	66.1 ± 18.5	75.9 ± 16.7	58.9	0.001
Vitality	60.2 ± 22.9	65.3 ± 20.6	10.1	0.002
Social Functioning	70.9 ± 24.4	79.1 ± 21.4	25.2	0.001
Role Emotional	57.9 ± 42.7	68.6 ± 39.5	12.4	0.001
Mental Health	65.6 ± 23.3	71.2 ± 19.6	19.5	0.001
Physical Composite Score	73.8 ± 15.0	80.8 ± 12.9	50.3	0.001
Mental Composite Score	63.9 ± 19.8	72.0 ± 18.1	34.3	0.001
Total Score	70.3 ± 17.1	77.9 ± 14.9	43.9	0.001

SF-36 – the 36-item Short Form Health Survey questionnaire.
SD – standard deviation; *p* – value for interaction presence of chronic diseases x Beck Depression Inventory score: 0.347.

Table 5

Linear regression models describing factors associated with health-related quality of life among Belgrade University students (n = 1,624)

Variable	Crude model	Basic model	Socio-demographic model	Behavior model	Full model
Presence of chronic diseases yes vs. no	-7.60 (-9.85, -5.35)*	-7.44 (-9.66, -5.21)**	-9.29 (-12.16, -6.42)**	-8.95 (-11.85, -6.04)**	-5.69 (-8.09, -3.28)**
Age		0.49 (0.01, 0.89)*	0.46 (-0.08, 1.01)	0.47 (-0.08, 1.03)	0.13 (-0.32, 0.59)
Gender females vs. males		-4.88 (-6.40, -3.36)**	-4.78 (-6.77, -2.78)**	-4.00 (-6.10, -1.90)**	-2.35 (-4.08, -0.62)**
Place of birth urban vs. rural			0.96 (1.67, 3.59)	0.81 (-1.82, 3.44)	1.01 (-1.15, 3.18)
Type of current residence with parents vs. other			-0.58 (-1.55, 0.39)	-0.31 (-1.29, 0.68)	-0.22 (-1.03, 0.59)
Household monthly income			2.76 (0.12, 4.85)**	2.03 (0.04, 4.21)**	1.39 (-0.48, 2.93)
Type of faculty social science vs. other			1.13 (0.10, 2.15)*	1.38 (0.34, 2.42)**	0.99 (0.14, 1.85)*
Grade point average			-0.64 (-2.21, 0.84)	-1.01 (-2.50, 0.47)	-1.19 (-2.42, 0.05)
Smoking yes vs. no				-3.47 (-6.02, -0.92)**	-0.50 (-2.61, 1.62)
Alcohol use yes vs. no				-2.79 (-5.60, 0.03)	-1.26 (-3.57, 1.06)
Ever drug use yes vs. no				-0.77 (-3.63, 2.08)	0.40 (-1.94, 2.75)
Physical activity yes vs. no				4.03 (1.20, 6.86)**	2.39 (0.06, 4.72)*
BDI score					-1.32 (-1.45, -1.19)**

BDI – Beck Depression Inventory; Values represent beta coefficients with corresponding 95% confidence intervals from linear regression models. *$p < 0.05$; **$p < 0.01$.

Discussion

In this cross-sectional study we sought to estimate the prevalence of chronic diseases in the University student population. The observed prevalence of chronic diseases was 16.5%. This prevalence was lower than the one observed among Slovenian (26.1%) [13] or British (33.5%) [4] University students. Castren et al. [14] reported that 72% of undergraduate students in Finland had one or more chronic diseases. However, their analysis included conditions such as refractive errors of the eye, dental caries and infection of wisdom teeth [14], which we did not take into consideration. Nevertheless, prevalence of asthma among the Belgrade and Finnish students seem to be quite similar (4.2% vs. 5.0%, respectively). Variations in chronic disease prevalence could be explained by different criteria for inclusion. Still, these variations could be attributed to other factors as well. For example, lower prevalence of chronic diseases among the Belgrade University students may have resulted from differences in perception on education and employment. It is possible that adolescents who suffer from chronic diseases decided to enter the job market, or opted to enroll in higher education institutions that offer programs of shorter duration (such as 4 or 5 semesters as opposed to at least 8 semesters at the University, depending on a type of faculty) to reduce academic stress, and yet develop certain professional skills.

We observed that students who suffer from chronic diseases did not have lower grade point average compared with healthy students. Crump et al. [6] reported that children and adolescents with chronic neurodevelopmental and seizure disorders had low school performance. This, however, was not observed among pupils with cardiovascular disorders or diabetes [6], which is in line with our results. Therefore, it seems that diseases other than neurodevelopmental disorders do not interfere with academic performance. On the other hand, we noted that students with chronic diseases reported ever drug use more frequently than their healthy peers. Ayvasik and Sümer [15] indicated that one of the predictors of drug use among college students is sensation-seeking and risk-taking. It is possible that, due to the presence of chronic diseases, these students have more propensity towards risk-taking. However, this study design limited us from defining whether or not having chronic diseases was associated with the previous use or initiation of illicit drug use at the University. Finally, we observed that the students with chronic diseases also had a higher BDI score when compared with healthy students. Depression was identified as one of the most common health problems in the college students [16]. Given this, it was suggested that most college health services in the US are able to manage it on-campus [1]. Still, it might be beneficial that the University students with chronic diseases are offered screening for depression as a part of the general health status assessment.

Overall, the HRQoL among the students with chronic diseases was worse than among healthy students and having chronic diseases remained associated with worse HRQoL across all regression models. Still, we observed that all dimensions of HRQoL were worse in the female students, whilst among the males, certain dimensions, including both physical and mental functioning, were not. Although psychosomatic complaints were strongly associated with self-reported health status across student populations [17], moderate-to-high sense of coherence was linked with lower frequency of health problems [18], indicating that mental health may have strong influence on overall health status. Studies showed that males were more likely to rate their health status as better [4, 5, 17, 19], while females "keep an eye" on their health more [4]. Females seem to report more often headaches, back pain or neck/shoulder pain [4], as well as fatigue, depression and anxiety [5]. In the survey of eleven faculties in Egypt, females reported more burden from studies, exams, assignments as well as from other responsibilities in addition to their academic duties [19]. In the Hong Kong study, by contrast, males seem more likely to accomplish effective stress management, particularly by "taking some time for relaxation each day" or by "other specific methods to control stress" [20], which may be a result of having more general resistance resources and coping strategies. In line with all previously mentioned, conformity to gender norms may play a role in various health indices [21].

Information bias should be acknowledged as a limitation in this study, because data on smoking, alcohol and ever drug use and physical activity were self-reported. Also, the sample size was drawn only from the Belgrade University, while students from other major four public Universities in the country were not included. It is possible that our sample included those students who had more health complaints and thus, were more likely to attend the Student Public Health Center. By including several other disorders, such as dental caries or refractory anomalies of the eye could have yielded a different disease prevalence. Although we aimed at reaching a representative sample of the Belgrade University student population, the relative size of our sample might have influenced the findings. Because of this, the study results may not entirely reflect the real-life situations. Finally, the associations based on the cross-sectional study design fail to take the direction of associations into account.

Conclusion

There is a lack of studies exploring HRQoL among University students with chronic diseases. Our study offered an insight into patterns of chronic diseases in the University student population and confirmed that, unlike other aspects, presence of chronic diseases is the consistent factor associated with worse HRQoL. Apart from the expected finding that students with chronic diseases had worse HRQoL compared to their healthy peers, our study identified that the males reported some dimensions of HRQoL as poor while the females perceive all dimensions of HRQoL as worse. The HRQoL measurement could be an informative tool in early recognition of physical and emotional well-being of young adults with chronic diseases in higher education institutions. To meet the needs of University students, the health care service should provide support in prevention, recognition and treatment of chronic dis-

eases. Because female students may be at higher risk of having worse health status, the importance of screening and health-related support could be crucial in providing a safe education environment for this population group.

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Morphological and histopathological heart changes in autopsies of heroin abusers

Morfološki i patohistološki nalaz na srcu obdukovanih korisnika heroina

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Abstract

Backround/Aim. Heroin is a semisynthetic opioid that may cause morphological and histopathological changes in heart: ventricular hypertrophy, myocardial fibrosis, hypertrophy of cardiomyocytes, myofibrils contraction band necrosis, loss of myocytes *nuclei* and cross-striation, perivascular bleeding, inflammatory cells infiltrate. The aim of the study was to show morphological and histopathological heart changes in autopsies of the long-time heroin abusers with positive toxicological analysis for 6-monoacetylmorphine (6-MAM) and morphine in blood and urine. **Methods.** Retrospective study was done at the Institute of Pathology and Forensic of the Medicine Military Medical Academy in Belgrade between 2010 and 2014 and included forensic autopsies of 27 examinees aged between 18 and 60. Heart ventricles thicknesses was analysed and histopathological myocard findings from processed material stained by hematoxyline-eosine (H&E) and trichrome stains (Masson) were examined. 6-MAM and morphine concentration in blood and urine using high-performance liquid chromatography coupled with photodiode (HPLC-PDA) and ultraviolet (UV) detector were analysed. **Results.** Heart ventricles thickness was increased in all persons (27/27; 100%) left 1.74 ± 0.17 cm and right 0.6 ± 0.09 cm. Myocardial fibrosis affected 27/27 (100%) of the examined persons including perivascular one in 24/27 (88.89%) and interstitial focal fibrosis in 3/27 (11.11%); hypertrophy of cardiomyocytes was present in 22/27 (81.48%); myofibril contraction band necrosis in 22/27 (81.48%); loss of myocytes *nuclei* and cross-striation in 10/27 (37.04%); fresh perivascular bleeding in 23/27 (85.19%); focal inflammatory cells infiltrate in 14/27 (51.85%). In toxicological findings, in 27/27 (100%), 6-MAM and morphine were found in urine. Both 6-MAM and morphine in blood were found in 3/27 (11.11%) and only morphine in blood in 16/27 (59.26%) persons subjected to an autopsy. **Conclusion.** Our results indicate both morphological (left and right ventricle hypertrophy) and histopathological heart changes (myocardial fibrosis, hypertrophy of cardiomyocytes, contraction-band necrosis, loss of myocytes *nuclei* and cross-striation, fresh perivascular bleeding and focal inflammatory infiltrate) in long-term heroin obusers. These changes are non-specific and could be caused either by long-term heroin abuse or by other factors. Having in mind a lack of medical histories of examined we could not exclude other factors besides long-term heroin abuse as cause of heart changes.

Key words:
heroin dependence; heart; myocardium; morphine; histological techniques; autopsy.

Apstrakt

Uvod/Cilj. Heroin je polusintetska droga iz grupe opioida, koja može izazvati morfološke i patohistološke promene na srcu: hipertrofiju komora, fibrozu miokarda, hipertrofiju kardiomiocita, izvijuganost miofibrila, gubitak jedara i poprečne ispruganosti miocita, perivaskularna krvarenja i zapaljenski infiltrat. Cilj rada bio je da se prikažu morfološke i patohistološke promene kod obdukovanih osoba sa pozitivnim toksikološkim nalazom metabolita heroina: 6-monoacetilmorfina (6-MAM) i morfina. **Metode.** Retrospektivna studija je rađena u Institutu za patologiju i sudsku medicinu Vojnomedicinske akademije u Beogradu na 27 osoba starosti od 18 do 60 godina obdukovanih u periodu od 2010. do 2014. godine. Analizirani su debljina zida komora i patohistološki nalaz isečaka miokarda, obrađenih standardnom procedurom i obojenih hematoksilin-eozin (H&E) i trihromnim bojenjem (Masson). U uzorcima krvi i urina analizirane su koncentracije 6-MAM-a i morfina primenom tečne hromatografije sa detektorom visokih performansi sa fotodiodom (HPLC-PDA) ultravioletnim (UV) detektorom. **Rezultati.** Debljina zidova komora srca bila je

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kod 27/27 (100%) obdukovanih veća od normalne i to leva 1,74 ± 0,17 cm, a desna 0,6 ± 0,09 cm; fibroza miokarda kod 27/27 (100%) i to perivaskularna kod 24/27 (88,89%), a fokalna intersticijalna kod 3/27 (11,11%) obdukovanih; hipertrofija kardiomiocita kod 22/27 (81,48%); izvijuganost miofibrila kod 22/27 (81,48%); gubitak jedara i poprečne ispruganosti miocita kod 10/27 (37,04%); sveže perivaskularno krvarenje kod 23/27 (85,19%); fokalni zapaljenski infiltrat kod 14/27 (51,85%). Toksikološkom analizom kod 27/27 (100%) obdukovanih su nađeni 6-MAM i morfin u urinu. Zajedno u krvi nađeni su 6-MAM i morfin kod 3/27 (11,11%), a samo morfin kod 16/27 (59,26%) obdukovanih. **Zaključak.** Našim istraživanjem utvrđene su morfološke (hipertrofija leve i desne komore srca) i patohistološke promene (fibroza miokarda, hipertrofija kardiomiocita, izvijuganost miofibrila, gubitak jedara i poprečne ispruganosti miocita, sveže perivaskularno krvarenje, fokalni inflamatorni infiltrat) kod obdukovanih heroinomana. Te promene su nespecifične i mogu biti uslovljene dugotrajnom upotrebom heroina, ali i nizom drugih faktora. Zbog nedostatka medicinske dokumentcije ispitanika ne možemo isključiti mogućnost da ostali faktori, pored dugotrajne upotrebe heroina, mogu biti uzrok opisanih promena na srcu.

Ključne reči:
zavisnost od heroina; srce; miokard; morfin; histološke tehnike; autopsija.

Introduction

In European population 0.6% of individuals aged between 15 and 64 are heroin abusers [1]. Heroin (3, 6-diacetylmorphine) is a semisynthetic opioid, morphine derivative synthetized by acetylation of two hydroxyl groups of morphine. It can be taken into body intravenously, intramuscularly, intranasally, subcutaneously and by smoking. Maximal blood concentration is reached 1 to 5 minutes after intravenous intake and smoking or 5 minutes after intranasal and intramuscular application of heroin [2]. Heroin is metabolized fast in the body to 6-monoacethylmorphine (6-MAM) which is converted to morphine by 6-acetyl group hydroxylation. The metabolic path of morphine includes glucuronidation to morphin-3-glucuronide and morphin-6-glucuronide in liver [3]. Heroin conversion into 6-MAM lasts 10 to 15 minutes and further conversion to morphine lasts few hours. 6-MAM half-life in urine is 0.6 hours and it can be detected in urine 2 to 8 hours after the intake [4]. Morphine is detected in urine up to 24 hours after heroin intake [5]. Histopathological changes in heart, lungs, liver, brain and other organs may appear due to heroin abuse [6]. Some of the common heart changes are myocardial fibrosis, ventricular hypertrophy and inflammatory cells infiltrate in myocardium [7]. Heart muscle changes in long-term opioid abusers increases the risk of sudden cardiac death after intravenous drug injection [8]. The aim of the study was to show the presence of morphological and histopathological heart changes observed in autopsies of long-term heroin abusers, who have positive toxicological analysis for heroin metabolites, 6-MAM and morphine in body fluids (blood and urine).

Methods

Retrospective study was done at the Institute of Pathology and Forensic Belgrade of the Medicine Military Medical Academy in Belgrade between 2010 and 2014 and included 27 forensic autopsies of examinees aged between 18 and 60. Heteroanamestic data from family members showed heroin abuse lasting more than 2 years. An average age of examinees was 35.11 ± 10.78 years. Most of the examined persons were males, 25/27 (92.59%), while there were 2/27 (7.41%) females. Forensic autopsies with positive toxicological analysis for heroin metabolites, 6-MAM and morphine, but without presence of other drugs, alcohol and other elements of abuse in body fluids (blood and urine) were analysed. In external autopsy examination fresh injection marks, scars and tattoos were searched as common findings for the population of drug abusers. During internal examination, left and right heart ventricle thickness was measured and parts of heart muscle were taken as material for further histopatholgical examination. Normal thickness value varies from 1.0 to 1.5 cm for left and 0.25 to 0.5 cm for right ventricle [9]. The material was processed by standard procedure, stained by hematoxylin-eosine (H&E) and trichrome stains by Masson. Light microscope Olympus BX 50 (×40) was used for histopathological examination of stained microscopic slides. Body fluids (blood and urine) were taken during the autopsy and the concentrations of 6-MAM and morphine were searched. Toxicological analyses were done using high-performance liquid chromatohgraphy coupled with photodiode (HPLC-PDA) with ultraviolet (UV) detection in the National Poison Control Centre, the Military Medical Academy in Belgrade and compared with standard library of spectrophotometry. Results were statistically analysed using descriptive statistics methods and non-parametrical tests by a statistical software package IBM SPSS Statistics 20. Non-parametrical test included Wilcoxon signed rank test, with confidence level at $p < 0.05$.

Results

Fresh injection marks were found during external examination in 17/27 (62.96%), tattoos in 14/27 (51.85%), scars in 18/27 (66.67%), both linear and circular scars in 4/27 (14.81%), only linear in 11/27 (40.74%) and only circular in 3/27 (11.11%) persons subjected to an autopsy. Neither scars and tattoos nor fresh injection marks were examined in 1/27 (3.70%) autopsies. All 27/27 (100%) of the examined had increased both left and right heart ventricles thickness in comparison to normal thickness values. An average left and right ventricles thickness of the examined was 1.74 ± 0.17 cm, and 0.6 ± 0.09 cm, respectively.

Findings of the histopathological examination of the heart muscle material were: myocardial fibrosis in 27/27

(100%), fresh perivascular bleeding in 23/27 (85.19%), hypertrophy of cardiomyocytes in 22/27 (81.48%), contraction band necrosis of myofibrils in 22/27 (81.48%), focal inflammatory cells infiltrate in 14/27 (51.85%) loss of myocytes *nuclei* and cross-striation in 10/27 (37.04%), cases (Figure 1). Perivascular myocardial fibrosis was found in 24/27 (88.89%) examined (Figure 2) and interstitial focal fibrosis in 3/27 (11.11%) (Figure 3). All 6 findings mentioned above were found in 2/27 (7.41%) examined people, 5 findings in 11/27 (40.74%), 4 findings in 7/27 (25.93%), 3 in 6/27 (22.22%) and 1/27 (3.70%) had 2 histopathological change.

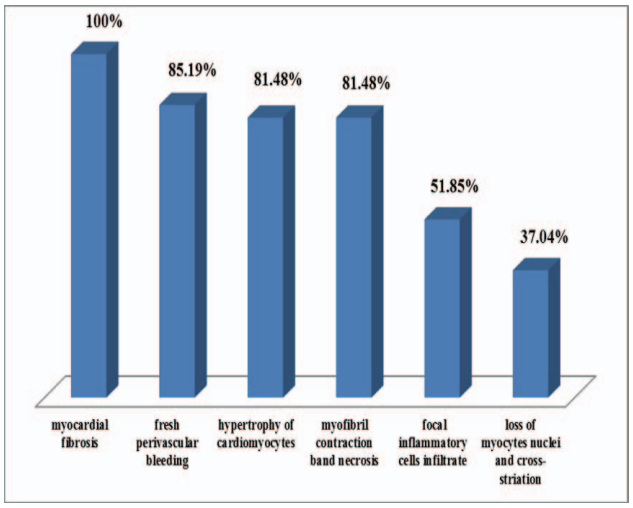

Fig. 1 – Histopathological changes in heart of heroin abusers.

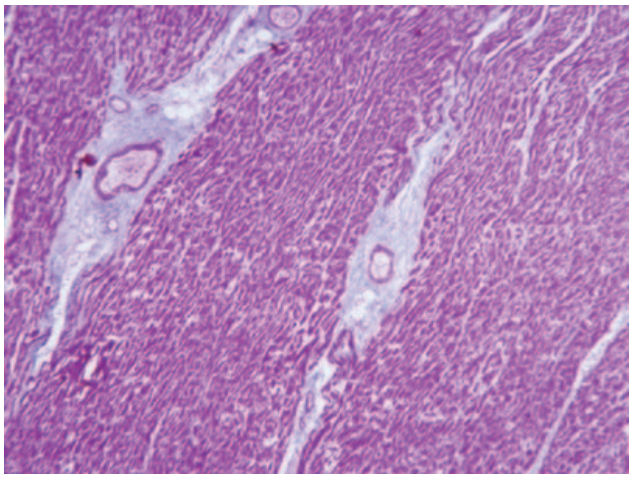

Fig. 2 – Perivascular myocardial fibrosis (trichrome staining by Masson, magnification ×40).

In all examined persons [27/27 (100%)], 6-MAM and morphine were found in urine. Both 6-MAM and morphine were found in blood of 3/27 (11.11%) and only morphine in blood of 16/27 (59.26%) examined. 6-MAM concentrations were 0.001–3.9 mg/L in urine, and 0.006–0.5 mg/L in blood. Morphine concentrations range was 0.015–12.72 mg/L in urine, and 0.004–0.8 mg/L in blood. There was not statistically significant difference between concentrations of 6-MAM in blood and urine (p = 0.109) but there was between concentrations of morphine in blood and urine (p = 0.002). There was no statistically significant difference between concentrations of 6-MAM and morphine in blood (p = 0.717), but there was in urine (p = 0.023).

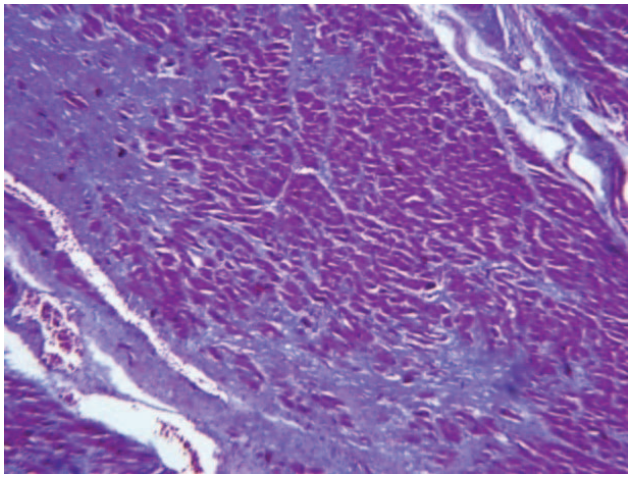

Fig. 3 – Interstitial focal myocardial fibrosis (trichrome staining by Masson, magnification ×40).

Discussion

Injection marks which were found during external examination were due to intravenous heroin intake. Skin scars were usually self-inflicted [10]. Tattoos are usually covering injection marks and scars as a try of covering the needle track of injections [11].

The frequency of heart diseases due to opioid consumption is still unknown. In one study it was published that only 10% of the examined had a heart disease (endocarditis), but also in another one it was reported that a heart damage was found in 100% of the examined people [2]. The long-term heroin or morphine abuse causes hypoxia that leads to myocardial hypertrophy and myocardial fibrosis [12, 13]. Respiratory centre depression and hard breathing as a consequence of lowered neuron sensibility in respiratory centre in brainstem after intravenous drug application is one of possible causes of myocardial hypoxia [1]. In our study, a morphological change in heart presented in all examined people during autopsies was left and right ventricle myocardial hypertrophy. Left ventricle myocardial hypertrophy is described also in other studies on autopsies of abusers as the most frequent finding among cardiac changes in opioid abusers [7]. It is shown in results of other studies that the frequency of this change increases with the age of abusers, leading to 2.1% between the age of 15 and 24 years and 10.7% in older than 44 years [7]. Myocardial hypertrophy increases the risk for hypoxia which can cause arrhythmias and cardiac arrest [12]. Hypoxia leads to apoptosis of myocytes similarly to myocardial infarction, where fibrosis is formed by remodelling in the region of death myocytes [13]. Multiplied fibrous tissue in heart muscle was present in all examined people in this study. Some authors report that fibrosis is a response to chronic hypertension that causes myocardial hypertrophy, and other au-

thors add the cellular growth factors as the reason of fibrosis [8, 12, 14]. It is shown in experimental studies that the absence of fibroblast growth factor 21 leads to myocardial hypertrophy and ischemia by activation of proinflammatory paths and oxidative stress and also by fibrosis and heart metabolism disruption [14]. Collagen accumulation in heart muscle leads to elasticity decrease, thickening and solidification of ventricle wall that complicates contractility. The other study shows that heroin and morphine intoxication causes myocardial contraction depression [7, 15].

Intravenous heroin abusers are exposed to many cardiotoxic factors that lead frequently to heart damage [11]. Coronary blood vessels vasoconstriction is caused by increased catecholamine level, especially noradrenaline and dopamine [16], detectable in blood and urine during the first day after heron intake [17]. Nowadays, investigations on experimental animals confirm previous theories of early heroin metabolism connection with catecholamine concentration increase [18]. Heron has systemic and direct effect on the heart [19–21]. Heart muscle rhabdomyolysis, hypoxia, acidosis and vasoconstriction lead to muscle necrosis and hypersensitive reaction to heroin [22]. Heroin has direct effect on coronary arteries causing its spasm or inflammation that may lead to occlusion [23]. It is written in studies that heroin has a direct effect on vasomotor centre with increased parasympathetic activation, decreased sympathetic activation and histamine production stimulation in mastocytes, with bradycardia and hypotension that may then cause myocardial infarction [24]. Increased parasympathetic activity may also play a role in coronary artery spasm initiation [25]. Bradycardia, tachycardia and atrial fibrillation are noted after heroin application. It was shown in experimental studies that morphine perfusion in sinoatrial node first caused tachycardia and then bradycardia which was explained as a consequence of vagal stimulation [11]. Fresh lesions, as perivascular bleeding in heart muscle and myofibril contraction band necrosis are not specific but can appear due to direct toxic and hypoxic heroin effects and can cause heart rhythm changes and sudden cardiac death.

Presence of heroin metabolites may initiate histamine release. Fresh perivascular bleeding in heart muscle is caused by histamine-induced increased blood vessel wall permeability [26].

Conclusion

Our results indicate left and right ventricle hypertrophy as morphological heart changes as well as histopathological heart changes: myocardial fibrosis, hypertrophy of cardiomyocytes, contraction-band necrosis, loss of myocytes *nuclei* and cross-striation, fresh perivascular bleeding and focal inflammatory infiltrate. These changes are non-specific and could be caused either by the long-term heroin abuse or by many other factors, for example, arterial hypertension and other drugs (stimulants) abuse. Having in mind a lack of medical histories of the examined and the fact that all the information we collected were based on heteroanamnestic data from family members, we could not exclude other factors besides the long-term heroin abuse as a cause of heart changes.

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Surgical treatment of symptomatic patellofemoral malalignment: Do we need an ideal patellofemoral congruency to solve the symptoms?

Hirurško lečenje simptomatske patelofemoralne inkongruencije: Da li je uspostavljanje idealnih odnosa u zglobu neophodno za rešavanje simptoma?

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Abstract

Background/Aim. The aim of this prospective nonrandomized study was to test functional results of different surgical strategies in the operative treatment of symptomatic patellofemoral malalignment. Our hypothesis was that immediate extensive surgery does not have serious advantage comparing to "step by step" procedure, regarding the main symptoms and functional end result. We wanted to check whether obtaining ideal surgical patellofemoral congruency is an essential prerequisite for subsidence of the major symptoms of patellofemoral malalignment. **Methods.** The study included 35 patients with patellofemoral malalignment who had persistant major symptoms: patellar pain and slipping, 3 months after nonoperative treatment. Divided into three groups, they all underwent the realignment surgery, but in different extent and sequence: immediate extensive surgery, step by step surgery, and only proximal realignment. Their overall functional scores as well as major symptoms were assessed at the beginning, after the surgery, and during the 3-years follow-up period and then, compared at the end. **Results.** There was no significant difference in the functional results among the groups, neither at the beginning ($p = 0.1318$) nor at the end of the study ($p = 0.3996$), but the results at the beginning compared to those at the end of the study showed a statistically significant difference in all three groups ($p1 = 0.005062$; $p2 = 0.011719$; $p3 = 0.000352$). The same result was in regard to the major symptoms. **Conclusion.** The study confirmed that insisting on immediate extensive surgery in order to achieve precise and complete congruency of the patellofemoral joint, did not prove its advantage over the less invasive, individual surgical approach concerning functional scores and major symptoms.

Key words:
patellofemoral pain syndrome; surgical procedures, operative; recovery of function.

Apstrakt

Uvod/cilj. Ova prospektivna nerandomizovana studija rađena je sa ciljem da se u lečenju simptomatske patelofemoralne inkongruencije nepodudarnosti uporede funkcionalni rezultati postignuti različitim hirurškim strategijama. Radna hipoteza bila je da se neposredom ekstenzivnom hirurgijom ne postižu značajno bolji rezultati u odnosu na dva glavna simptoma i krajnji funkcionalni skor u poređenju sa umerenijom hirurškom strategijom. Nameravali smo da proverimo da li je postizanje idealnih geometrijskih odnosa u patelofemoralnom zglobu opsežnom hirurgijom neophodno za rešavanje glavnih simptoma patelofemoralne inokngruencije. **Metode.** Studija je obuhvatila ukupno 35 bolesnika sa patelofemoralnom inkongruencijom, kod kojih su i posle tri meseca neoperativnog lečenja i dalje postojali glavni simptomi: bol u prednjem delu kolena i osećaj isklizavanja patele. Svi bolesnici su podvrgnuti hirurškim centražnim procedurama, ali su u zavisnosti od obima i redosleda tih intervencija podeljeni u tri grupe: prva sa jednokratnom ekstenzivnom hirurgijom, druga, gde su proksimalne i distalne centražne procedure vremenski razdvojene i treća kod koje su rađene samo proksimalne procedure. Funkcionlni rezultati, kao i dva glavna simptoma, ocenjivani su na početku lečenja, posle poslednje hirurške intervencije i tokom trogodišnjeg praćenja, i na kraju međusobno upoređeni. **Rezultati.** U pogledu funkcionalnih rezultata, između grupa nije bilo statistički značajne razlike ni na početku

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($p = 0.1318$), ni na kraju ($p = 0.3996$) lečenja, ali su u sve tri grupe postojale statistički visoko značajne razlike pri poređenju rezultata na početku, sa onim na kraju lečenja ($p1 = 0.005062$; $p2 = 0.011719$; $p3 = 0.000352$). Isti odnos dobijen je i za dva glavna simptoma. **Zaključak.** Studija je pokazala da u hirurškom lečenju simptomatske patelofemoralne inkongruencije, u pogledu funkcionalnih rezultata i rešavanja dva osnovna simptoma, postizanje idealnih odnosa u patelofemoralnom zglobu jednokratnom ekstenzivnom hirurgijom ne donosi značajnu prednost u poređenju sa manje invazivnim, sekvencijalnim, individualnim hirurškim pristupom.

Ključne reči:
patelofemoralni bolni sindrom; hirurgija, operativne procedure; funkcija, povratak.

Introduction

Malalignment of the patellofemoral joint, besides morphological, involves also a dynamic incongruence of the joint surfaces of trochlea and patella. The dynamic aspect of the patellar malalignment, according to Grelsamer [1], is presented by translational (subluxation) and/or rotational (tilt) deviation of the patella related to one of patellar axis. There are two major symptoms of the patellofemoral malalignment: patellar slipping and anterior knee pain. Patellar slipping that occurs during flexion and extension of the knee, sometimes also presented as catching and pseudo locking, is regarded as a subjective interpretation of the clinical phenomena known as patellar subluxation. Both morphologic and dynamic disorders of the patellofemoral joint, in various reciprocal relations, represent the source of patellar slipping. Those disorders could be constitutional, acquired or the combination of those two [2,3]. The anterior knee pain that originates from the patellofemoral joint is controversial in many ways. Patellofemoral malalignment is one of the principal etiologic factors for that pain, but certainly not the only one. Histological and functional changes in small parapatellar nerves, especially of the lateral retinaculum, also contribute substantially to occurrence of the pain syndrome [4]. Degenerative changes in subchondral bone as well as fibrous synovial plicae of the knee are also responsible for the pain. Some authors hypothesize that a number of patients might have individual trigger for the onset of the pain [1].

Treatment of the symptomatic patellofemoral malalignment should always start nonoperatively [5]. The cornerstone of this treatment is a physical therapy, based on knee extensor and hip abductor muscles strengthening, appropriate patella bracing and taping, foot orthotics, and modification of life activities associated with bending of the knee. Although it obviously does not address the knee extensor alignment significantly, forementioned therapy shows good results in terms of subsidence and sometimes complete elimination of the major complaints [2, 6, 7]. Unacceptable result of the nonoperative treatment, that was performed not less than three months continually, leads to preparation for the surgery [8]. Surgical treatment of the symptomatic patellofemoral malalignment is mainly based on the knee extensor realignment. Proximal alignment procedures are focused on passive and dynamic balance of the parapatellar soft tissues: medial and lateral retinaculum as well as muscles attached to the patella. Those procedures could be combined mutually as well as with distal ones, performed either immediately or step by step. Since rotational component of patellar malalignment tilts patella laterally with consecutive shortening of lateral retinaculum, proximal alignment presumes open or arthroscopic lateral release [9]. On the opposite side, reparation, reefing and reinforcing of the medial patellofemoral ligament (MPFL) is common in the acute and subacute disorders either arthroscopicaly [10, 11] , or as an open surgery [12,13], whilst in chronic cases, reconstruction of the MPFL is regarded as standard procedure, sometimes with advancing distal fibers of the *vastus medialis obliquus* muscle [14–16]. Attitude to immediate lateral release and reparation or reconstruction of the MPFL, according to literature, is noticeably different and obviously controversial [17–20]. Distal alignment procedures have represented basis of the surgical treatment of patellofemoral malalignment since 1938 when Hauser [21] introduced his technique of tibial tubercle transfer. They are mainly aimed to correct the Q angle, and, to a certain extent, patellar height and contact area between patella and trochlea. It presumes translocation of the tibial tubercle together with patellar ligament insertion, or partial translocation of the ligament, performed as a classic open surgical procedure [22, 23]. Besides the knee extensor, the surgical treatment of the patellar malalignment could be focused on deepening the trochlear sulcus [24], removal or refixation of the loose osteocartilaginous body, high tibial osteotomy to correct varus/valgus knee alignment, removal of synovial tissue, and peripatellar soft tissues denervation. The combination of proximal and distal procedures as well as arthroscopic and open surgery enables immediate and precise correction of marked patellofemoral malalignment and accomplishment of almost ideal congruency. Despite that fact, in the reviewed literature, postoperative outcomes, concerning patellar pain, slipping and overall functional results, apparently were not always ideal [18, 24–27]. On the other hand, nonoperative treatment, even without noticeable correction of the alignment, in a number of patients, establishes good functional control of the knee extensor, significant reduction and sometimes even complete subsidence of the major symptoms. In some cases, where surgical treatment of patellofemoral malalignment was planned as two steps procedure (first arthroscopic or arthroscopicaly assisted proximal alignment, and second distal procedure), even proximal alignment alone, significantly decreased or completely eliminated major symptoms, providing also acceptable and good functional results. Long-term follow-up of those patients showed that, in most of the cases, there was no need for further surgical treatment, besides the fact that, neither clinically nor using imaging methods, physiological patellofemoral alignment and congruency were not achieved.

The aim of this study was to test whether establishing ideal geometrical congruency and alignment of the patellofemoral joint by means of extensive one-stage surgery, provided a significant advantage in treatment of the symptomatic patellofemoral malalignment. We compared extensive one-stage surgery with two other, less invasive surgical strategies, evaluating their functional results and improvements of the major symptoms.

Methods

This prospective nonrandomized study included 35 patients with patellofemoral malalignment and complaint on anterior knee pain and patellar slipping, without improvement of the symptoms after a 3-month program of nonoperative treatment. After the program, their two major symptoms were both still present and evaluated according to the Tegner and Lysholm scale [28]: for the anterior knee pain each of them scored less than 15 (max. 25), and for the patellar slipping less than 10 points (max. 15). Other inclusion criteria were presence of at least two out of 3 quantitative factors of patellofemoral malalignment [29]: "Q angle", according to Brattstroem [30], higher than 15°, "Laurin angle" (measure of patelar tilt) less than 20°, and "Merchant's angle" (measure of patellar subluxation) more than +6°. The positive clinical test of provoked patellofemoral pain [31] was also obligatory. The exclusion criteria were: previous knee surgery, bilateral knee symptoms and x-ray signs of arthritis of the patellofemoral joint.

There were 28 female, and 7 male patients, whose age ranged from 16 to 46 years (mean 28.9). Initially, the patients were divided into two groups. In the first group, there were 10 patients, all of them with both numerical predictors of patellar subluxation: Q angle more than 15°, and Merchant's angle more than +6°. For that reason, those 10 patients underwent immediate proximal and distal alignments. In the second group, with the rest of 25 patients, we planned to perform the same surgical scenario, but in two separate steps, at least 4 months apart: first step arthroscopic or arthroscopicaly assisted proximal alignment, and second – open distal realignment. But four months after the proximal alignment, 17 of those 25 patients were satisfied with the result. In all those cases, functional Tegner-Lysholm score exceeded 65 points and assessment of the patellar pain and slipping showed at least "good" results, so, further surgical treatment was stopped. Therefore, 4 months after the proximal alignment, the patients from the initial second group were subdivided into two more groups, those 17 whose surgical treatment was terminated and the rest of 8 who underwent delayed distal alignment. Finally, the study had three groups of patients: the first (10 patients) with immediate proximal and distal alignment, the second (8 patients) where proximal and distal alignment were performed in two steps four months apart from each other and the third (17 patients) where only proximal alignment was performed. For the assessment of functional status of their knees and major symptoms, we had to use one of the validated knee scoring systems. Most of the available functional knee scores are designed for specific pathology of the knee joint. Yet, some of them are modified, so that they could be used more extensively. The Kujala Anterior Knee Pain Scale [32] is designed for patellofemoral disorders, but since it includes some of the activities such as running and jumping, that most of our patients excluded from their everyday activities even before the onset of symptoms we decided to go for the Tegner-Lysholm Knee Scoring Scale, a modification of the classic Lysholm-Gillquist knee test [33], that was more appropriate for usual activities of our patients. The Tegner and Lysholm test utilizes eight major symptoms, findings and activities related to the knee, predominantly patellofemoral part, for evaluation of its functional status with maximal score of 100 points. The score is graded as follows: less than 65 is poor, 65–83 fair, 84–90 good and more than 90 is excellent. The first measurement was made after completion of physical therapy (before the surgery), the second – 4 months after the surgery, the third – a year later, and the fourth – 3 years later, with the total follow-up of 40 months after the surgical treatment.

By means of the Kolmogorov-Smirnov test, we confirmed that data obtained during the study did not belong to a normal distribution. Consequently, statistic analysis was accomplished using the nonparametric tests for rank analysis: Kruskal-Wallis *H* test and Wilcoxon signed rank *T* test. We used statistic program "Statistics 6.0 by StatSoft Inc". To assess whether our groups at the beginning as well as at the end of our study originated from the same distribution regarding either functional status or major symptoms, we used the Kruskal-Wallis test by ranks. For that test, the *p* values less than 0.05 indicated that differences among the groups were so large that they were unlikely to occurred by chance.

To compare functional results and major symptoms at the beginning to those findings at the end of the treatment, we used the Wilcoxon signed rank test for comparing two related, matched samples. We used the same Wilcoxon test for our repeated measurements, to compare every two subsequent phases of treatment, to estimate uniformity of improvement during the treatment. For both purposes, the Wilcoxon test was significant if the *p* values were less than 0.05.

Results

All 35 patients included in this study were divided into three groups in order to compare their functional results and major symptoms (patellar pain and slipping) during the follow-up period, using the Tegner and Lysholm scale. Basic statistic parameters of the obtained functional results are shown throughout the groups and all four measurements completed during follow-up (Table 1).

Mutual comparison of the groups, based on the results of the functional scores of the patients before any operative treatment, showed that there was no statistically significant difference between the groups at the beginning of the study according to the Kruskal-Wallis *H* test by ranks ($n = 35$; $df = 2$; $H = 4.05346$; $p = 0.1318$), providing hence a sound basis for evaluation of different modalities of treatment. At the end of our follow-up, functional status between the groups was also compared using the same Kruskal-Wallis *H* test again, without significant statistical difference between them ($N = 35$; $df = 2$; $H = 1.834619$; $p = 0.3996$).

Table 1

Functional status of all 3 groups throughout all 4 measurements

Group	FSM[1]	n	Median	Min.	Max.	25%	75%
1st	FSM 1	10	66.00	33.00	73.00	60.00	69.00
	FSM 2	10	77.50	63.00	86.00	73.00	82.00
	FSM 3	10	84.00	75.00	91.00	80.00	88.00
	FSM 4	10	83.50	81.00	91.00	83.00	88.00
2nd	FSM 1	8	65.00	55.00	76.00	61.50	71.50
	FSM 2	8	76.00	45.00	83.00	73.00	80.50
	FSM 3	8	82.50	63.00	92.00	76.50	86.50
	FSM 4	8	83.00	65.00	96.00	76.00	90.00
3rd	FSM 1	17	71.00	48.00	81.00	65.00	75.00
	FSM 2	17	78.00	55.00	85.00	72.00	80.00
	FSM 3	17	83.00	62.00	97.00	78.00	88.00
	FSM 4	17	89.00	62.00	97.00	80.00	93.00

FSM – functional score measurement; FSM 1 – preoperative; FSM 2 – 4 months after the surgery; FSM 3 – 1 year later; FSM 4 – 3 years later; n – sample size.

On the other hand, the functional results of all analysed patients in all three groups at the beginning, compared to those at the end of the treatment (40 months after the last operation), using the Wilcoxon matched pair test, showed a statistically significant difference (Table 2).

It is confirmed that all three surgical strategies applied in this study resulted in a significant functional improvement.

Using the same nonparametric analysis of the ranks (Wilcoxon test) where the matched pairs were successive measurements of the functional scores in each of the three groups of our patients, we have tested functional status between every two successive steps of the treatment, to estimate whether the improvement was smooth and consistent during the whole observed period, or irregular, limited to some of the phases (Table 3). To do so, we divided the whole follow-up period in three phases of the treatment as follows: first (I), from the preoperative measurement until 4 months after the last operation, second (II), beginning 4 months after the last operation and the year ahead and third (III) between 1 and 3 years after the second measurement.

In the 1st group, statistically significant changes of the functional scores occurred during first two phases of the treatment ($p < 0.05$), while, in the third, there was no significant change.

The median values in the first two groups (Table 1) altered to the same direction: showing an increase during the first two phases and stagnation during the third.

In the 3rd group, statistically significant changes in the functional scores were noticed during each phase of the treatment: improvement was consistent throughout the whole observed treatment. A continual increase of the median values in this group, shown in Table 1, were in accordance with the conclusion.

In the 2nd group, a statistically significant change in the functional score occurred only in the second phase ($p < 0.05$), while in the first, the p values were close to indicate a significant (0.068), and in the third phase there was no significant change.

Table 2

Comparison of functional results at the beginning to those at the end of the study

Group of patients	Period (months)	n	T	Z	*p*
1st	0–40	10	0.00	2.803060	0.005062
2nd	0–40	8	0.00	2.520504	0.011719
3rd	0–40	17	1.00	3.574027	0.000352

Wilcoxon matched pair test: statistically significant for $p < 0.05$; n – sample size; T – referent critical value; Z – standard score; *p* – probability.

Table 3

Comparison of functional results between every two successive steps of treatment

Group	Phase of treatment	n	T	Z	*p*
1st	I	10	0.00	2.80	0.005062
	II	10	0.00	2.80	0.005062
	III	10	15.00	0.89	0.374260
2nd	I	8	5.00	1.82	0.068704
	II	8	0.00	2.37	0.017961
	III	8	6.50	0.84	0.401679
3rd	I	17	12.00	2.54	0.011008
	II	17	0.00	3.62	0.000293
	III	17	4.00	3.18	0.001470

Wilcoxon matched pair test: statistically significant for $p < 0.05$; n – sample size; T – referent critical value; Z – standard score; *p* – probability.

Summing up the data, in the first two groups, we obtained similar results. A significant change of the functional score occurred after the extensive surgery and lasted until the end of first postoperative year, followed by flat, insignificant alteration during the third phase. In the third group we had even-handed, significant improvement during all three phases.

Two most important symptoms in patellofemoral malalignment, patellar pain and slipping, were observed separately. Basic satistic parameters of the major symptoms, in all three groups, preoperatively and during follow up, are shown in Tables 4 and 5.

Median values for patellar slipping, showed the most impressive and constant increase during the whole follow-up period and reached maximum only in the first group in which the patients underwent immediate extensive surgery (Table 4). On the contrary, the median values for the patellar pain were almost equal between all groups, with the even and slow increase, but non of them reached maximum (Table 5).

Comparison between the groups at the beginning of the treatment regarding both symptoms, using rank analysis (Kruskal-Wallis *H* test), showed no statistical difference (for pain: n = 35 df = 2; $H = 2.534385$, $p = 0.2816$, and n = 35 df = 2; $H = 2.461491$, $p = 0.2921$ for patellar slipping). The same results came at the end of the treatment: there was no statistically significant difference neither for pain nor patellar slipping (n = 35; df = 2; $H = 1.032605$, $p = 0.5967$ and n = 35; df = 2; $H = 1.642500$, $p = 0.4399$, respectively). So, concerning two major symptoms of patellofemoral malalignment, before our treatment, all three groups were equivalent, which was a good starting point for later comparison. At the end of the treatment, the results related to the patellar pain and slipping between all groups, also belonged to the same distribution pattern.

Comparison of the results, in the beginning and at the end of the treatment, for both symptoms, patellar pain and slipping, in all patients and groups, using the nonparametric rank analysis for successive measurements (the Wilcoxon matched pairs test), showed statistically significant differences: for pain $p = 0.000005$ (n = 35; $T = 11.00$; $Z = 4.555887$), and for slipping $p = 0.000015$ (n = 35; $T = 13.00$; $Z = 4.326570$). This confirmed equal results of the treatment, concerning both essential symptoms, regardless of surgery strategy.

Table 4

Patellar slipping in all three groups throughout the follow-up measurements

Group	PSSM	n	Median	Min.	Max.	25%	75%
1st	PSSM 1	10	6.00	2.00	10.00	6.00	6.00
	PSSM 2	10	10.00	10.00	10.00	10.00	10.00
	PSSM 3	10	12.50	10.00	15.00	10.00	15.00
	PSSM 4	10	15.00	10.00	15.00	10.00	15.00
2nd	PSSM 1	8	8.00	6.00	10.00	6.00	10.00
	PSSM 2	8	10.00	10.00	15.00	10.00	10.00
	PSSM 3	8	12.50	10.00	15.00	10.00	15.00
	PSSM 4	8	10.00	10.00	15.00	10.00	15.00
3rd	PSSM 1	17	6.00	2.00	15.00	6.00	10.00
	PSSM 2	17	10.00	6.00	15.00	10.00	10.00
	PSSM 3	17	10.00	10.00	15.00	10.00	15.00
	PSSM 4	17	10.00	10.00	15.00	10.00	15.00

Patellar slipping score measurement (PSSM): PSSM 1 – preoperative; PSSM 2 – 4 months after the surgery; PSSM 3 – 1 year later; PSSM 4 – 3 years later.

Table 5

Patellar pain in all three groups throughout the follow-up measurements

Group	PSM	n	Median	Min.	Max.	25%	75%
1st	PSM 1	10	10.00	5.00	20.00	10.00	10.00
	PSM 2	10	15.00	10.00	20.00	10.00	15.00
	PSM 3	10	15.00	15.00	20.00	15.00	20.00
	PSM 4	10	15.50	10.00	20.00	15.00	20.00
2nd	PSM 1	8	10.00	5.00	10.00	5.00	10.00
	PSM 2	8	12.50	5.00	15.00	10.00	15.00
	PSM 3	8	15.00	5.00	15.00	10.00	15.00
	PSM 4	8	15.00	5.00	20.00	12.50	15.00
3rd	PSM 1	17	10.00	0.00	15.00	5.00	10.00
	PSM 2	17	10.00	0.00	20.00	10.00	15.00
	PSM 3	17	15.00	0.00	25.00	10.00	15.00
	PSM 4	17	15.00	0.00	25.00	10.00	20.00

Pain score measurement (PSM): PSM 1 – preoperative; PSM 2 – 4 months after the surgery; PSM 3 – 1 year later; PSM 4 – 3 years later.

Discussion

Combinations of various surgical techniques applied on different levels of extensor apparatus of the knee as well as within the patellofemoral joint, using up to date diagnostic and surgical devices, enables complete correction of almost all diagnosed types of patellofemoral malalignment. However, postoperative functional scores, symptoms and objective findings were not equally satisfactory: some of widely accepted surgical techniques, after prolonged follow-up, showed disadvantages [25–27]. The other ones did not show major improvement over the nonoperatively treated patients [18], and some studies report significant differences between subjective records and objective findings [24]. Therefore, some authors advocate restricted and highly controlled surgery after precise definition of origin of the particular disorder [34].

In this study, the surgical treatment of the symptomatic patellofemoral malalignment included standard methods of proximal and distal alignment of the extensor apparatus of the knee. In all three groups, as the first surgical step, patients were submitted to arthroscopic, or arthroscopically assisted proximal alignment. The difference between the groups, besides geometric parameters of the patellofemoral joint and extensor apparatus of the knee, was also quantity of operations, and time frame of successive surgical procedures.

Comparison of the treatment results was based on assessment of the knee functional score of each patient as well as two major symptoms: patellar pain and slipping.

Since the functional score of the patients of all three groups was assessed preoperatively as equal, changes in the score at the end of the follow-up, could be regarded as outcome of the treatment. Statistically significant difference between functional scores at the end of the follow-up compared to the beginning of treatment, in all observed patients, pointed out equal end results of all 3 modalities of surgical treatment applied in this study. However, comparison of the functional scores within the groups, between succeeding phases of the treatment, showed a discontinuous increase in the first two groups where more extensive surgery was applied: during the first and second phase of treatment, which included period from the surgery until one year afterwards, the improvement was considerable unlike the third phase, which represented a period from 1 until 3 years postoperatively. So, in the first and second group, we achieved the successful functional results more rapidly than in the third group in which the amount of surgery was substantially smaller. But having in mind that at the end of the follow-up, the functional scores among all three groups did not show statistically significant difference and more rapid improvement of function after extensive surgery, it could be concluded that it did not provide better end functional result. Furthermore, the median values of the functional scores, at the end of the treatment, were the highest in the third group, where the least invasive surgical strategy was applied.

Launching definitive conclusions regarding results of surgical treatment of symptomatic patellofemoral malalignment and patellar instability, based only on evidence of the total functional score, might lead to inaccurate assessment of the treatment. Several articles confirm that, especially in cases with marked patellar instability, in spite of significant improvement of the functional score of the knee after the surgery, percentage of persistent instability was unacceptably high [18, 19, 35]. For that reason, in this study, besides the functional status, we tested separately two major symptoms of patellofemoral malalignment, patellar pain and slipping. There were no statistically significant differences concerning those two symptoms, among the observed groups, neither at the beginning nor at the end of the follow-up. Yet, patellar slipping, basically a biomechanical symptom, according to the median values, definitely showed more significant improvement after the extensive immediate surgery than in the second and third group. On the other hand, a pattern of the median values increase for the second major symptom, the pain, during the overall follow-up, was almost equal in all three groups. Nevertheless, all observed patients within all three groups, showed statistically significant difference and improvement, for both symptoms at the end of the follow-up, comparing to the beginning of the treatment. So, all three surgical strategies for the treatment of symptomatic patellofemoral malalignment, applied in this study, ended up with equal success concerning two major symptoms of the disorder.

Conclusion

The results obtained in this study proved that extensive surgery in the treatment of symptomatic patellofemoral malalignment did not confirm decisive role. On the other hand, the persistent and equally good functional results, after prolonged follow-up, were obtained in the patients who underwent only arthroscopic or arthroscopically assisted proximal realignment in the “step by step” surgery without insisting on immediate ideal congruency. Therefore, if a complex and extensive surgery, based on objective criteria, seems to be inevitable, two steps surgery should be considered by all means.

This study also implicates that achieving morphological and dynamic congruency of the patellofemoral joint in a surgical treatment of symptomatic patellofemoral malalignment, using combined proximal and distal procedures, gives substantial improvement of functional results promptly, but may not always result in complete solving of major symptoms. Nevertheless, equally good results in a surgical treatment of the same disorder could be obtained without insisting on ideal congruency.

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Relationship between increased body weight and oral health in children

Povećana telesna masa i oralno zdravlje dece

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Abstract

Backgraund/Aim. Increased body weight in childhood may have negative effects on many tissues and organs in the body. The aim of this study was to determine whether the state of oral health in children with increased body mass is different from the children with normal body weight. **Methods.** The study included 190 children, aged 6 to 15 years. Assessment of nutritional status of subjects was performed by the use of Body Mass, and the respondents were divided into a group of increased body weight children (IWC) and a group of normal body weight children (NWC). Hard dental tissue state of health was assessed by the decayed, missing and filled teeth (DMFT) index. The gingival health was assessed using gingival index (GI). Community Periodontal Index (CPI) was used for the assessment of periodontal tissue. The oral hygiene was assessed using the Simplified Debris (Plaque) Index Greene-Vermilion. **Results.** Average DMFT value in the IWC group was 5.01 ± 2.4, and in the NWC 4.43 ± 2.0; ($p > 0.05$). GI values in the IWC group was 0.64 ± 0.37, while in the NWC group it was 0.55 ± 0.35 (the difference was not statistically significant). Average CPI index values were 1.33 ± 0.49 in the IWC group and 0.77 ± 0.61 in the NWC group and statistically significant differences were observed concerning periodontal tissue state of health. The GV index values in the IWC group were 1.01 ± 0.49, and in the NWC group 0.89 ± 0.45; it was not statistically significant. **Conclusion.** Results of this research do not indicate that children with increased body weight have more affected teeth. However, they have a worse condition of periodontal tissue in comparison to normal weight children.

Key words:
body weight; child; body mass index; obesity; periodontal index; oral hygiene; tooth.

Apstrakt

Uvod/Cilj. Povećana telesna masa u dečijem uzrastu može negativno da utiče na mnoga tkiva i organe u organizmu čoveka. Cilj ove studije bio je da se ustanovi da li se stanje oralnog zdravlja dece sa povećanom telesnom masom razlikuje u odnosu na decu koja imaju normalnu telesnu masu. **Metode.** U studiju je bilo uključeno190 dece, uzrasta od 6 do 15 godina. Procena stepena uhranjenosti ispitanika izvršena je pomoću Indeksa telesne mase, a ispitanici su podeljeni u grupu dece sa povećanom telesnom masom i decu sa normalnom telesnom masom. Stanje zdravlja tvrdih zubnih tkiva ocenjeno je pomoću KEP indeksa (ukupan broj karoiznih, ekstrahovanih i plombiranih zuba). Stanje zdravlja gingive procenjeno je pomoću loe Gingivalnog indeksa (GI). Za procenu parodontalnih tkiva upotrebljen je *Community Periodontal Index* (CPI). Stanje oralne higijene utvđeno je uz pomoć Plak indeksa po Greene-Vermilion-u. **Rezultati.** U grupi djece sa povećanom telesnom masom prosečna vrednost indeksa KEP bila je 5,01 ± 2,4 a kod dece sa normalnom telesnom masom 4,43 ± 2,0; ova razlika nije bila statistički značajna. Vrednost GI u grupi gojazne dece bila je 0,64 ± 0,37, a u grupi dece sa normalnom telesnom masom 0,55 ± 0,35; ni ova razlika nije bila statistički značajna. Prosečne vrednosti CPI u grupi gojazne dece bila je 1,33 ± 0,49, a u grupi dece sa normalnom telesnom masom 0,77 ± 0,61 što je bilo statistički značajno ($p < 0.05$). Vrednosti plak-indeksa nisu bile statistički značajno različitie u obe ispitivane grupe dece. **Zaključak.** Rezultati istraživanja ne ukazuju da deca sa povećanom telesnom masom imaju više obolelih zuba. Međutim, ona imaju teži stepen obolenja parodontalnih tkiva u odnosu na decu koja imaju normalnu telesnu masu.

Ključne reči:
telesna težina; deca; telesna masa, indeks; gojaznost; periodontalni indeks; usta, higijena; zub.

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Introduction

Excessive nutrition is a growing problem in modern age population. Excess body weight has a negative impact on many tissues and organs in human body. Excessive nutritional status in childhood is one of the main risk factors that may lead to the occurrence of obesity in adulthood. The World Health Organization (WHO) defines obesity as abnormal or increased accumulation of fat in the adipose tissue to the extent that damages health and leads to the development of numerous health complications [1].

Obesity may occur in all age groups and is increasingly evident in children. In 2006, the European Commission reported that 22 million of children within the European Union are overweight or obese, with an increase in incidence of 400,000 new cases per year [2]. It can be said that obesity presents a chronic health problem that reduces quality of life and has a significant impact on morbidity and mortality [3]. In obesity, uncontrolled chronic inflammation of low intensity keeps the body in a constant state of stress, which leads to peripheral tissues insulin insensitivity, insulin secretion disorders, underutilization of glucose by the liver and muscles, what may further lead to metabolic disorders development, damage to the heart and blood vessels as well as locomotor apparatus [3].

Lifestyle factors such as a lack of physical activity, changes in eating habits and social changes, have been considered as crucial factors for the global spread of obesity [4] and they, at the same time, present risk factors for oral diseases occurrence, too. In order to reduce the obesity incidence, it is necessary to promote and improve coordination between various primary health care services as a priority. Contacts between pediatric dentists and patients are relatively common; therefore, dentists are able to identify obesity risk patients even at the early age [5]. Many previous studies that have investigated the impact of obesity on population general health, suggest that everyone responsible for primary care of children (pediatricians, dentists and dental hygienists) should join together to achieve a common goal [6–8].

Literature generally provides data about impact of obesity on the general health, while data about impact of obesity on the teeth, gingiva and periodontal tissue states of health are very scarce. As oral health is an important part of overall health, the aim of this study was to determine whether oral health of increased body weight children is different from that of normal body weight children.

Methods

The study was designed as an academic cross sectional study. The study included 190 subjects (122 boys and 68 girls) aged 6 to 15 years. Children from four cities in the Republic of Srpska, Bosnia and Herzegovina (Višegrad, Rogatica, Čajniče, Foča) were examined in this study. The study was conducted in accordance with the Declaration of Helsinki and principles of Good Clinical Practice. Parents were informed about the purpose of the study and signed an informed consent form. The study protocol was approved by the Ethical Committee of the Faculty of Medicine, University of East Sarajevo (No. 01-8/38).

All participants underwent dental examination with the use of standard dental diagnostic instruments. Dental caries was determined by the DMFT index (decayed, missed, filled teeth) for permanent dentition, according to the World Health Organization (WHO) standards [9]. The gingival health was assessed using the gingival index (GI), as described by Löe [10]. Community Periodontal Index (CPI) was used for periodontal tissues state of health assessment [9]. As participants of this study were less than eighteen years old, modified version of this index, corresponding to their age, was used. The oral hygiene was assessed using the Simplified Debris (Plaque) Index Greene Vermilion (GV) [11]. Since the all the aforementioned indices cannot be applied to deciduous dentition, in accordance with the determining criteria, only permanent teeth were assessed in this study and state of health of permanent dentition was analyzed.

Assessment of nutritional state of the respondents was conducted using Body Mass Index (BMI). All participants underwent basic anthropological examination, i.e., body weight (kg) was measured and height (cm), on the basis of which BMI was determined. BMI was calculated as the body weight (in kg) divided by the square of height (in meters). Percentiles defined the position of a particular index value compared to children and adolescents of the same sex and age. According to the WHO growth references for children and adolescents, BMI between the 5% and 85% was categorized as "normal weight" children and adolescents, those with a BMI between the 85% and 95% were classed as "at risk of overweight", and those with a BMI greater than the 95% as "overweight" [12–14]. The children and adolescents with BMI < 5% were excluded from this study. Based on the BMI value, the respondents of this study were evenly divided into two groups: children with increased body weight (IWC) and children with normal body weight (NWC). The IWC group consisted of 95 patients (61 boys and 34 girls) with increased body weight (BMI > 85%) who visited the Pediatric Dental Clinic, the Faculty of Medicine in Foča, or the Pediatric Ward of the University Hospital in Foča. The NWC group consisted of the same number of normal weight respondents (BMI 5–85%), which were matched with the IWC group by gender, age and city they lived in. Both groups included children without any chronic or systemic disease.

Methods of descriptive and analytical statistics were used for results description. The used descriptive statistics method parameters were: mean value, standard deviation and percentages. The differences of individual parameters between and within the studied groups, depending on the distribution, were tested with the Mann-Whitney test, Fisher test and χ^2-test. The statistical data analysis was performed in SPSS 11.5 (SPSS Inc., Chicago, IL, USA). The obtained results were presented in Tables, with the level of significance set at 0.05.

Results

The study included more boys (64.2%) than girls (35.8%), that was of highly statistically significant difference ($p < 0.05$; χ^2-test). The average age of the IWC group was 10.7 ± 1.6 years, while the average age of the NWC group was 10.2 ± 1.4 years. No statistically significant difference

between the groups was observed related to the age structure ($p > 0.05$, χ^2-test).

In the overall sample, 16.04% of children had all permanent teeth healthy. In the IWC group, percentage of healthy permanent teeth was 15.75%, while 17.84% teeth were in the NWC group; the difference between the groups was not statistically significant ($p > 0.05$, χ^2-test).

The average value of DMFT for the entire population studied was 4.64. In the IWC group average value of DMFT was 5.01 ± 2.4 while in the NWC group the index value was 4.43 ± 2.0. The difference between observed groups was not statistically significant ($p > 0.05$; χ^2-test). DMFT index distribution is presented in Table 1.

Table 1

Structure of DMFT in the study groups

Index	Parameters of DMFT	IWC (%)	NWC (%)
DMFT	D	60.0*	52.8*
	M	9.5	11.9
	F	30.4	34.9

IWC – increased body weight children; NWC – normal body weight children; DMFT – decayed, missing, filled teeth; *$p < 0.05$, Mann-Whitney test.

Concerning percentage of decayed teeth (%D), the results showed that children in the IWC group had a higher percentage of decayed teeth compared to children in the NWC group, which was significant difference ($p < 0.05$; Mann-Whitney test). The maximum number of decayed teeth in one respondent from the IWC group was 13 teeth, while their number in the NWC group was 9 teeth. The average number of untreated teeth per respondent was 2.31 in the IWC group, and 1.58 teeth in the NWC group. When observing percentage of extracted teeth (%E), results showed that the percentage of extracted teeth was slightly higher in the NWC group. The maximum number of extracted teeth at one respondent in the IWC group was 3 teeth, while that number in the NWC group was 7 teeth. The average number of extracted teeth per respondent was 0.23 in the NWC group and 0.19 in the IWC group. The percentage of filled teeth (%F) was higher in the NWC group. The maximum number of filled teeth at one respondent of the IWC group was 12 while that number in the NWC group was 15. In the IWC group, the average number of tooth fillings per respondent was 1.68 while that number in the NWC group was larger and was 1.79. Statistically significant differences were not observed in relation to %E and %F teeth between the two study groups ($p > 0.05$; Mann-Whitney test).

The average value of the GI for all respondents was 0.60 ± 0.36. GI values were slightly higher in the IWC group 0.64 ± 0.37 compared to the NWC group of respondents (0.55 ± 0.35), but the difference between the groups was not statistically significant (Table 2). The respondents from both groups, in the highest percentage, had mildly inflamed gingiva. In this study, there were no patients with severely inflamed gingiva because the maximum value of the index was 1.71 which corresponds to moderate inflammation.

Table 2

Gingival state of health (gingival index – GI) [10] in the study groups

GI	IWC (%)	NWC (%)
Normal gingiva	31.9	30
Mild inflammation	64.5	66.8
Moderate inflammation	3.6	3.2
Severe inflammation	0	0

IWC – increased body weight children; NWC – normal body weight children.

The average value of the CPI for all respondents was 1.04 ± 0.57. The average values of this index were higher in the IWC group 1.33 ± 0.49 compared to the NWC group of the respondents (0.77 ± 0.61). There was a statistically significant difference when it comes to the periodontal tissue state of health between the groups ($p < 0.05$; Mann-Whitney test). Distribution CPI index percentages are presented in Table 3. Calculus deposits were more frequently observed in the IWC children than in the normal body weight children.

Table 3

Community periodontal index (CPI) distribution in the study groups

CPI	IWC (%)	NWC (%)
0	38.3	62.2
1	54.5*	31.5*
2	17.2*	6.3*

CPI – community periodontal index: 0 – healthy periodontium; 1 – gingival bleeding after probing; 2 – calculus; IWC – increased body weight children; NWC – normal body weight children.
***$p < 0.05$, Mann-Whitney test.**

The average value of soft debris index (Greene-Vermillion, GV), for all subjects involved in this study, was 0.95 ± 0.48. The obtained values indicate that soft deposits along the marginal edge of the gingiva were present in most respondents from both the observed groups. The values of this index in the children with increased body weight were 1.01 ± 0.49, and 0.89 ± 0.45 in the normal body weight children, but statistically significant difference between the groups was not observed ($p > 0.05$; Mann-Whitney test). Distribution of the GV percentages in the observed groups is given in Table 4.

Table 4

Oral hygiene presented by GV distribution in the study groups

Hygiene	IWC (%)	NWC (%)
Excellent	11	12.4
Good	31.3	31.6
Fair	48.7	50.4
Poor	9	8.8

IWC – increased body weight children; NWC – normal body weight children; GV – simplified debris (plaque) Greene-Vermilion.

Discussion

This study investigated the relationship between increased body weight in children and state of permanent dentition health. Oral health affects the proper development of orofacial system, chewing, speech and swallowing functions, and has a great importance to the psychological and social aspects of human life. Also, it has a significant impact on the aesthetic appearance and therefore a sense of personal satisfaction [15]. Although obesity and oral health are globally leading health problems in children and adolescents, possible relationship between obesity and the periodontal status or frequency of caries in children has been neglected [16].

Excessive nutritional status and oral diseases count as multifactorial diseases due to the fact that they have a common „risk factors". For example, both diseases are associated with negative eating habits, with lower economic status and irresponsibility toward personal health [17].

The results of our study indicate that about 16% of the respondents had all permanent teeth healthy. Obtained data is worrying when having in mind that respondents were up to 15 years old, their permanent teeth have relatively recently emerged or even just formed at that age. The average score of DMFT per respondent was 4.64, indicating that we are still far from the objectives set by the WHO.

When observing the average DMFT index values between the observed groups, results of this study did not indicate association between excessive nutrition and affected teeth in children. However, after analyzing the individual DMFT components, it was observed that the %D was more prevalent in children with increased body weight compared to normal body weight children and the difference between the groups was statistically significant. The results of this study are consistent with previous research conducted by Sharma and Hegde [18].

Review of the literature indicates that available results, from increasing number of studies that deal with this issue, are different. Some studies demonstrated that overweight and obese children were more affected by tooth decay, in deciduous as well as permanent dentition, compared to normal weight children [7, 19–22]. In contrast, there are also studies that reported different results [23–25]. In a study from Turkey, which included 5–9 years old subjects, it was concluded that children with reduced body weight have a higher risk of tooth decay than overweight or obese children [26].

It should also be pointed that all of these studies used the BMI as a parameter for obesity assessment. However, the study conducted in Italy in 2011, also used BMI for nourishment assessment, but the dual-energy x-ray absorptiometry DXA index as well (i.e., percentage of body fat determined by McCarthy classification). When the authors classified patients according to BMI, no statistically significant difference between the groups and observed parameters was found. However, when patients were classified according to McCarthy classification, it was shown that obese children had a higher number of deciduous and permanent decayed teeth, compared with normal body weight children [5].

In our study, the normal body weight children had more fillings and extracted teeth, which might suggest that children with increased body weight neglect both general and oral health.

During physiological teeth replacement, as normal phenomenon, eruption gingivitis occurs and may mask gingival state of health. In this study, gingival state was assessed around completely emerged permanent teeth. Differences in gingival state of health between the children with increased body weight and the normal body weight children were not observed in our study. In contrast to this, some studies reported that overweight children had higher degree of gingival inflammation than normal body weight children [16].

Periodontal diseases are caused by dental plaque microorganisms, while the severity of periodontal disease may be associated with the mouth and teeth health. The results of our research indicate that the respondents with the increased body weight had worse condition of periodontal tissue. The main findings of this study demonstrate a positive association between increased body weight and periodontal risk indicators in children and adolescents, which, in the long term period, may lead to chronic systemic inflammation. Our results are in line with other recently reported data [16, 17, 27, 28]. The average CPI value in this study indicates that it is necessary to motivate and train the patients on how to regularly and properly maintain oral hygiene as well as to remove supra-gingival, sub-gingival deposits and inadequate fillings. None of the respondents from either of the groups needed mechanical debridement of periodontal pockets or surgical treatment. A survey, conducted in Finland, demonstrated higher prevalence of periodontal disease in the increased body weight group of respondents as well as the presence of periodontal pockets in the normal weight group of respondents [17].

Oral hygiene state analysis did not show differences among the examined groups related to the presence of soft debris. However, the results of some studies suggest that children [29] reported that there was a difference in bacterial plaque composition between obese and normal body weight children as well as that the larger sum of bacterial cells in subgingival biofilm was significantly associated with obesity.

Conclusion

The results of this study do not suggest that children with increased body weight have more affected teeth than normal body weight children; however, they have a worse periodontal tissue state of health. Due to this, it can be stressed that excessive body weight may affect state of health of individual oral structures. Therefore, application of preventive measures, proper and regular oral hygiene, proper selection of oral hygiene maintenance tools and if needed application of chemical prophylactic means, can prevent the occurrence or further development of oral diseases.

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Asthma and periodontal health in children

Astma i parodontalno zdravlje kod djece

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Abstract

Background/Aim. Oral health is an important part of overall health. Good oral health is important for oral diseases prevention and health maintenance of respiratory system. The aim of the study was to evaluate oral hygiene and periodontal health parameters of asthmatic children and to compare them with children without asthma as well as to evaluate those parameters according to type of used medications and time of taking medications in children with asthma. **Methods.** This epidemiological study included 68 children with asthma and 68 children without asthma or any other chronic disease aged from 6 to 16 years. Parameters used in this study were Greene-Vermillion index, Löe-Silness gingival index and Community Periodontal Index (CPI). **Results.** Good oral hygiene (31.1%) was more present in children without asthma whereas poor hygiene (20.0%) was more frequent in children with asthma ($p < 0.001$). Healthy gingiva was more frequent in children without asthma (25%) while mild (58.8%) and moderate gingival inflammation (5.9%) were more frequent in the group of children with asthma ($p < 0.01$). Mean CPI values were higher in children with asthma ($p < 0.001$). Mean values of Plaque Index, Gingival Index and CPI did not show statistically significant difference in relation to type of administered medication. However, taking medications in the afternoon was related to higher mean values of Plaque Index and Gingival Index ($p < 0.05$) within the group of children with asthma. **Conclusion.** Children with asthma had poorer oral hygiene and were diagnosed with greater values of oral hygiene and periodontal indices compared with the group of children without asthma. For this reason, it is necessary to promote oral health and establish good oral hygiene habits in asthmatic children.

Key words:
asthma; child; adolelscent; oral hygiene; periodontal index.

Apstrakt

Uvod/Cilj. Oralno zdravlje je važan deo opšteg zdravlja. Dobro oralno zdravlje je važno kako za prevenciju oralnih oboljenja tako i za održavanje zdravlja respiratornog sistema. Cilj istraživanja bio je da se proceni oralna higijena, stanje zdravlja parodontalnih tkiva dece obolele od astme, te da se te vrednosti uporede sa pronađenim vrednostima kod dece bez astme, kao i da se dobijene vrednosti procene u odnosu na vrstu i vreme upotrebljenog leka u grupi dece sa astmom. **Metode.** U epidemiološku studiju je uključeno 68 dece sa astmom i 68 dece bez astme ili bilo koje druge hronične bolesti, uzrasta od 6 do 16 godina. Parametri korišćeni u studiji su bili plak indeks po Greene-Vermillionu, Löe-Silnessov gingivalni indeks i Indeks stanja parodoncijuma u zajednici (*Community Periodontal Index* – CPI). **Rezultati.** Dobra oralna higijena bila je prisutnija kod dece bez astme (31,1%), dok je loša češće bila zastupljena kod dece sa astmom (20,0%) ($p < 0,001$). Zdrava gingiva je bila zastupljena češće kod dece bez astme (25%), dok je blaga (58,8%) i umerena (5,9%) upala desni bila prisutnija u grupi dece sa astmom ($p < 0.01$). Prosečne vrednosti CPI indeksa bile su veće kod dece sa astmom ($p < 0.001$). Prosečne vrednosti plak indeksa, gingivalnog indeksa i CPI nisu ukazale na značajnu razliku u odnosu na vrstu primenjenog leka. Međutim, uzimanje lekova u popodnevnim satima je bilo povezano sa višim vrednostima plak i gingivalnog indeksa ($p < 0,05$) u grupi dece sa astmom. **Zaključak.** Deca sa astmom su imala lošiju oralnu higijenu i kod njih su utvrđene veće vrednost plak i parodontalnih indeksa u poređenju sa grupom dece bez astme. Iz tog razloga neophodno je promovisanje oralnog zdravlja kao i uspostavljanje dobrih oralno higijenskih navika kod dece sa astmom.

Ključne reči:
astma; deca; adolescencija; usta, higijena periodontalni indeks.

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Introduction

Asthma is the most frequent chronic disease and represents not only a health but a social problem as well. It appears relatively early, in childhood, and its symptoms are not often recognized and/or are not treated in a proper way which might present aggravating circumstance for these patients.

Asthma is the leading cause of hospitalization in children. Children who are hospitalized for an illness may have lethargy or malaise, so that their oral hygiene may be neglected. Having recognized this problem, Blevins proposed oral health protection program for these children based on the fact that pediatric nurses can as well encourage children with asthma to care about regular oral hygiene and promote the importance of good oral health [1].

Parents are poorly aware of potential oral diseases in asthmatic children and preventive actions that results in increased incidence of oral diseases. It is proved that if parents are less educated, their children are more exposed to oral diseases [2].

The dental plaque control is the basis for good oral hygiene. Good oral health is important not only to prevent oral disease but also to maintain healthy respiratory system. The evidence also suggest that complete removal of the dental plaque may lead to the improvement of the respiratory function in children with asthma [3].

Children with asthma usually prefer to consume sweet soft drinks and food while neglecting adequate oral hygiene, the use of fluorides and regular dental checks. Also, some of the drugs used for treatment of asthma usually contain artificial sweeteners [1] that may affect teeth, other oral tissues and promote presence of oral diseases. Poor oral hygiene followed by soft and hard tooth debris accumulation leads to gingivitis that not necessarily has to turn into periodontal diseases. However, it has been reported that it may progress to severe periodontal disease and suggested that high-risk group identification should be given greater attention [4].

Scannapieco and Ho [5] emphasized the connection between chronic respiratory disease and participants with poor oral hygiene. The oral cavity bacteria might have an important part in exacerbations of chronic obstructive pulmonary diseases, as the dental plaque might serve as storage for respiratory pathogenic microorganisms [5].

Recent studies have considered very seriously a relationship between oral pathogens, which cause gingivitis and periodontal diseases and asthma [6, 7]. On the other hand, chronic disease (like asthma) may be considered as a risk factor for the periodontal diseases [8] as well.

The aim of the study was to evaluate oral hygiene and periodontal health parameters of asthmatic children and to compare it with children without asthma as well as to evaluate oral hygiene and periodontal health parameters according to type of used medications and time of taking medications in children with asthma.

Methods

Study population

This one-year long study was conducted according to Declaration of Helsinki of 1975, as revised in 2000.The study was approved by the Ethical Committee of our Institution (No. 01-8/37). Before the examination, the parents and children involved in the study were informed about the aim of the study and applied methodology. The parents were asked to sign written approval for the participation of their children in this study and children were permitted to leave process in any time during study.

The study included two groups of children, aged from 6 to 16 years. The first group consisted of children with asthma (AG), without any other systemic disease who had 2 symptoms of asthma and were treated or examined in the University Hospital Foca or Primary care facility in Foca. On the basis of presented symptoms of asthma AG group was split into two subgroups [9]. The division was conducted by specialist in pediatric pulmonology. The first subgroup consisted of children with good controlled asthma (GCA), while the second subgroup consisted of children with partly controlled asthma (PCA). Asthma is well controlled if: children have symptoms no more than 2 days a week, these symptoms do not wake them from sleep more than 1 or 2 nights a month; they can perform all usual activities; they take quick-relief medicines no more than 2 days a week; they do not have more than one asthma attack a year that requires taking systemic steroids and their peak flow does not drop below 80 percent of their personal best value. Asthma is partly controlled if: children have symptoms more than 2 days a week; they cannot perform normal physical activities and they have nocturnal symptoms; they have more asthma attack a year that requires taking asthma drugs and their peak flow drop below 80 percent of their personal best values.

The second group consisted of children without asthma (NAG) or any other chronic disease and was matched by gender and age to the children with asthma.

Examination

Prior to the examination, general data along with medical history data were recorded. The planned epidemiologic examination was performed according to the World Health Organization recommendation for epidemiologic explorations of the National Oral Health Survey [10]. All participants were examined at the Department of Pediatric and Preventive Dentistry, the Faculty of Medicine in Foca. The dental examination was performed by the use of dental or periodontal probe, mirror and artificial light. Data were recorded according to plan prepared for this study.

Clinical measurements

Oral hygiene was evaluated by Greene-Vermillion index for soft deposits or "plaque index" (PI) [11]. The presence of the plaque was noticed on the certain surfaces of completely erupted representative teeth.

The Löe-Silness gingival index (GI) was used for the evaluation of gingival status [12]. The clinical examination of the gingiva according to mentioned index included the evaluation of gingival status by inspection and probing at vestibular, mesial, oral and distal side of each present tooth.

The Community Periodontal Index (CPI) [10] was used to evaluate the periodontal state of health, following the World Health Organisation (WHO) recommendation for participants under 15 years of age.

Statistical analysis

The study analysis was carried out by the Statistical Package for Social Sciences (SPSS version 19.0 for Windows, SPSS Inc., Chicago, IL, USA) using χ^2-test, Independent-samples *t* Test, Mann-Whitney test and one-way Anova, in accordance with particular parameters. The values of $p < 0.05$ were considered statistically significant.

Results

The research included 136 children, aged between 6 and 16 (10.5 ± 3.3) years, divided into the AG group (n = 68; age 10.5 ± 3.3) and the NAG group (n = 68; age 10.5 ± 3.3). In this study, there was a statistically significant ($p < 0.05$) higher percentage of male patients (77.9%) comparing to female ones (22.1%).

The AG group consisted of the GCA subgroup which was 64.7% of the AG group (mean age 10.3 ± 3.5) and the PCA subgroup which was 35.3% of the AG group (mean age 10.9 ± 2.8). There was no statistical significance related to age between two subgroups ($p > 0.05$) within the AG group.

The results revealed that 63.0% of participants were using inhalator steroids, 13.2% bronchodilators and 23.5% combination of inhaler steroids and bronchodilators.

The average value of the PI was 1.2 in the AG group (Table 1). Excellent hygiene (19.7%) was more present in the NAG group whereas poor hygiene (47.5%) was more frequent in the AG group (Table 2). The obtained values showed no statistical difference in relation to the applied medicine (Table 3). However, the PI values showed a statistically significant difference related to the time of the day of asthma drugs administration (Table 4).

Table 1

The arithmetic mean and standard deviation (SD), the values of Plaque index (PI), Gingival index (GI) and community periodontal Index (CPI) in the observed groups

Indices	AP (mean ± SD)	AG (mean ± SD)	NAG (mean ± SD)	GCA (mean ± SD)	PCA (mean ± SD)	*p*
PI	0.9 ± 0.6	1.2 ± 0.7	0.7 ± 0.5	1.1 ± 0.6	1.2 ± 0.8	< 0.001* NS†
GI	0.4 ± 0.4	0.5 ± 0.4	0.3 ± 0.3	0.5 ± 0.4	0.5 ± 0.5	< 0.001* NS†
CPI	0.5 ± 0.7	0.8 ± 0.7	0.3 ± 0.5	0.8 ± 0.8	0.8 ± 0.7	< 0.001* NS†

***statistical significance between the AG and NAG; †statistical significance between the GCA and PCA. AP – all participants; AG – children with asthma; NAG – children without asthma; GCA – good controlled asthma; PCA – partly controlled asthma; *p* – the level of a statistical significance (Independent samples *t*-test), NS – nonsignificant.**

Table 2

Comparison of difference in oral hygiene according to the plaque index (PI)

Plaque Index	AP (n = 121) %	AG (n = 60) %	NAG (n = 61) %	GCA (n = 37) %	PCA (n = 23) %
Excellent hygiene	14.9	10.0	19.7	5.4	17.4
Good hygiene	23.1	15.0	31.1	16.2	13.0
Poor hygiene	51.2	55.0	47.5	62.2	43.5
Very poor hygiene	10.7	20.0	1.6	16.2	26.1
p		< 0.001*		NS†	

***statistical significance between the AG and NAG; †statistical significance between GCA and PCA. AP – all participants; AG – children with asthma; NAG – children without asthma; GCA – good controlled asthma; PCA – partly controlled asthma; n – number of subjects; % – percentage of subjects;**
***p* – the level of a statistical significance (Mann-Whitney test), NS – nonsignificant.**

Table 3

The Plaque index (PI), Gingival index (GI) and Community periodontal index (CPI) in relation to a type of administered medication

Type of applied medications	PI (mean ± SD)			GI (mean ± SD)			CPI (mean ± SD)		
	AG (n = 60)	GCA (n = 37)	PCA (n = 23)	AG (n = 68)	GCA (n = 24)	PCA (n = 24)	AG (n = 60)	GCA (n = 37)	PCA (n = 23)
Inhalator steroids	1.2 ± 0.6	1.1 ± 0.6	1.2 ± 0.7	0.5 ± 0.4	0.5 ± 0.4	0.5 ± 0.4	0.9 ± 0.7	0.9 ± 0.8	0.8 ± 0.7
Bronchodilators	1.2 ± 0.5	0.7 ± 0.4	1.6 ± 0.3	0.7 ± 0.5	0.4 ± 0.4	1.2 ± 0.3	1.1 ± 0.8	1.0 ± 1.2	1.3 ± 0.5
Combination of drugs	1.2 ± 0.7	1.3 ± 0.7	0.9 ± 1.2	0.4 ± 0.4	0.4 ± 0.3	0.5 ± 0.7	0.5 ± 0.6	0.5 ± 0.7	0.4 ± 0.5
p	NS	NS	NS	NS	NS	< 0.05	NS	NS	NS

AG – children with asthma; GCA – good controlled asthma; PCA – partly controlled asthma; n – number of subjects; SD – standard deviation; *p* – the level of statistical significance (One-Way ANOVA), NS – nonsignificant.

Table 4

The Plaque index (PI), Gingival index (GI) and Community periodontal index (CPI) in relation to time of medication administration

Time of taking medication	PI (mean ± SD)			GI (mean ± SD)			CPI (mean ± SD)		
	AG (n = 60)	GCA (n = 37)	PCA (n = 23)	AG (n = 68)	GCA (n = 44)	PCA (n = 24)	AG (n = 60)	GCA (n = 37)	PCA (n = 23)
Only in the morning	1.0 ± 0.6	0.9 ± 0.6	1.5 ± 0.1	0.5 ± 0.4	0.4 ± 0.4	0.6 ± 0.2	0.8 ± 0.9	0.8 ± 1.0	1.0 ± 0.0
Only in the afternoon	2.3 ± 0.5	0.0 ± 0.0	2.3 ± 0.5	1.1 ± 0.1	0.0 ± 0.0	1.1 ± 0.1	2.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
In the morning and evening	1.1 ± 0.7	1.2 ± 0.6	0.9 ± 0.8	0.4 ± 0.4	0.5 ± 0.4	0.4 ± 0.5	0.6 ± 0.7	0.7 ± 0.7	0.5 ± 0.6
Before sleeping	1.4 ± 0.4	1.3 ± 0.4	1.5 ± 0.3	0.7 ± 0.5	0.6 ± 0.4	1.0 ± 0.5	1.1 ± 0.7	1.2 ± 0.8	1.0 ± 0.7
p	< 0.05	NS	< 0.05	< 0.05	NS	NS	NS	NS	< 0.05

AG – children with asthma; GCA – good controlled asthma; PCA – partly controlled asthma; n – number of subjects; SD – standard deviation; *p* – the level of statistical significance (One-Way ANOVA); NS – nonsignificant.

Table 5

Comparison of difference in gingival health status according to the Gingival index (GI)

GI	AP (n = 136)	AG (n = 68)	NAG (n = 68)	GCA (n = 44)	PCA (n = 24)
Healthy gingiva	21.3	17.6	25.0	13.6	25.0
Mild gingivitis	64.0	58.8	69.1	68.2	41.7
Moderate gingivitis	14.7	23.5	5.9	18.2	33.3
p		< 0.01*		NS†	

Note: Results are given as percentage of subjects.
***statistical significance between AG and NAG; †statistical significance between GCA and PCA.**
AP – all participants; AG – children with asthma; NAG – children without asthma; GCA – good controlled asthma; PCA – partly controlled asthma; n – number of subjects; % – percentage of subjects; *p* – the level of statistical significance (Mann-Whitney Test), NS – nonsignificant.

Table 6

Comparison of difference in periodontal status according to the Community periodontal index (CPI)

CPI	AP (n = 121)	AG (n = 60)	NAG (n = 61)	GCA (n = 37)	PCA (n = 23)
0	58.7	38.3	78.7	40.5	34.8
1	29.8	41.7	18.0	37.8	47.8
2	10.7	18.3	3.3	18.9	17.4
3	0.8	1.7	0	2.7	0
p		< 0.001*		NS†	

Note: Results are given as percentage of subjects
***statistical significance between AG and NAG; †statistical significance between GCA and PCA.**
AP – all participants; AG – children with asthma; NAG – children without asthma;
GCA – good controlled asthma; PCA – partly controlled asthma; n – number of subjects;
***p* – the level of statistical significance (Mann-Whitney Test), NS – nonsignificant.**

Healthy gingiva was more present in the NAG group (25%), while the presence of mild (58.8%) and moderate (5.9%) inflammation was more frequent in the AG group (Table 5). Statistical significant difference in the GI values was found between the AG (0.5) and the NAG groups (0.3) (Table 1). The GI values were higher in the AG group who were using anti-asthmatic therapy in the afternoon hours (Table 4). The PCA children showed higher values of the GI when treated with bronchodilators (Table 3).

As far as periodontal health of the experimental group concerns, the average CPI value was 0.8 (Table 1). The healthy periodontal tissue was found in 78.7 % of the NAG group (Table 6). The presence of hard debris on teeth, prominent filling edges or the presence of periodontal pockets with the depth of 4–5 mm, were more frequent in the AG group. The values of the average CPI values were higher in the PCA children who were using the therapy in the afternoon (Table 4).

Discussion

This study included elementary school age children. This various group might indicate whether asthma or the drugs used in asthma treatment can affect health of periodontal tissues. In addition, evaluation of PI and CPI could have been recorded against completely erupted permanent teeth and GI against all present teeth, which could present limiting factor of this study.

Some authors support finding that children with asthma have higher values of the plaque index and poorer oral hygiene [13–17] which is in accordance with the results of this study that speaks in favor of the close connection between poorer hygiene and the presence of asthma. In the present study, three quarters of the AG group participants had poor and very poor oral hygiene. That was even more frequent in children who were using anti-asthmatic drugs in the afternoon as well as in the PCA group. Anandhan et al. [18] reported that children with advanced forms of asthma had poorer oral hygiene and that girls had better oral hygiene than boys.

However, Ehsani et al. [19] did not notice any difference in the amount of the plaque between children with asthma and children without asthma.

Apart from the statements mentioned above, there is a group of authors who pointed that a lower PI was registered among the asthmatic children [20, 21], thanks to good oral hygiene, frequent parental surveillance and regular diet [21]. Having in mind that the diet has an important role in the dental plaque formation, Mazzoleni et al. [20] reported that children with asthma showed to have similar dietary habits, but better oral hygiene in comparison to children without asthma of the same age.

Saliva has an important role in the limitation of periodontal diseases. However, drugs prescribed for asthma treatment that can cause reduced salivary secretion, greatly affect the severity of periodontal diseases of these group of patients [22]. The higher average values of the GI in the participants with asthma compared with the healthy group were found in Attavar, India [13] and Jonkoping, Sweden [23, 24] that is in accordance with the results of our study.

The protective mechanisms within saliva balance the interactions between bacterial and immunological factors and help to maintain periodontal health. Mouth-breathing, frequently observed in asthmatic patients due to the obstruction of respiratory system [25], along with a decrease in salivary flow caused by the long-term use of asthma medication, reduce saliva protective qualities, and therefore place the patient at a greater risk of developing periodontal disease [26].

Asthma is a disease, which prevails among young people, whereas periodontal diseases are more frequent in older people. Some authors consider that as a reason why periodontal diseases are rarer in young people with asthma, while other studies showed that periodontal diseases were more frequent in people with asthma than in healthy people [8].

This study revealed poorer state of health of the periodontal tissues in children with asthma and 18.3% of them were diagnosed with the presence of incorrect dental works, hard tooth debris, while the presence of shallow periodontal pockets were recorded in 1.7% of the participants. Considering that the most of the children with asthma suffered from gingival bleeding after probing, which is a clear sign of gingivitis, a necessary treatment which consists of instructions on proper and regular oral hygiene and motivation/remotivation of the patients is required. The higher values of

observed indices were recorded with children who used drugs in the afternoon hours what might be explained by inadequate oral hygiene, larger sweet drinks consumption and reduced salivation as well.

Positive correlation between asthma and the presence of the periodontal diseases in children and adolescents (age of 13–17) were not found in some studies [27, 28]. Shulman et al. [27] think that these results may be false due to hormonal status of the observed groups, the fact that the depth of pockets is not measured on children younger than the age of 13 years as well as the fact that the majority of the studied group was consisted of adolescents with mild and moderate types of asthma.

Belgian scientists confirmed that there was no difference in the gingival health status in children with or without asthma, regardless duration of asthma, or type of used drugs [28]. The absence of difference in gingival or periodontal status of the participants with or without asthma was confirmed in the studies in Iran [19] and Italy [29].

Conclusion

Results of this study demonstrated that children with asthma had poorer oral hygiene and poorer state of health of the periodontal tissues compared with children without asthma, and that the PI and GI values obtained within the group of children with asthma were higher in the patients who were taking their asthma drugs in the afternoon hours. However, results of previously mentioned studies are somewhat conflicting, dental clinical protocols for prevention of periodontal tissue deterioration in children with asthma do not exist and the literature does not provide sufficient data on preventive programs achievements. Due to complexity and importance of this matter, further studies that might provide sufficient data, better understanding and would help in creating the most efficient protocol for prevention and dental treatment of asthmatic child patients in future are needed.

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The effects of three months long continuous glucose monitoring in children with type 1 diabetes on multiple daily insulin injections

Efekti tromesečnog kontinuiranog praćenja glukoze kod dece sa dijabetesom tipa 1 koja primaju više dnevnih doza insulinskih injekcija

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Abstract

Background/Aim. The Professional System of Continuous Glucose Monitoring, the *i*Pro®2 CGM System (Medtronic) is designed to be worn together with a glucose sensor with an electrode inserted into the subcutaneous tissue, up to 7 days, without insight into the current level of glycemia. After reading data from the iPro®2 device, a realistic picture of the glycemia movement during the period of wearing the device is obtained. The aim of the study was to examine whether objective measurement information collected through the use of professional continuous glucose monitoring (CGM) contribute to improved metabolic control in children with type 1 diabetes mellitus who are on the multiple daily insulin injections (MDI). **Methods.** The study was conducted on 24 patients (14 girls) aged 5 to 18 years, with an average age 12 ± 3.3 years, in the period from June to December 2016 in the Clinic of Pediatrics, University Clinical Center of the Republic of Srpska in Banja Luka. Glycated hemoglobin (HbA1c) was measured in the laboratory at the start of the trial and 3 months afterwards in order to determine the effect of wearing professional *i*Pro®2 on metabolic control, and then three months later, to test for the long-lasting effects in the absence of iPro®2 monitoring. **Results.** The initial HbA1c was 7.78 ± 1.17% (min: 5.50%; max: 10.00%). After 3 months, HbA1c showed a statistically significant decrease to 7.34 ± 0.84% (min: 5.60%, max: 8.90%). At the six-month follow-up visit, without implementing professional CGM in the meantime, a significant increase in HbA1c was reached, with the average value of 7.68% ± 0.83% (min: 5.50%, max: 9.10%). **Conclusion.** This study shows that carrying a professional CGM for 7 days per month, 3 months continuously is associated with certain improvement of metabolic control in children with diabetes who are on MDI without increasing risks of hypoglicemia.

Key words:
diabetes mellitus, type 1; child; bosnia and herzegovina; drug monitoring; insulin; blood glucose self monitoring.

Apstrakt

Uvod/Cilj. Profesionalni sistem kontinuiranog monitoringa glikemije, *i*Pro®2 (Medtronic), je dizajniran za nošenje u kombinaciji sa glukoznim senzorom, čija elektroda je insertovana u potkožno tkivo, do sedam dana, bez uvida u trenutni nivo glikemije. Nakon očitavanja podataka sa *i*Pro®2 uređaja dobija se realna slika kretanja glikemije tokom perioda nošenja uređaja. Cilj ispitivanja bio je da se proveri da li informacije dobijene objektivnim merenjem putem profesionalnog kontinuiranog monitoringa glikemije doprinose poboljšanju metaboličke kontrole dece sa dijabetes melitusom tip 1 koji su na intenziviranom pen režimu insulinske terapije. **Metode.** Istraživanje je obuhvatilo 24 ispitanika (14 devojčica) uzrasta od 5 do 18 godina, prosečne starosti 12 ± 3.3 godina, u periodu jun-decembar 2016. godine na Klinici za dečije bolesti, Univerzitetski Klinički Centar, Banja Luka. Laboratorijski je izmeren glikozilirani hemoglobin (HbA1c) na početku ispitivanja, i nakon tri meseca kako bi se utvrdio efekat nošenja profesionalnog *i*Pro®2 na metaboličku kontrolu, te nakon još tri meseca kako bi se proverili dugoročni efekti u odsustvu *i*Pro®2 praćenja. **Rezultati.** Početni HbA1c bio je 7,78 ± 1,17% (min: 5,5%; max: 10%). Nakon tri meseca HbA1c pokazao je statistički značajno sniženje na 7,34% ± 0,84% (min: 5,60%; max: 8,90%). Na kontrolnom pregledu nakon šest meseci, bez upotrebe profesionalnog kontinuiranog monitoringa glikemije u međuvremenu, došlo je do značajnog porasta HbA1c na 7,68% ± 0,83% (min: 5,5%, max: 9,1%). **Zaključak.** Ova studija pokazuje da je nošenje profesionalnog kontinuiranog monitoringa glikemije sedam dana u mesecu, tri meseca u kontinuitetu, povezano sa određenim poboljšanjem metaboličke kontrole kod dece obolele od dijabetesa koja su na višednevnim insulinskim injekcijama, bez povećanja rizika od hipoglikemija.

Ključne reči:
dijabetes melitus, insulin-zavisni; deca; bosna i hercegovina; lekovi; monitoring; insulin; glukoza u krvi, samopraćenje.

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Introduction

Continuous glucose monitoring (CGM) is an important mean for determining the adequate insulin therapy and it is rapidly becoming the standard for the care of patients with diabetes [1]. Despite advances in insulin therapy (the introduction of short and long acting insulin analogs) and delivery systems (insulin pumps) only 30% children with type 1 diabetes meet the recommended international glycemic target of glycated hemoglobin (HbA1c) level of 7.5%. Postprandial hyperglycemia and glycemic variability are a trigger and the first step in the pathogenesis of micro and macrovascular complications in diabetes. Patients still most commonly adhere to the traditional glycemic control at least 4 to 6 times per day. Many studies have demonstrated the association of lower HbA1c to greater number of glycemic self-monitoring, but the exclusive focus on HbA1c may miss important fluctuations in glucose that are easily identified using continuous glucose monitoring [1–8]. Continuous glucose monitoring with iPro®2 is a holter monitor type that allows automatic glucose measurement in the subcutaneous interstitial tissue using a glucose sensor. The sensor measures the glucose level in the interstitium continuously every 10 seconds, and the mean measurement values are sent every 5 minutes to the iPro®2 device that keeps the data for the entire duration of the sensor life span. According to the manufacturer, the life span of the sensor is approximately 144 hours; after this time passes, the data are collected with CareLink iPro®2 applicational software which produces relevant statistical data including graphics for better understanding of glucose variability.

Such data can help both doctors and patients to determine adequate insulin therapy. There are studies that demonstrate the usefulness of the device for the CGM in real time by increasing the number of patients with target HbA1c, reducing glycemic variability, and reducing the risk of severe hypoglycemia. Patients who use multiple daily insulin injections (MDI) experience same benefit from CGM as well as patients using an insulin pump, and when they are compared in the studies, MDI regimen works the same or even better than insulin pump.

There are only a few studies exploring iPro®2-to monitor metabolic control in children with diabetes mellitus type 1 on MDI, although it is clear that it provides an excellent insight for all interested parties in the therapy context. An example of its successful use would be a recent study examining the effects of physical activity during the day on hypoglycemia during the night [9–14]. Thus, one of the aims of our study is to broaden research employment of this relatively novel tool for measuring glucose control.

Methods

The study was conducted in the period from June to December 2016 at the Clinic of Pediatrics, University Clinical Centre (UCC) of the Republic of Srpska in Banja Luka, Bosnia and Herzegovina. The study was approved by the Ethics Committee of Human Experimentation in Bosnia and Herzegovina. The study involved 24 children with diabetes mellitus type 1; 10 boys and 14 girls, aged 5 to 18 years (average age 12 ± 3.3 years and with average diabetes duration 2.5 years) (Table 1). All the patients were using ultra short-acting insulin before meal, and long-acting insulin once a day such as intensive insulin therapy. The criteria for inclusion in the study were: duration of type 1 diabetes mellitus more than one year; understanding and consent to follow prescribed protocols as well as regular visits to the doctor. Understanding and consent for monitoring protocol meant wearing iPro®2 device 7 days per 3 months, tracking and writing down the self-monitored blood glucose concentration for at least 4 times a day. Hypoglycemia was defined as a blood glucose concentration less than 3.9 mmol/L, while hyperglycemia was considered as a blood glucose concentration higher than 7.8 mmol/L. All participants, both parents and children, after they got to know the criteria, plan and protocol of this study committed to implement consistently the protocol during the self-monitoring at home and at regular medical visits. All the patients were followed up at the Clinic at baseline, during 3 and 6 months by the same investigator. Both demographic and clinical data were collected using a standardized data collection form.

Table 1

Demographic characteristics of the study participants

Characteristics	Baseline	Time spent on CGM (iPro®2)			After 3 months	After 6 months
		1st usage	2nd usage	3rd usage		
Mean age (years)	12.0 ± 3.3					
Duration of diabetes (years)	2.5 (min.:1; max.:14)					
Mean weight (kg)	153.83 ± 18.28					
Mean height (cm)	45.97 ± 14.88					
BMI (kg/m^2)	18.80 ± 2.90				19.00 ± 3.08	19.04 ± 3.18
Total insulin per kg (IU/kg)	0.65 ± 0.25	0.65 ± 0.25	0.63 ± 0.24	0.65 ± 0.28	0.65 ± 0.28	0.70 ± 0.25
HbA1c (%) – laboratory measure	7.78 ± 1.17				7.34 ± 0.84	7.68 ± 0.83
HbA1c (%) – estimated on *i*Pro®2		7.74 ± 1.30	7.39 ± 1.34	7.39 ± 1.06		

Note: Values are presented as arithmetic mean ± standard deviation, except for the duration of diabetes which presented as median with minimum and maximum values.
CGM – continuous glucose monitoring; BMI – body mass index; HbA1c – glycated hemoglobin.

The beginning of the study was marked as the first visit (Figure 1) when both laboratory measurements of HbA1c were conducted (performed at the UCC Banja Luka on Cobas E601, the Roche apparatus) and when iPro®2 device was activated in the patients for seven days. After receiving the data, they were discussed with the patients and therapy was changed, if deemed necessary. The second visit was done after one month with another reading of the 7-day iPro®2 device with the subsequent consultation regarding therapy. The third visit was done after the third iPro®2 device reading when HbA1c measurement was performed again. After 6 months from the beginning of the study, the third laboratory measurement of HbA1c was performed.

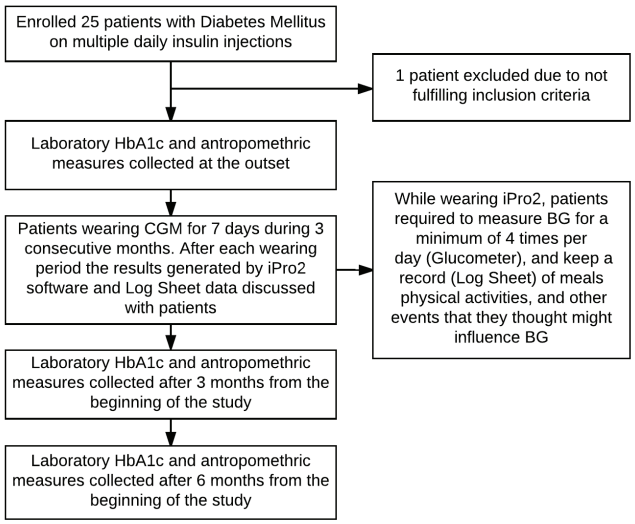

Fig. 1 – Flowchart of continuous glucose monitoring study completion. HbA1C – glycated hemoglobin; CGM – continuous glucose monitoring; BG – blood glucose.

Thus, within 3 months the participants wore a device for seven days per month. The iPro®2 device is a recorder that collects data from the sensor during the wearing period, and after taking off the device, the summarized data is generated through CareLink iPro®2 software in the form of relevant statistical results and graphics. It is important to note that the participants did not have an insight into the current glycemic values during the time that they carried the device, so they could see the results of the readings after 7 days. During the wearing of the device, the participants were obliged to measure the glycemic value using standard glucometers (Accu-Chek, Roche Diagnostic) for at least 4 times a day, and to keep records of the important events. The events reported by the participants required the report of: doses and times of insulin delivery, meals eaten (descriptive), the amount of carbohydrates per meal, and the type of physical activity and its duration. After each of the readings was collected, they were discussed with the investigators (the first two authors) when they attempted to determine the dependence on a particular event on the value of glycemia with the goal to educate the patients.

Based on the mean value of each measurement, CareLink iPro®2 software also estimates the HbA1c values that the respondent would have if she/he continues to behave according to the same patterns. Based on the analysis of the results after reading the data using a computer retrogradely, insulin therapy of patients was corrected.

Data analysis

When it comes to statistical analysis, categorical variables were analyzed by showing the distribution of the absolute and relative frequencies, while, for the numerical variables, the measures of central tendency and variability were used. Changes between the measurements were tested using the analysis of variance for repeated measures. To control for the inflated experiment-wise error rate, we used Bonferroni corrections when calculating *p*-values to test the differences between the measurement pairs. These differences were tested by both paired sample *t*-tests and its nonparametric alternative, Wilcoxon signed-rank tests. Wherever the tests gave the congruent results with regard to the statistical significance, we provided only the *t*-test results.

Results

The average initial measured value of HbA1c was 7.78% ± 1.17% (min: 5.5%, max: 10.0%). The results showed that after 3 months of using iPro2 devices value of HbA1c decreased to 7.34% ± 0.84% (max: 8.9%; min: 5.60%) ($\Delta M = 0.45$, $t = 2.67$; $P_{bonf} = 0.041$). At the final visit, after the three-month period without wearing the iPro®2 device, the HbA1c increased to 7.68% ± 0.83% (max: 9.1%; min: 5.5%) ($\Delta M = -0.35$; $t = -2.81$, $P_{bonf} = 0.3$). This final value is negligibly lower than the initial HbA1c value ($\Delta M = 0.1$, $t = 0.69$, $p_{bonf} = 1.00$).

In addition to the clinical measurements, the HbA1c values were estimated by iPro®2 devices once a month for three subsequent months after wearing the device for 7 days. The value for the last measurement 3 months after the start of the study was approximately equal to its time-related clinical measurements of HbA1c ($\Delta M = -0.05$, $t = -0.28$, $p = 0.779$), although the correlations between these measures were not exceptionally high ($r = 0.61$, $p < 0.001$) (Table 1).

A multiple daily insulin injections with short-acting analogue insulin before a meal and a basal analogue of long-acting were recorded for all subjects during the study. The average total insulin dose for all patients yielded 0.65 ± 0.25 IU / kg of body mass. After 3 months, there was no clinically significant increase neither in basal, nor in bolus insulin dose compared to the start of the study. The average dose of insulin after 3 months remained virtually identical to the initial insulin dose of 0.65 ± 0.28 IU/kg, while there was a significant improvement in metabolic control. For the last 3 months without the use of iPro®2, the total insulin dose somewhat increased to 0.70 ± 0.25 IU / kg of body weight, but metabolic control deteriorated (Table 2). None of the changes related to the insulin therapy reached the level of a statistical significance.

Table 2

Dosage (in IU/kg) of total, basal and bolus insulin during the study

Use of continous monitoring	Total insulin dose	Basal insulin dose	Bolus insulin dose
Baseline	0.65 ± 0.25	0.28 ± 0.13	0.38 ± 0.18
1st usage of iPro®2	0.65 ± 0.25	0.28 ± 0.13	0.38 ± 0.18
2nd usage of iPro®2	0.63 ± 0.24	0.27 ± 0.12	0.36 ± 0.16
3rd usage of iPro®2	0.65 ± 0.28	0.28 ± 0.12	0.38 ± 0.17
After 3 months from baseline	0.65 ± 0.28	0.28 ± 0.12	0.38 ± 0.17
After 6 months from baseline	0.70 ± 0.25	0.29 ± 0.11	0.41 ± 0.18

Note: values are presented as mean ± standard deviation.

Table 3

Results from iPro®2 7 days a month, during the three months period

Variables	Time on iPro2		
	1st usage	2nd usage	3rd usage
Number of sensor values	1,712.67 ± 259.65	1,841.83 ± 51.52	1,815.00 ± 122.30
Number of valid calibration	31.80 ± 8.66	35.54 ± 8.03	35.83 ± 7.38
% of time above target glycemia range (> 7.8 mmol/L)	60.21 ± 18.74	54.38 ± 19.54	56.88 ± 18.64
% of time in target glycemia range (3.9–7.8 mmol/L)	33.08 ± 17.47	38.71 ± 15.39	37.38 ± 17.40
% of time below glycemia range (< 3.9 mmol/L)	6.71 ± 7.09	6.92 ± 6.82	5.75 ± 5.57
Recording time (min)	8,563.33 ± 1298.27	9,209.17 ± 257.59	9,079.38 ± 611.48
Highest measured sensor glycemic value (mmol/L)	19.55 ± 3.12	19.38 ± 3.12	19.93 ± 2.89
Lowest measured sensor glycemic value (mmol/L)	2.83 ± 0.83	2.78 ± 0.61	2.73 ± 0.48
Average measured sensor glycemic value (mmol/L)	9.75 ± 2.04	9.18 ± 2.15	9.19 ± 1.69
Average time spent in one hyperglycemic episode (min)	375.50 ± 184.59	322.08 ± 209.69	322.70 ± 142.81
Average time spent in one hypoglycemic episode (min)	72.83 ± 50.38	63.13 ± 43.23	63.63 ± 41.28
AUC above limit	2.90 ± 1.67	2.43 ± 1.66	2.35 ± 1.31
AUC below limit	0.06 ± 0.09	0.04 ± 0.05	0.04 ± 0.06

Note: values are presented as mean ± standard deviation.
AUC – area under the curve.

Measurements using iPro®2 devices

According to the data retrieved from iPro®2 devices, the average number of readings from the sensor in the first measurement was 1712.67 ± 259.65. The number rose at the second and third measurement to 1,841.83 ± 51.52 and 1,815.00 ± 122.30, respectively. This was accompanied with the increase in the average number of calibrations (glycemic self-monitoring) at the second and third reading compared to the first carrying of the sensor (Table 3).

The results showed that the use of iPro®2 devices moderately reduced the average percentage of time spent in hyperglycemia in the second (54.38 ± 19.54%), and in the third measurement (56.88 ± 18.64%) compared to the initial reference value (60.21 ± 18.74%). In the second measurement, we observed, on average, 9.6% less hyperglycemia compared to the initial measurement ($t = 2.06$; $p_{bonf} = 0.153$), while in the third measurement this improvement was also present, but of somewhat smaller magnitude representing a decrease of 6% compared to the initial average value, ($t = 0.86$, $p_{bonf} = 1.00$). In addition, the readings showed a significant clinical shortening of the average time spent per one hyperglycemic episode, although the effect did not reach the limit of statistical significance presumably due to the small sample size. At the initial measurement the average time spent per one hyperglycemic episode was 375 minutes (6 hours and 15 minutes), whereas in the second measurements it was 322 minutes (5 hours and 22 minutes) (Table 3). In other words, this decrease of duration yielded on average 53.4 minutes (a decrease of 14.2%; $p_{bonf} = 0.351$). Similarly, in the third measurement, the average duration of a hyperglycemic episode was 322.7 minutes, which amounted to the reduction of 52.8 minutes compared to the baseline (decrease of 14.1%; $p = 0.624$) (Table 3).

Compared to the baseline (6.71 ± 7.09%), the percentage of time spent in hypoglycemia–slightly increased in the second measurement (6.92 ± 6.82%) but we observed a statistically significant reduction in the third reading (5.75 ± 5.57%). Consequently, the time spent in normoglycemia rose on average both in the second (38.71 ± 15.39%) and the third measurement (37.38 ± 17.40%) compared to the baseline (33.08 ± 17.47%).

Reducing the average duration of a hypoglycemic episode was similar to reducing the average durations of hyperglycemic episodes. The episodes of hypoglycemia were reduced, on average, by 9.7 minutes in the second measurement where the duration dropped from 72.8 minutes to 63.1 minutes (a reduction of 13.3%, $p_{bonf} = 1.00$). The almost identical situation was observed when comparing the initial and third measurement. The percentage change of time spent in hypoglycemia is negligibly higher in the second measurement (3.1% change compared to the initial measurement, $p_{bonf} = 1.00$), while in the third measurement it is slightly lower than in the initial measurement (by 14.3%; $p_{bonf} = 1.00$) (Table 3). Most importantly, no patient experienced severe hypoglycemia or ketoacidosis throughout the study.

With regard to the variability of glycemia measured in critical periods during the day, the conspicuous changes were related to periods of breakfast and lunch. In particular, after the first visit, there was a significant reduction in glycemia before lunch from 10.26 ± 3.59 mmol/L in the first measurement down to 7.79 ± 1.90 mmol/L after the third measurement. There was also a significant fall in glycemia after lunch from the initial 10.03 ± 3.00 mmol/L to 8.72 ± 1.91 mmol/L in the third measurement. Pre-breakfast glycemic values increased from 8.55 ± 2.51 mmol/L to 9.30 ± 2.69 mmol/L during the period of examination. It is also important to note that overnight glycemia was maintained at a satisfactory level, although the average values rose from 8.21 ± 2.19 mmol/L at the baseline to 8.90 ± 2.31 mmol/L in the third measurement (Table 4).

Table 4

Sensor glycemic values (mmol/L) in critical periods during the day

Period of a day	Usage of iPro®2		
	1st usage	2nd usage	3rd usage
Evening (23:00–03:00)	8.85 ± 1.96	8.61 ± 2.67	8.95 ± 2.16
Sleeping (03:00–06:00)	8.21 ± 2.19	8.13 ± 2.65	8.90 ± 2.31
Before breakfast	8.55 ± 2.51	8.67 ± 2.38	9.30 ± 2.69
After breakfast	12.87 ± 4.11	11.42 ± 3.68	11.13 ± 3.39
Before lunch	10.26 ± 3.59	9.32 ± 3.36	7.79 ± 1.90
After lunch	10.03 ± 3.00	9.81 ± 3.52	8.72 ± 1.91
Before dinner	8.70 ± 3.30	9.34 ± 3.15	8.32 ± 2.73
After dinner	9.76 ± 3.69	9.36 ± 3.12	9.26 ± 2.42

Note: values are presented as mean ± standard deviation.

Discussion

Our study with children with diabetes mellitus type 1 on a therapy of multiple daily insulin injections, showed that the use of the iPro®2 professional device for the CGM over period of 3 months was associated with several positive changes: a significant reduction in HbA1c, shortening the time spent in hyper and hypoglycemia, and clinically significant reduction of glycemic variability after 3 months, which was similar to other studies exploring the effects of wearing CGM devices [1]. This seems to be an important finding, having in mind that intensified insulin bolus-basal therapy regimen is rigid in comparison to insulin pump therapy, knowing that the success in lowering HbA1c is harder to achieve in patients with pen therapy [11, 13].

Nevertheless, some of the positive changes proved to be only short-term and the mechanism of change is not clear. For example, the reduction of HbA1c in the second measurement after 3 months might be a result of an increase in the average number of calibrations (glycemic self-monitoring) during the second and third wearing compared to the first wearing of the sensor [1]. On the other side, the deterioration of HbA1c after 3 months without the iPr®2o device indicates that 3 months of wearing the device may not be enough to gain a true insight into glycemic movements for a longer period of time. A longer use of this device could be a strategy in the treatment of children with diabetes.

One another extremely useful feature of iPro®2 devices for both the doctor and the patient is its ability to provide anticipated HbA1c after wearing the device for only 7 days. Such a measure indicates what can be expected if a patient continues with the same insulin therapy and habits related to glycemic control. It should be, however, noted that in our study correlations between the iPro®2 estimated and laboratory measures of HbA1c were not exceptionally high, hence although these HbA1c estimates obtained through iPro®2 are helpful - they should be used in a combination with clinical assessment. Future research should clarify the possible causes of the differences that result from such measures.

With the above in mind, iPro®2 estimated values of HbA1c also showed a significant improvement during the second and third wearing of the device compared to the first wearing. The second and third values of HBA1c were identical, which suggested that after getting the device the patients became more consistent with regard to the adherence to the guidelines and recommendations given, which was also observed in our clinical consultations. Indeed, the improvement of HbA1c values does not necessarily need to be a sign of a positive change, but in our study it was observed along with reduced duration of hypo- and hyperglycemia and reduced glycemic variability, which indicates the improvement.

Namely, the data collected showed that the percentage of time spent in hyperglycemia in both the second and third measurements were reduced relatively to the baseline. Simultaneously, the iPro®2 device showed that more time was spent in normoglycemia, and what is of particular importance, time spent in hypoglycemia was reduced on average for 9–10 minutes. Although these results were not statistically significant, this reduction in time spent in hypoglycemia might be of a clinical significance by reducing the likelihood of serious hypoglycemia. We learned in our sessions with the patients that the fear of a hypoglycemic event at school was a cause of elevated glycemia before and after lunch spotted during the first wearing. Our instructions targeted this behavior and we noticed a significant drop in glycemia before and after lunch during the third device wearing. Such results indicate that by using iPro®2 devices we can more precisely affect the changes of poor metabolic control by making the right decisions to change the insulin therapy or a lifestyle of a child with diabetes.

We also observed some reduction in glycemic variability during the later device wearing compared to the first wearing which is an additional benefit. The ability to monitor the dynamics of glycemic changes is on itself an important feature of such devices, and it is promising that effective diabetes monitoring could be achieved in a short time of using them.

Indeed, numerous studies have confirmed a significant influence of continuous glycemic monitoring on improvement of the metabolic control [1, 15–21]. However, Telo et al. [22] found that only 28% of children with diabetes mellitus type 1 that were offered a CGM accepted a possibility to use it.

Only motivated patients expressed that they would take more than one device (i.e. both the sensor and the insulin pump) on themselves for a few days. That said, we think that wearing iPro®2 system for 7 days, would be acceptable for most patients on MDI in terms of the length of time needed. This would be enough to bring a number of useful information to doctors, especially those information that the patient is unable to express, which subsequently could help in the more appropriate dosing of insulin therapy. The advantage of this device compared to the classical CGM devices with the real-time visibility of glycemia on the screen is that the doctor can see the usual pattern of glycemic events and hence can suggest an appropriate insulin treatment while eating and during the physical activity. By implementing numerous corrections of glycemic events during the day, based on explicitly monitored glycemic values, the practitioners lose the ability to get insight into the truer image of the pattern of behavior of the child with diabetes.

This study had several limitations. First of all, due to objective reasons, the number of patients was small, so we had a low statistical power for the tests conducted, and we were not able to include the control group in our design. Furthermore, the diverse age of the patients weakens the generalizability of the result. Such a small sample size also made it unrealistic to even consider to reliably model the effects of covariates on the outcomes. In addition, the duration of the study was limited to 3 months of the usage of the iPro®2 device or 6 months in terms of monitoring, due to the limited number of devices available to the investigators. Nevertheless, patients expressed the satisfaction with consultations they received during the study, because these consultations helped them to target bad eating habits during school and improve physical activities. As practitioners, we were also more confident in our advice since they were based on comprehensive information of individual glycemia variability.

Conclusion

This study showed that wearing a professional continuous monitoring of glycemic events for 7 days per 3 month led to some short-term and some long-term improvements in the metabolic control in children with diabetes mellitus type 1 who use multiple daily insulin injections. The results indicate that the iPro®2 system could be helpful in maintaining better metabolic control as the part of daily clinical practice, since glycemic self-monitoring has significant limitations and does not provide sufficient information for the doctor in order to give suggestion for adequate insulin therapy to improve the metabolic control in child with type 1 diabetes mellitus. A professional iPro®2 device for continuous monitoring of glycemia worn for 7 days per month, would provide the doctor with much richer insight into the habits and behaviors of a child with diabetes through retrogradely monitored glycemic variability during the day and night. In our clinical experience, we noticed that visual demonstrations of this variability are easily grasped by both patients and their parents, which in turn help them to draw better conclusions on the influence of important events, such as physical activity and carbohydrate intake. With this device, all interested parties have an insight into the real events throughout 7 days and nights, and in addition to the estimated HbA1c, this should be used to correct the therapy in advance and prevent the occurrence of high HbA1c. Because of all above said, future studies are necessary to confirm the long-term effectiveness of iPro®2 devices for metabolic control.

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Adjunctive effect of the colloidal silver ions solution in the treatment of chronic periodontal disease: a preliminary clinical study

Dopunski efekat koloidnog rastvora jona srebra u lečenju hronične parodontopatije: preliminarna klinička studija

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Abstract

Background/Aim. Bacteria play the most important role in the development of periodontitis and chlorhexidine (CHX) is a "gold standard" in its treatment. Silver ions are also strong antiseptics, being used in dentistry for a long time. Therefore, the aim of this study was to compare the efficacy of CHX and colloidal silver ions solution (SSI) in the treatment of patients with chronic periodontitis. The additional aim was to ascertain silver ions, tolerability and efficacy comparing to CHX. **Methods.** Twenty-nine examinees of both sexes (the average age 50.6) participated in this study and were divided into two groups. The patients in the first group (n = 15) suffering from a generalized moderate chronic periodontitis, after scaling and root planning (SRP), were treated by rinsing of periodontal pockets with 0.2% solution of CHX. The patients of the second group (n = 14), in addition to the treatment of periodontal pockets, were treated with a 5 mg/mL colloidal SSI. **Results.** During the periodontal treatment, the mean values of all clinical parameters (except clinical attachment loss – CAL), in the both groups of patients were statistically significantly lower ($p < 0.001$) in relation to the initial values. The greater reduction of periodontal bleeching on probing (BOP) depth after one month was found in the SSI treated group (0.97 mm) in relation to the CHX group (0.65 mm). The local application of CHX and SSI led to statistically significant reduction of gingival parameters (gingival index – GI and BOP) in the groups after the treatment (GI for 0.65 and 0.87; BOP for 0.31 and 0.33, respectively). **Conclusion.** The results of our study showed that colloidal SSI was at least equally effective in the treatment of patients with periodontal disease as the solution of CHX. Additionally, the SSI is simple for use which speaks in favor of its more extensive use in dentistry including chronic periodontal disease.

Key words:
periodontal diseases; periodontal index; chlorhexidine; silver.

Apstrakt

Uvod/ Cilj. U nastanku parodontopatije bakterije imaju vodeću ulogu, a hlorheksidin (CHX), predstavlja „zlatni standard" među antisepticima u terapiji ove bolesti. Joni srebra, takođe jaki antiseptici, koriste se odavno u stomatologiji. Imajući to u vidu, cilj ovog rada bio je da se uporedi efikasnost CHX i koloidnog rastvora jona srebra (SSI), kao adjuvantnih antiseptika u okviru kauzalne terapije *scaling and rooth planing* (SRP) kod obolelih od hroničnog oblika parodontopatije. Dodatni cilj bio je da se utvrdi da li je SSI podnošljiviji za pacijente, a jednako efikasan rastvor kao i CHX. **Metode.** U studiji je učestvovalo 29 ispitanika oba pola (prosečne starosti 50.6 godina), podeljenih u dve grupe. Prvu grupu (n = 15) sačinjavali su ispitanici oboleli od hroničnog oblika parodontopatije, kojima su nakon SRP parodontalni džepovi ispirani 0,2% rastvorom CHX. U drugoj grupi (n = 14), nakon završene kauzalne terapije, parodontalni džepovi su ispirani rastvorom SSI koncentracije 5 mg/mL. **Rezultati**. Tokom parodontalne terapije, srednje vrednosti svih kliničkih parametara (osim gingivalnog džepa), kod obe grupe pacijenata su se statistički značajno smanjile u odnosu na početne vrednosti. Najveće smanjenje dubine parodontalnih džepova, nakon mesec dana, zabeleženo je u SSI grupi (0,97 mm) u poređenju sa CHX grupom (0,65 mm). Lokalna aplikacija rastvora CHX i SSI takođe je dovela do statistčki značajnog smanjenja gingivalnih parametara [gingivalni indeksi (GI) krvarenja na dodir (BOP)] u grupama posle lečenja (GI za 0,65 i 0,87); (BOP za 0,31 i 0,33). **Zaključak.** Rezultati su pokazali da je koloidni SSI najmanje jednako efikasan u terapiji kod osoba sa hroničnom parodontopatijom kao i CHX. Pored toga, CHX je jednostavan za upotrebu, bez neželjenih efekata, što ga preporučuje za širu upotrebu u stomatologiji, uključujući i adjuvantnu terapiju hroničnog oblika parodontopatije.

Ključne reči:
periodontalne bolesti; periodonatlni indeks; hlorheksidin; srebro.

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Introduction

The problems caused by periodontitis have been identified as serious health issues in many populations for a long time [1]. Chronic periodontitis has a slow to moderate rate of disease progression, which may be associated with local predisposing factors, such as dental plaque, subgingival calculus deposits, some iatrogenic factors and systemic diseases, such as diabetes mellitus [2]. The severity and extent of periodontal tissues damage vary from person to person and depend largely on the individual immune responses to microorganisms [3, 4]. Through their products, such as acids, endotoxins, antigens, the microorganisms cause changes in the periodontium, ranging from gingivitis, inflammation of the alveolar bone, and formation of periodontal pockets, to the terminal destruction of alveolar bone and loss teeth [5].

Nowadays, a very efficient and widely accepted periodontal therapy is a mechanical removal of bacterial biofilm and bacterial toxins from the teeth surfaces [6]. This kind of therapy including scaling and polishing the teeth surfaces and periodontal pocket curettage is also known as a causal therapy (Scaling and Root Planing – SRP). SRP is applied in the first phase of the treatment for all patients with periodontitis, regardless of the type and severity of the disease as well as the future therapy direction and course.

Routine dental checks show significantly better results of the treatment when local antiseptics combined with a causal therapy were used, compared to the cases when causal therapy was implemented without the additional therapy [7]. Chlorhexidine (CHX) is certainly one of the most widely studied antiseptic with the outstanding plaque inhibitory properties and as a such, is considered the „gold standard“ for the adjuvant treatment of patients with periodontal disease [8]. It provides a constant bactericidal and fungicidal effect for more than six hours [9]. Depending on concentration and sensitivity, CHX can act both as bacteriostatic and bactericidal agent [10] and can be used in a form of a solution spray or perio-chips [11]. Some studies suggest the adverse effects of CHX, such as discoloration of teeth (dark brown pigmentation), numbness of the tongue dorsal surface and some taste disturbances. More serious side effects include an extensive erosion of the oral mucosa and swelling of parotid glands [12].

On the other hand, silver is a non-toxic, very powerful disinfectant, which can significantly reduce bacterial infection [13]. The use of silver as a strong antiseptic is very frequent today, but it has not been tested sufficiently in the field of periodontics. The effects of silver ions are mostly based on three mechanisms: firstly, interaction with the DNA of bacteria, secondly, the destruction of the cell membrane, and thirdly, the blocking of essential enzymes that regulate transport of electrons [14]. Silver has a long lasting bacteriostatic effect because it is bind to proteins tissue and chlorides. The resulting compounds gradually release silver ions. Higher concentrations have a caustic effect by depositing proteins [15].

Some recent studies have provided data on the use of silver nitrate solution in aphthous stomatitis treatment during the past years [16]. Additionally, a strong antimicrobial effect of silver nitrate solution (0.5 μg/mL) on microorganisms that cause periodontal disease is described in some *in vitro* studies [17]. Some authors investigated the effect of long-term release of silver ions from resorptive periochips soaked in 12% silver nitrate, set in periodontal pockets [18]. Recently, tests have been conducted on the impact of silver zeolite enriched with extracts of polyphenols from an alga *A. nodosum* investigating the potential role of this solution in the prevention of periodontal disease [19].

According to our electronic data base search, there was no information in the existing literature on the effects of aqueous silver ions solution on subgingival dental plaque microorganisms and periodontal tissue during the causal therapy phase for chronic generalized periodontitis. Therefore, the aim of this study was to compare the efficacy of CHX as a “golden standard” in the treatment of patients with periodontitis with silver ions, which have not been used so far in this respect. The assumption was that silver ions, in addition to better tolerability, would be at least equally effective as CHX.

Methods

A randomized prospective clinical study was conducted at the Periodontology Department and Implantology Department of the Clinic for Dental Diseases, the Military Medical Academy in Belgrade. Twenty-nine examinees of both sexes (26 men and 3 women, the average age 50.6) participated in the study and they were divided into two groups. The patients from the first group (n = 15) were treated by rinsing with 0.2% solution of CHX after SRP of periodontal pockets. The second group (n = 14), in addition to the treatment of periodontal pockets, was treated with a 5 mg/mL colloidal silver ions solution (SSI).

The parameters for inclusion of patients into the study were as follows: clinical diagnosis of generalized chronic periodontitis with radiographic confirmation of the presence of alveolar bone resorption (≥ 30%), patients who had a sufficient number of teeth in the upper and lower jaw (≥ 20) and those who had ≥ 5 sides of the teeth with periodontal pockets whose depth was ≥ 5mm. The study excluded the patients whose treatment of periodontitis was conducted more than 12 months ago, the presence of systemic diseases that may affect the treatment of periodontal disease, the use of antibiotics and anti-inflammatory drugs in the last 6 months, pregnancy, lactation and the use of contraception drugs as well as systemic infections of the oral cavity.

After taking a medical history and making diagnosis and indications for the treatment of the periodontal disease, the examinees were offered a form of voluntary consent for participation in the study for which we received the approval of the Ethics Committee of the Military Medical Academy in Belgrade.

All teeth were measured (third molars were not included) with a graduated probe (the community periodontal index of treatment needs – CPITN: US, Williams, Pro-Dentec, Batesville, Ark). For the assessment of oral hygiene the following indices were used: Plaque index (PI), measured on four sides (mesio-vestibular, vestibular, disto-vestibular,

oral), whose score was marked with values 0–3 [20]; Gingival index (GI), measured on the same four sides, whose score is also marked with 0–3 [21]. The GI score of 2 and 3 indicated bleeding on probing (BOP). In order to assess the periodontal status, the clinical attachment level (CAL) – the distance from the cement-enamel junction to the bottom of the periodontal pocket, was measured in mm, and the periodontal pocket depth (PPD) in mm – the distance from the free gingiva margin to the bottom of the periodontal pocket.

After the measurements were completed, the causal therapy was performed using a method of periodontal pockets curettage per a quadrant (in the following four days one quadrant was processed). After the curettage was conducted around each of the designated teeth, the application of a particular adjuvant antiseptic (SSI or CHX) was carried out by injecting 10 mL into the periodontal pockets. The patient was asked to mouthwash a given amount of antiseptic for 60 seconds and not to take food in a following hour after the treatment.

Each patient, depending on the allocated group, was given the same solution for home use for the following 10 days, with precise usage instructions. A month after scaling and root planning, including a particular adjuvant antiseptic, the control measurement of all parameters was performed in order to compare the values to the baselines.

Complete statistical analysis of data was done with the statistical software package, SPSS Statistics 18. Variables were presented as mean value ± standard deviation (SD). The Kolmogorov-Smirnov test was used for the evaluation of distribution of clinical data. A statistical significance within and between groups was tested by *t*-test for the paired and independent samples. All the analyses were estimated at $p < 0.05$ level of statistical significance.

Results

The results of the study are shown in Table 1 and Figure 1.

Table 1 shows the mean values of clinical parameters before and one month after the treatment in both groups of patients. During the SRP treatment, the mean values of all clinical parameters (except CAL), in both groups of patients, were statistically significantly lower ($p < 0.001$) comparing to the initial values. The greater reduction of periodontal probing depth after one month was found in the SSI treated group (0.97 mm) comparing to the CHX group (0.65 mm), however, without a statistical significance ($p = 0.420$).

The values of CAL inside the studied groups after the treatment were little lower in relation to their initial values, without statistically significant difference.

A local application of CHX or SSI led to the statistically significant reduction of gingival parameters (GI and BOP) in the groups after the treatment. There were no differences found between those two groups of patients after the treatment ($p = 0.492$ and $p = 0.918$, respectively). The index of oral hygiene (PI) was statistically significantly lower in both groups of patients after the treatment ($p = 0.001$), but it was also lower in the SSI group in relation to the CHX group ($p < 0.05$).

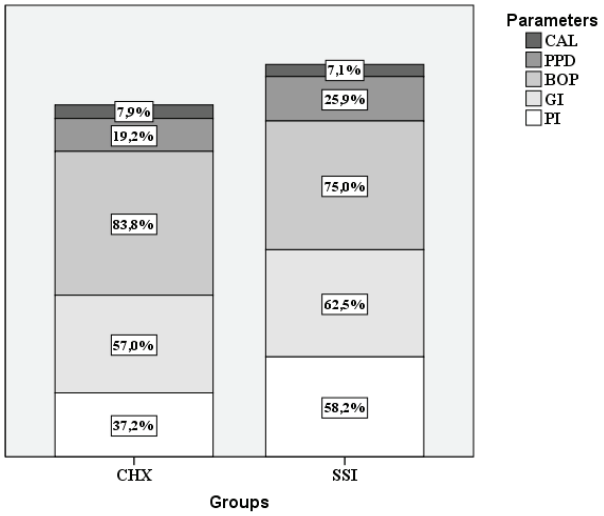

Fig. 1 – The percentage values of clinical parameters shown in Table 1 (see abtreviations) one month before and after the SRP treatment in the groups in which the solution of CHX and SSI were applied.

Table 1

Mean values of clinical parameters one month before and after the treatment in both groups of patients

Groups (before/ after treatment)	PI mean ± SD	GI mean ± SD	BOP mean ± SD	PPD mean ± SD	CAL mean ± SD
CHX (n = 15)					
baseline	0.86 ± 0.08	1.14 ± 0.46	0.37 ± 0.24	3.39 ± 0.67	3.64 ± 1.43
month 1	0.54 ± 0.27	0.49 ± 0.30	0.06 ± 0.07	2.74 ± 1.01	3.35±1.37
p_1	< 0.001	< 0.001	< 0.001	< 0.001	< 0.109
SSI (n = 14)					
baseline	1.22 ± 0.34	1.39 ± 0.34	0.44 ± 0.26	3.74 ± 0.74	3.65 ± 1.02
month 1	0.51 ± 0.37	0.52 ± 0.36	0.11 ± 0.16	2.77 ± 1.02	3.39 ± 1.14
p_1	< 0.001	< 0.001	< 0.001	< 0.001	0.069
p_2	< 0.05	0.492	0.918	0.420	0.913

p_1 – significance within the groups before and after the treatment; p_2 – significance between the groups after the treatment; PI – plaque index; GI – gingival index; BOP – bleeding on probing; PPD – periodontal probing depth; CAL – clinical attachment level; CHX – chlorhexidine; SSI – solution silver ions.

Figure 1 shows that the highest reduction of percentage values among clinical parameters was found concerning the BOP. In the CHX group, it was 83.8% lower, while in the SSI group it was somewhat even more lower (75%). The reduction of the percentage values in the GI values was also favorable in both groups as well as the PI values after the application of SSI (62.5% and 58.2%, respectively). It was distinct in relation to the CHX group amounted to 57% and 37.2%, respectively. The periodontal parameters also had lower values after the SRP treatment in both groups in which the corresponding solutions were applied. The higher percentage reduction was found in PPD, amounting in the CHX group of patients to 19.2%, and in the SSI group even more significant reduction (25.9%). The value of CAL was approximately equally lowered in both groups of patients (CHX = 7.9%; SSI = 7.1%).

Discussion

Our results showed that the CHX and SSI as adjuvant antiseptic within the SRP have an equal, statistically significant effect on all studied clinical parameters PI, GI, BOP, PPD, CAL in the patients with a chronic form of periodontal disease. The most significant result was a reduction in the PPD – it was reduced in the SSI group to 0.97 mm compared to the CHX group where the reduction was 0.65 mm. Very good results in reducing the PPD were also obtained by other authors who used CHX as a control with the SRP. The PPD values for CHX ranged from 6.28 mm to 4.90 mm one month after the therapy [22]. A significant improvement in the PPD reduction was shown by Krück et al. [21], whereas for the same parameters Cobb [22] obtained the value of 1.29 mm. These results were explained primarily by the antiseptic influence on the growth of aerobic bacteria and the reduction of the total number of anaerobic bacteria which may consequently affect the anti-inflammatory processes and the impaired maturation of plaque after conducting the causal therapy with antiseptics irrigation.

In our study, we demonstrated that the SSI led to a significant reduction in PPD because of its successful antibacterial efficiency. This efficacy against periodontal pathogenic species was also shown in some other studies. The compounds containing silver are attractive because of their strong antimicrobial activity, high stability and wide-spread antibacterial spectrum [23]. The results of Reise et al. [24] indicate that silver compounds 3 and 4 represent the new possible antibacterial agents to be used in different therapeutic procedures of periodontitis, caries and endodontic diseases.

The values of the CAL within our tested groups were slightly lowered after the treatment, and thus there was not statistically significant difference whithin group as well as between the CHX and SSI group. In the study [25] that compared the CHX effects with other antiseptic, there were also no statistically significant differences in terms of this parameter. Their studies showed that 15, 30 and 60 days after the treatment, no statistically significant effect on the CAL level was noted ($p = 0.21$).

For the assessment of oral hygiene, the PI was used, which was statistically significantly reduced after the treatment in both of the tested groups ($p = 0.001$); however, it was significantly lower in the SSI group compared to the CHX group ($p < 0.05$). We can explain these results by the fact that silver ions prevent adhesion of bacteria and thus influence the prevention of biofilm formation which precedes creation of dental plaque [26]. It is also important to emphasize that testing of silver activity against periodontal pathogens from the biofilm has been increased recently. The study of Lu et al. [25] also focused on the antibacterial effect of silver nano-particles of various sizes on anaerobic bacteria – the smallest tested nano-particles (5 nm) showed greater antimicrobial effects compared to larger particles (15–55 nm).

Yilmaz and Bayindir [26] got almost identical results at the level of testing the CHX effects on the PI a month after the therapy, with a significant reduction in the PI value. They explained this as the CHX ability to penetrate into the areas that are inaccessible for mechanical instruments. The CHX ability to inhibit the plaque growth and its formation, in the first hours after mouthwashing, is reflected in its high affinity for the oral cavity tissues and a tooth surface [27, 28]. Otherwise, according to the CHX chemical definition, it is a positively charged cationic bisbiguanide, which has a very high ability to attract negatively charged areas, including mucous membrane, salivary dental pellicle as well as various biofilm components on a tooth surface such as bacteria, extracellular polysaccharides and glycoproteins [29, 30]. Thus, CHX represents an excellent antimicrobial agent of a broad spectrum which significantly reduces growth and development of both facultative and obligate anaerobic bacteria inside the dental plaque [31]. This was reflected in our results as the positive clinical effect on GI and BOP which were statistically significantly lower one month after the treatment ($p < 0.001$); however, similar result was noticed for the SSI, too.

Considering the CHX effect in the case of these parameters, similar results were obtained in the study of Sağlam et al. [30], where a month after the treatment, BOP was decreased to approximately 79% and GI to 0.66 mm. However, CHX has a high cytotoxic effect on a human periodontal ligament content by inhibiting the double-stranded nucleic acid molecules, protein synthesis and mitochondria activity [32]. Pucher and Daniel [33] also demonstrated that CHX is cytotoxic for human fibroblasts through inhibition of protein synthesis.

Following the positive antibacterial effects of different sizes of silver nanoparticles on anaerobic bacteria, the assumption is that this effect stems from silver adstrigent potential associated with the direct or even indirect antibacterial effects of silver ions [34]. Passing through the bacteria cell wall, silver ions prevent replication of the DNA molecule. In this way, the interactive inactivation of bacterial proteins occurs and as a result of the catalytic activity of silver**,** oxygen radicals are released, which cause structural damages inside the bacteria. This phenomenon leads to damage or even the death of bacteria.

Conclusion

The use of adjuvant antiseptics within the SRP for patients with chronic forms of periodontal disease has been justified. In our study, the tests showed that the CHX and SSI as adjuvant antiseptics as a part of the SRP, have practically the same statistically significant effect on all the studied clinical parameters. In the SSI group, a month after the therapy, a significant reduction of GI and PI was obtained compared to the CHX group. This is of great importance, because the SSI was not used as the adjuvant antiseptic in this category of patients and, as the CHX was accepted as a "gold standard" of this type, it seems that silver ions should be re-evaluated for adjuvant treatment within the causal therapy of patients with chronic periodontal disease.

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CURRENT TOPIC

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Dried blood spot: utilising dry blood for pharmacokinetic investigations – an old method with great future for therapeutic drug monitoring

Osušena krvna mrlja: upotreba osušene krvi za farmakokinetička istraživanja – stara metoda sa velikom budućnošću za terapijsko praćenje lekova

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Key words:
dried blood spot testing; chromatography, liquid; mass spectrometry; pharmacokinetics; therapeutic equivalence; metabolic diseases; neonatology; hiv.

Ključne reči:
krv, osušena mrlja, testiranje; fragmentografija mase; farmakokinetika; terapijska ekvivalentnost; metabolike bolesti; neonatologija; hiv.

Introduction

Dried blood spot (DBS) is a biological sampling of the full blood. Although it was initially described back in 1910s, this technique started to be explored during 1960s by Guthrie and Susi [1] for neonatal screening for phenylketonuria [2–5]. Since then, DBS has been used in screening for metabolic disorders, neonatal human immunodeficiency virus (HIV) infections and therapeutic drug monitoring (TDM). In 2009, DBS was implemented in development program in the US, for pediatric anti-HIV campaign (MK-8931) due to its minimal invasiveness and potential use as the sole matrix for phase 3 studies for Alzheimer's disease [6].

Using aseptic technique, blood is withdrawn from patients using puncture by micro blood lancet. The first sample has to be discarded because of potential contamination with tissue fluid. The following sample is then transferred to previously marked area on a specially designed filter paper and dried for defined time at room temperature [6, 7]. Drying is done on non-adsorbing area and presents an important step because humidity increases a risk of microbiological contamination of the sample. After drying, paper is stored in a waterproof plastic bag, ideally with desiccant and humidity indicator, and the sample is ready for further processing [2].

Various methods for dried blood spot sample processing and analysis

Various techniques have been used for DBS sample processing and analysis. Sample dilution, filtration and centrifugation are commonly used [6]. For sample analysis, immunoassays are used and they normally require long incubation, gas chromatography and sample derivatization. Modern techniques such as desorption electrospray ionization and direct analysis in real time combined with mass spectrometry have shown promising results, but sensitivity of analysis is not as good, and may compromise findings. Loss of sensitivity may be attributed to the absence of chromatographic extraction and efficiently detectable functional groups. New technologies, based on liquid chromatography with mass spectrometry (LC-MS) have better sensitivity, selectivity and speed. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) showed best results and its use is increasing worldwide [5].

Coupled plasma mass spectrometry is used for determination of metals in traces, mainly essential metals including human immunodeficiency virus cuprum, zinc, molybdenium and selenium (Cu, Zn, Mo and Se, respectively), and non-essential metals including arsenic, cadmium, mercury and lead (As, Cd, Hg and Pb, respectively) in DBS sample. Thus, as

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the DBS methodologies are refined further, they have the potential to be used in epidemiological studies for detection of metals in traces in newborns [9].

Potential uses of dried blood spot

The DBS applications are becoming more common in clinical trials, especially epidemiological ones. A study by Norwegian Breast Cancer Screening Program (NBSCP) included 4,597 women to whom questionnaires and kits for DBS and saliva were delivered and results showed to be promising. The aim of the study was to determine concentration of vitamin D and carotenoids (lutein, zeaxanthin, β-cryptoxanthin, α-caroten, β-caroten and lycopene) and to compare results with results obtained using different analytical methods. Seventy-one percent of 4,597 women returned their DBS samples, and 93% of those analyses showed similar results. High-performance liquid chromatography (HPLC) with the UV detection and liquid chromatography with mass spectrometry were used for vitamin D and carotenoids detection**.** Total number of 381 samples, chosen according to selected criteria (age, energy intake and body mass index) were analyzed [7].

In order to optimise accuracy of the DBS results, the value adjustment may be required. This will accommodate the difference between full blood and serum and plasma levels of specific biomarkers or drugs. One way to obtain plasma concentrations is by multiplying the DBS values by two. This took into consideration that hematocrit value in adult women is approximately 50%. When using the value adjustment, one study of vitamin D and carotenoids concentrations in plasma showed that values in plasma were similar to results obtained in DBS. Accordingly, the DBS technique can be used efficiently in large epidemiological studies. Considering that patients perform sampling by themselves and then send the samples via mail, a huge reduction of costs represents an important advantage which could, after additional studies and validations, be encouraging factor in wider use of this technique [7].

Study on 10 patients admitted to the Urgent Care Center of the Hospital del Mar in Barcelona, due to acute intoxication with psychoactive substances, had DBS sampling and analysis of 23 psychoactive substances and their metabolites by using ultra-high performance liquid chromatograph with mass spectrometry (UHPLC-MS). Analysis was completely validated. Results indicated a possibility of DBS sampling use in noninvasive monitoring for the presence of psychoactive substances or intoxication [4]. The analysis is possible even post-mortem [10].

The possibility of the DBS use in pharmacokinetic studies is interesting. As most clinical studies in phase I include plasma sampling, because of the advantages of DBS, its potential use should be further studied. Certain studies are ongoing and with proper communication with regulatory authorities the aim is to improve and validate methods which would enable the use of DBS in clinical pharmacokinetics/pharmacodynamics studies [6]. In case DBS is validated for the use in pharmacokinetic studies, it can be used in monitoring of tacrolimus in renal transplant recipients on triple immunosuppressive therapy, since the data showed that monitoring of tacrolimus blood dose is necessary in the early post-transplant days [11–13].

It has also been shown that the detection and genotypisation of hepatitis C virus is possible on the basis of the DBS samples [14, 15].

Therapeutic monitoring of antiepileptic drugs contributes to individualization and optimization of the treatment. This is important due to intra- and inter-individual variabilities in concentrations which consequently affect frequency and severity of adverse events. Development and validation of specific protocols for quantification of certain antiepileptic drugs are needed for the routine implementation of DBS in TDM of antiepileptic drugs [16] and monitoring of the adherence [17].

Methotrexate (MTX) is an antirheumatic drug often used in therapy of juvenile idiopathic arthritis (JIA) and juvenile dermatomyositis (JDM) in children. Inter-individual concentrations vary and thus affect profile of adverse events. Monitoring of adherence is very important, but also difficult because 95% of the MTX dose is metabolized within 24 hours after administration. This is possible by monitoring methotrexate polyglutamates (MTXPG) [18]. MTXPG are synthesized intracellularly by γ- linked sequential addition of glutamic acid residues to MTX mediated by enzyme folylpolyglutamate synthase and can be found in erythrocytes long after MTX was cleared from plasma. The LC-MS method for the analysis of MTXPG from the DBS sample was described and validated, and it showed linearity and precision. It enables the detection of lower MTX concentrations, which is important because MTX doses in JIA and JDM treatment are significantly lower than in cancer treatment. Along with LC-MS/MS, its use is possible when there are very small volumes (12 μL of the full blood) [19]. A validated LC-MS/MS method was developed for the determination of MTX and MTXPG in Caco-2 cells exposed to MTX. This method showed to be more rapid and more sensitive compared with previously reported assays for MTX and MTXPG in other matrices [20, 21].

The insufficiencies of dried blood spot use

DBS has certain insufficiencies, which need detailed defining for better understanding, improvement and wider use of this technique. They are: due to small volumes of the sample, repeating the analysis is usually not feasible [22], some drugs are not stable at the room temperature, light and humidity influence of hematocrit [3, 23, 24].

While the repeatability of the small sample analysis can be solved by using more sophisticated analytical technique and stability can be improved by upgrading the storage containers for the DBS sample, the influence of the hematocrit attracted especial attention and it is the most investigated obstacle to the use of DBS. There are two main reasons why hematocrit influences analysis after the DBS sampling: blood viscosity, which is in proportion with hematocrit value, affects volume of the blood on the filter paper of precisely de-

fined diameter, ratio of blood cells count and plasma in the sample affects relative concentration of the analyte.

Considering that DBS covers analysis of the full blood, hematocrit value will consequently affect concentration of analyte in regard to plasma. By using the equation shown below (1.0), concentration of the analyte in plasma can be predicted.

$$C_{plasma} = \frac{C_{blood}}{(1 - \mathrm{Hct}) + \mathrm{Hct} * f_u * \rho} \quad (1.0)$$

In case f_u is constant, the equation (1.0) can be presented in the following form :

$$C_{plasma} = \frac{C_{blood}}{1 - Hct} * f_\rho \quad (1.1)$$

where C_{plasma} is the concentration of the analyte in plasma, C_{blood} is the concentration in full blood, Hct is hematocrit value, f_u is unbound fraction in plasma, ρ is erythrocyte-to-plasma concentration ratio and f_ρ is the fraction in plasma.

In 2013, the authors Capiau et al. [25] showed linear correlation between potassium concentration and hematocrit in a range of 0.19–0.63, with acceptable accuracy and precision. By measuring concentration of potassium in the DBS sample, hematocrit can be easily determined, and by using above described equations, concentration of the analyte in regard to plasma can be easily calculated . The main lack of this method is the use of part of the DBS sample for analysis of potassium concentration which also requires separate preparation of the sample. To overcome this issue, Capiau et al. [25] showed that measuring hematocrit in the DBS sample can be done via noncontact diffuse reflectance spectroscopy. In this case, the whole quantity of the sample is preserved and a possibility of an error is decreased because the preparation of the sample is not needed.

Advantages of dried blood spot over venipuncture liquid blood sample storage containers as blood sample collection and storage method

Due to numerous advantages, this sampling technique became interesting and its potentials drew investigators attention. The advantages of DBS over venipuncture are: minimally invasive technique, less painful than venipuncture, smaller volumes of blood are sampled (15 ± 5μL per one marked area of the filter paper) and therefore it is more convenient for the use in newborns and small children [3,10, 22], cost-effective [3], patients can perform self-sampling at home after proper instruction and send it to a laboratory via mail [7, 26], suitable transport and storage during the transport of the DBS samples of the patients infected with HIV or HCV, a possibility of contamination is minimal and this kind of samples can be sent even via mail [2–4, 10], good stability of analyte in sample [3, 10, 27]; use of DBS in preclinical trials supports ethical approach to animals because sampling from tail vein is possible [22].

Conclusion

Using optimised sample preparation and analysis, the DBS technique has significant potential applications in preclinical and clinical trials, therapeutic and toxicological drug and poison monitoring as well as large epidemiological trials. DBS represents a cost-effect model of drug analysis and can provide much needed pharmacokinetic results in an efficient and robust manner.

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CASE REPORTS

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The role of a captopril renal scintigraphy in examination of children with hypertension – A case report

Uloga kaptoprilske scintigrafije u ispitivanju dece sa hipertenzijom

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Abstract

Introduction. Secondary hypertension is a relatively common form of hypertension in childhood with renovascular hypertension being responsible for 5%–10% of all arterial hypertensions in children. An early diagnosis of renovascular hypertension is important when considering an appropriate treatment of hypertension that may prevent or slow further progression of kidney damage. To validate the usefulness of a captopril renal scintigraphy in hypertensive children, we report a case of a 16-year-old female patient with a history of hypertension as a result of fibromuscular dysplasia. **Case report.** The patient was asymptomatic with elevated blood pressure revealed by a routine physical examination. Laboratory tests showed the increased levels of plasma renin activity with the normal levels of aldosteron. The renal ultrasound was normal. The Doppler of the renal arteries showed no significant differences of resistive index. A renal captopril scintigraphy was performed, including two day study protocol, the baseline study followed by another captopril study several days later. The scintigraphy showed the abnormal baseline and captopril renogram curve of the right kidney with significant cortical retention of radiotracer after angiotensin-converting enzyme inhibition. Following the captopril scintigraphy a renovasography was obtained confirming the presence of a 2 mm long circular narrowing of the right renal artery. It was immediately treated resulting in a significant expansion of the lumen. **Conclusion.** The captopril renal scintigraphy allows non-invasive functional testing in a selected group of hypertensive children, which can either confirm or rule out the existence of hemodynamically significant renal artery stenosis.

Key words:
hypertension, renovascular; child; diagnostic techniques and procedures; radionuclide imaging; captopril; renal circulation.

Apstrakt

Uvod. Sekundarna hipertenzija je najčešći oblik hipertenzije kod dece i renovaskularna hipertenzija je odgovorna za 5%–10% svih hipertenzija. Pravovremeno otkrivanje renovaskularne hipertenzije je važno jer omogućuje specifičan tretman koji vodi do odgovarajućeg lečenja hipertenzije, čime se utiče na prevenciju nastanka i dalje progresije parenhimskog oštećenja bubrega. U cilju potvrde korisnosti kaptoprilske scintigrafije bubrega u skriningu hipertenzivne dece prikazujemo slučaj šesnaestogodišnje devojčice sa istorijom hipertenzije uzrokovane fibromuskularnom displazijom. **Prikaz slučaja.** Bolesnica je bila bez simptoma. Povišen krvni pritisak je otkriven prilikom rutinskog sistematskog pregleda. Laboratorijske analize su otkrile povišene vrednosti reninske aktivnosti plazme uz vrednosti aldosterona koje su bile u granicama referentnih vrednosti. Ultrasonografski nalaz bubrega bio je uredan. Dopler krvnih sudova bubrega nije ukazivao na prisustvo signifikantne razlike u vrednostima indeksa otpora. Urađena je kaptoprilska scintigrafija bubrega, koja uključuje bazalnu studiju dinamske scintigrafije bubrega, a potom ponovljenu dinamsku scintigrafiju bubrega sa premedikacijom kaptoprilom. Kaptoprilska scintigrafija ukazala je na patološki nalaz bazalne i kaptoprilske renografske krivulje desnog bubrega sa značajnom kortikalnom retencijom radioobeleživa nakon inhibicije angiotenzin konvertujućeg enzima. Nakon kaptoprilske scintigrafije, renovazografija je potvrdila prisustvo cirkularnog suženja desne renalne arterije u dužini od 2 mm. Neposredno nakon potvrde stenoze desne bubrežne renalne arterije urađena je balon dilatacija i postignuto je značajno proširenje lumena. **Zaključak.** Kaptoprilska scintigrafija bubrega omogućava neinvazivno funkcionalno ispitivanje u selektovanoj grupi hipertenzivne dece koja može potvrditi ili isključiti postojanje hemodinamski značajne stenoze renalne arterije.

Ključne reči:
hipertenzija, renovaskularna; deca; dijagnostičke tehnike i procedure; scintigrafija; kaptopril; bubreg, cirkulacija krvi.

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Introduction

According to several epidemiological studies, the prevalence of hypertension in children is approximately 1%–3%, increasing with obesity in older children [1]. Secondary hypertension is a relatively common form of hypertension in childhood and renovascular hypertension (RVH) is responsible for 5%–10% of all arterial hypertensions in children [2]. RVH is defined as hypertension caused by renal hypoperfusion, usually due to anatomic renal artery stenosis (RAS) and the activation of the renin-angiotensin aldosterone system (RAAS). The critical degree of stenosis leads to a reduction in renal perfusion pressure below the range of autoregulation while the experimental hemodynamic studies indicate that measurable changes in perfusion pressure do not develop until the stenotic lesion cross section area is reduced by at least 70%–75%. The most common causes of RAS are fibromuscular dysplasia (FMD) in younger population and atherosclerosis in elderly population [2–3].

A captopril renal scintigraphy (CRS) represents a non-invasive functional test aiming to demonstrate both the RAAS activation and the lateralization of renin secretion by the kidney affected by a "hemodynamically significant" RAS. The captopril scintigraphy is based on the fact that the reduced renal perfusion pressure, due to renal artery stenosis, results in an angiotensin II-mediated efferent arteriolar vasoconstriction, as described by Gates [4]. The following rise of the transglomerular pressure gradient allows the kidney to maintain the glomerular filtration rate (GFR) even in the condition of the reduced perfusion pressure to the glomerulus. Premedication with an angiotensin-converting enzyme (ACE) inhibitior interrupts the renin–angiotensin system reducing the compensatory vasoconstriction of the efferent arteriole. The consequent dilatation lowers the glomerular transcapillary pressure, leading to a decrease in GFR. The decrease of the individual kidney GFR, induced by inhibition of ACE, can be evaluated by the captopril scintigraphy with the use of tubular agents, such as technetium-99m mercaptoacetyl triglycine (MAG3) or technetium-99m ethylenedicysteine (EC) or by glomerular agent, such as technetium-99m diethylene triamine penta-acetic acid (DTPA) [5].

Diagnosis of RVH is important since it enables a specific treatment leading to the cure of arterial hypertension that may prevent or slow progression of kidney damage. Nowadays, many tests are available for the evaluation of RVH [6]. The conventional angiography represents the gold standard used to diagnose renal artery stenosis mainly in adults. However, this is an invasive test with potential risks, usually requiring hospitalization and general anesthesia, principally in pediatric population. For these reasons, it is not universally performed for the evaluation of pediatric hypertension. Several non-invasive methods have been recommended for evaluation of RVH in children, but their specificity and sensitivity varies widely [7–9].

To validate the usefulness of the captopril renal scintigraphy in hypertensive children, we report a case of a 16-year-old female patient with a history of hypertension as a result of FMD.

Case report

A 16-year-old female with a history of high blood pressure (BP) was referred to our department by a pediatric nephrologists, for the renal captopril scintigraphy. The patient was asymptomatic with elevated blood pressure revealed by a routine physical examination. The family history of hypertension was positive (father suffering from hypertension and receiving antihypertensive therapy). Her physicial examination revealed a blood pressure of 170/95 mmHg (multiple readings taken from both arms).

During hospitalisation at the Pediatric Nephrology Department, a high BP was confirmed, with the value of 145/95 mmHg (which is more than 95% for her age, gender and height). Body mass index (BMI) was recorded to be 20.2 kg/m^2. Laboratory tests showed the increased levels of plasma renin activity with the normal levels of aldosteron. The blood urea nitrogen, creatinine and uric acid values were within the normal limits.

Urin analysis reported the elevated albumin/creatinin ratio and the patient tested positive for proteinuria and microalbuminuria (24 hour urine collection). The renal ultrasounds was normal. The doppler of the renal arteries showed no significant differences in resistive index (RI on *aa.arcuata* left was 0.6 and RI on right *aa. arcuata* was 0.56). Evidence of *retinopathia hypertensiva* gradus I–II was discovered during a fundus examination. There was no carotid, abdominal or femoral arterial bruits. The electrocardiography (ECG) was normal but the echocardiography showed left ventricular hypertrophy. The suggested therapy for her hypertension was a calcium channel blocker.

A two-day study protocol was performed including a baseline scintigraphic study followed by another dynamic scintigraphy several days later, the latter with the captopril premedication (25 mg of captopril) one hour before the test. The patient was hydrated with 10 mL of water per kg of body weight half an hour before both studies. The blood pressure (BP) was measured prior to the captopril intake and after 60 minutes of rest.

The study protocol for the basal and captopril scintigraphy implies the patient being in the supine position with the gamma camera facing the lower back of the patient, with the kidneys, heart and bladder in the field of view. Starting immediately after the intravenous injection of 99mTc-MAG3 (Mallinckrodt Ltd.) at a dose according to an international scale, serial 10-second/frame images were obtained for 30 min. A single-headed large field of view gamma camera (Symbia, Siemmens) equipped with a general-purpose parallel-hole collimator was used. The analyses were made using the software for renal scintigraphy. Regions of interest (ROI) were manually drawn around the whole kidney and perirenal background as well. The renographic curves of the right and left kidneys were analysed separately and the relative function of each kidney (in percentage) was calculated as well as the functional renal indices [10].

The CRS showed the abnormal baseline and captopril renogram curve of the right kidney with significant cortical retention of radiotracer after the ACE inhibition. During the

baseline study, the functional renal indices of the right kidney (Tmax and the 20 min/peak min count ratio) had pathological values, showing significant deterioration after the ACE inhibition (Figures 1 and 2). The relative kidney function of the right kidney was 45%. According to the diagnostic criteria based on the consensus report, these findings were indicative of a high probabillity of renal vascular hypertension [11].

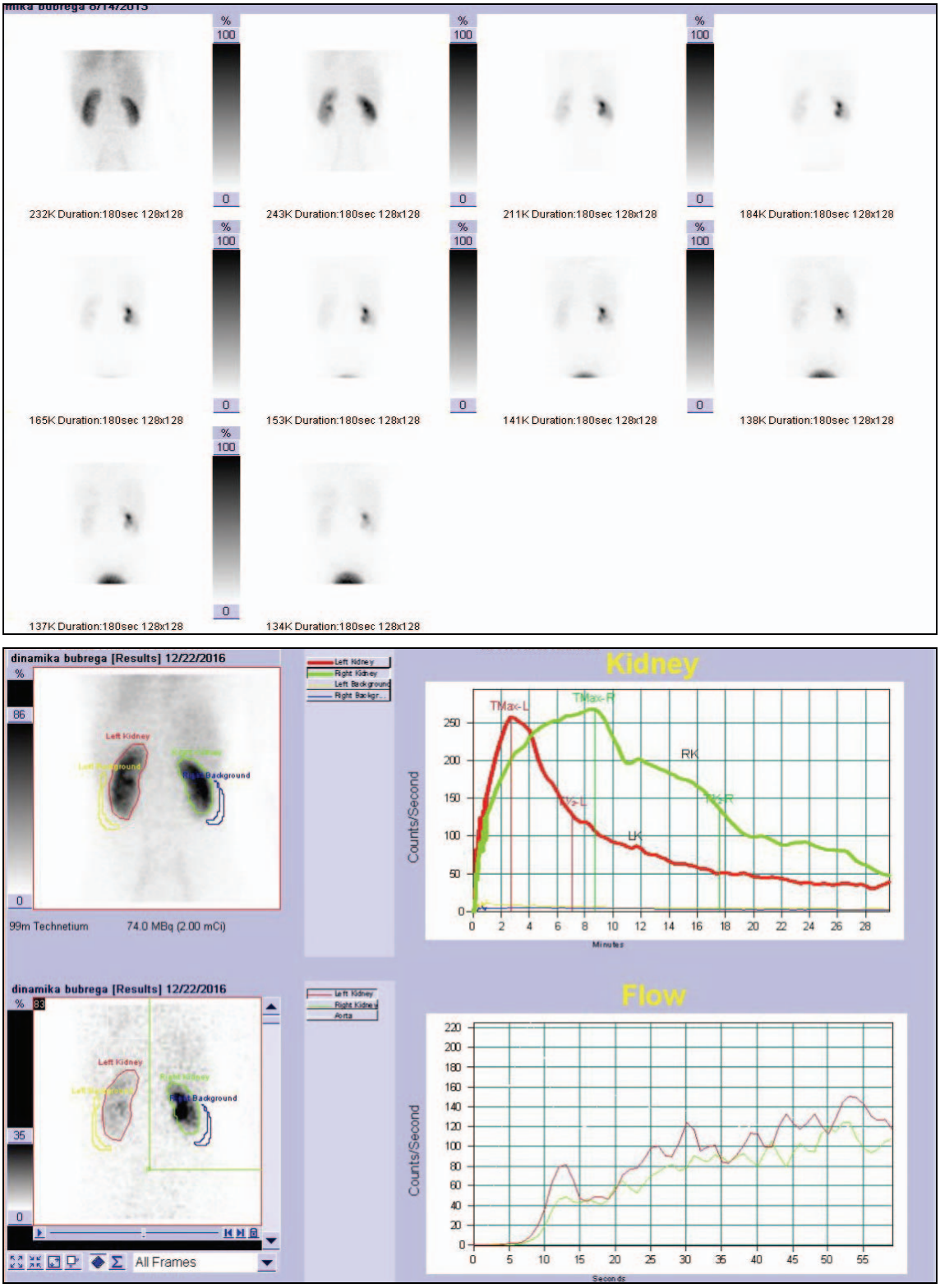

Fig. 1 – Baseline study – unilateral retention of radiotracer in the right kidney with the abnormal baseline renogram curve. The time to maximum counts of the right kidney was 8.6 minutes and to the left kidney was 2.6 minutes. A 20 min/peak min count ratio of the right kidney was 0.39 – prolonged transit time. A 20 min/peak min count ratio of the left kidney was 0.15 – normal transit time.

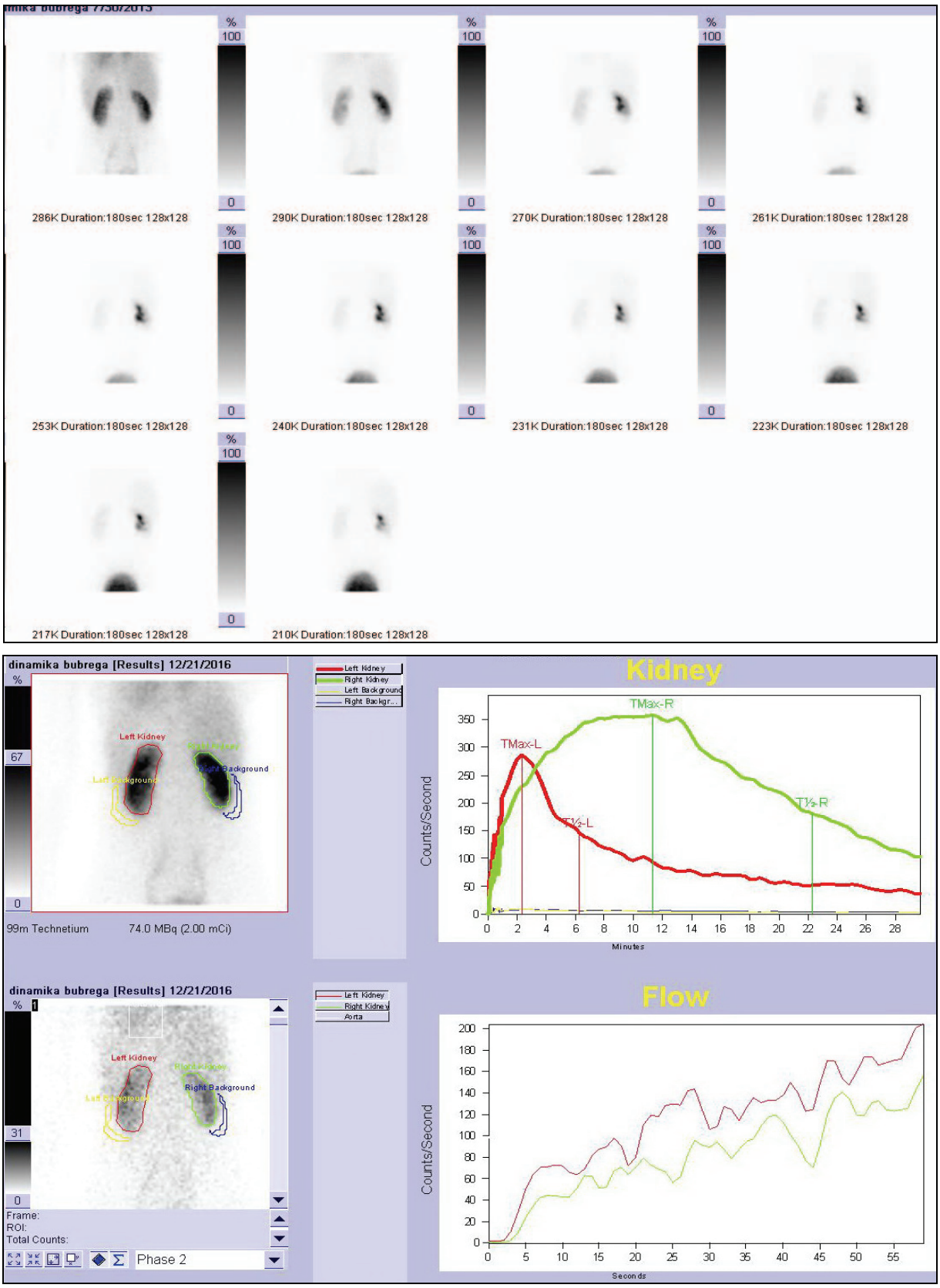

Fig. 2 – Captopril study – marked parenchymal retention after angiotensin-converting enzyme (ACE) inhibition of radiotracer and the abnormal renogram curve of the right kidney. Time to maximum counts of the right kidney after ACE inhibition was 11.6 minutes and 20 min/peak min count ratio of the right kidney was 0.65 – a significant increase in T max and prolongation of transit time from the baseline study.

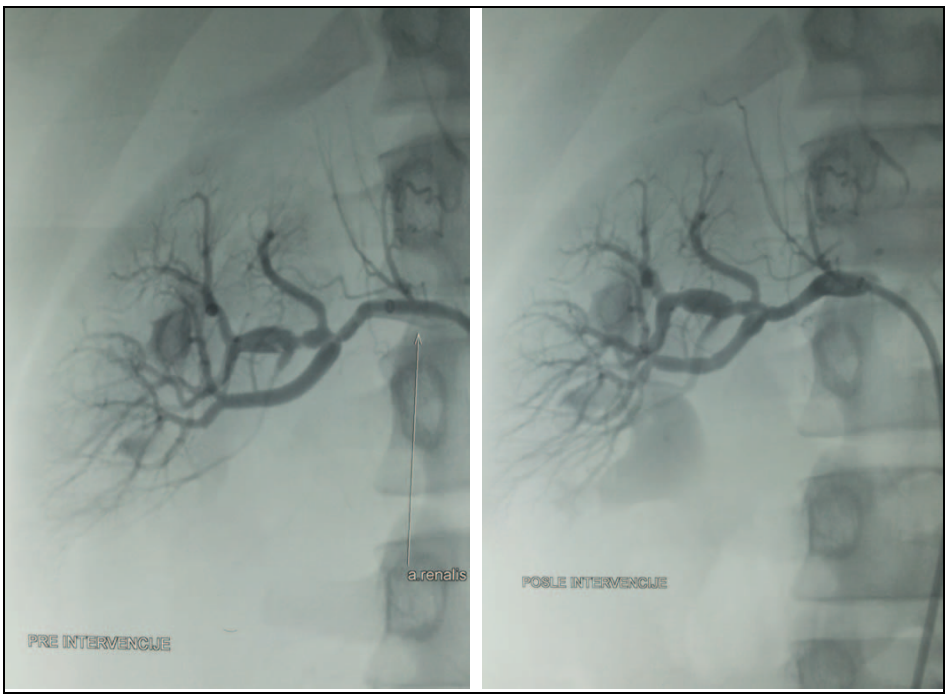

Fig. 3 – The renal angiography: Before the procedure, the presence of a circular narrowing of the right renal artery; After a percutaneous balloon dilatation, a significant expansion of the lumen was achived.

Following the captopril scintigraphy a renovasography was performed, confirming the presence of a 2 mm long circular narrowing of the right renal artery. It was immediately treated by a percutaneous balloon dilatation of the stenosis on the bifurcation of the renal artery and two segmental branches resulting in a significant expansion of the lumen (Figure 3).

The follow-up captopril renal scintigraphy showed significantly improved parenchymal indicators of the right kidney without any signs of cortical retention of radiotracer (Figure 4). The repeated renin activity plasma test was within the normal limits. The patient's blood pressure normalized over a period of several months resulting in the discontinuation of antihypertensive therapy. The patient's BP is currently within the normal limits and she actively plays sports.

Discussion

Arterial hypertension poses a significant risk factor for cardiovascular, renal and cerebrovascular diseases. In childhood, arterial hypertension is usually of the secondary origin, mainly due to a renal disease. Since RVH is potentially treatable, it is essential to identify these patients.

As discussed above, conventional angiography is considered the gold standard in diagnosis, but it is invasive, relatively expensive and exposes the kidney to a contrast load. Therefore, it is less useful as a screening test. A number of screening tests have been developed. Being less invasive and carrying the least possible radiation burden, the captopril renal scintigraphy and Doppler ultrasonography are suggested to be the initial tests in the evaluation of RVH in younger population [12]. It is also suggested that several factors need to be considered when choosing an appropriate diagnostic test; in the first place, the clinical index of suspicion, then the presumed etiology of RVH, the presence or absence of renal insufficiency, the risk of complications from conventional contrast angiography and the use of antihypertensive medications that affect the RAS. Taking all these factors into account, the captopril scintigraphy is considered to be the primary non-invasive test in patients with a moderate clinical suspicion of FMD (or suspected uncomplicated atheromatous disease) with a normal renal function as presented in our case [13].

A number of studies, mainly referring to adult population, have shown different sensitivity and specificity of the captopril scintigraphy in the detection of renovascular disease [14]. The research results in the adult population as well as in the pediatric ones were quite inconsistent. The sensitivity and specificity of the captopril scintigraphy greatly depended on the selected group of subjects. In the group of subjects with the preserved renal function, the sensitivity and specificity were approximately 90%, while in the group of patients with the impaired renal function the tests showed lower specificity [11, 14, 15]. In addition, some case studies point out the potential role of the captopril scintigraphy in the evaluation of the effects of percutaneous transluminal dilatation of renal artery stenosis in hypertension [16]. In the presented case, the captopril scintigraphy, performed immediately after the endovascular procedure, indicated significantly improved parenchymal indicators of the right kidney which corresponded to a complete normalization of the patient's blood pressure occurring several months after the scintigraphy. This may indicate not only the importance of the captopril scintigraphy in RVH screening but also its potential role in further monitoring of patients following endovascular procedures and its prognostic significance.

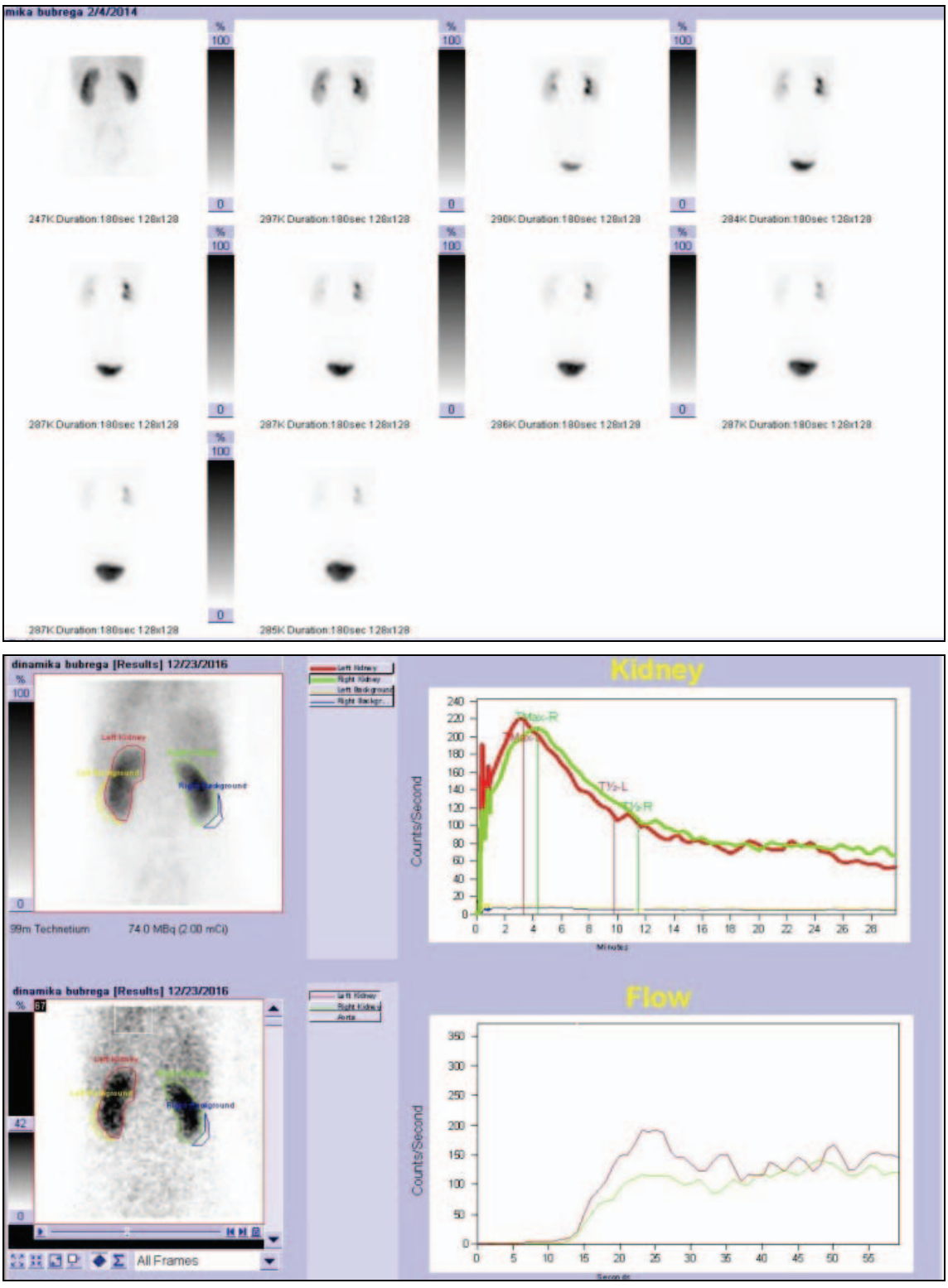

Fig. 4 – The captopril renal scintigraphy after the procedure – a significant improvement of the renogram curve of the right kidney. The time to maximum counts of the right kidney was 4.3 minutes and 20 min/peak min count ratio of the right kidney was 0.33.

Conclusion

It could be said that the captopril renal scintigraphy allows non-invasive functional testing in a selected group of hypertensive children which can either confirm or rule out the existence of hemodynamically significant renal artery stenosis.

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CASE REPORT

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Autoimmune manifestation of hepatitis C virus infection as a risk for late virological relapse after pegylated interferon and ribavirin therapy

Autoimunska manifestacija infekcije virusom hepatitisa C kao rizik od kasnog virusološkog relapsa posle terapije pegilovanim interferonom i ribavirinom

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Abstract

Introduction. We are aware of the risk of late virological relapse (LVR) years after sustained viral response (SVR) by pegylated interferon and ribavirin alfa (PegIFNα + RBV) of chronic hepatitis C viral (HCV) infection. We presented three patients with LVR, treated by PegIFNα and ribavirin 5 years after the SVR was established. **Case report.** We analysed 129 (38.8% female, 61.2% male, mean age 37.02 ± SD 11.99) patients treated for chronic HCV with PegIFNα + RBV, with at least 5 years from the establishment of SVR. In addition to the biochemical parameters of liver function, the qualitative HCV RNA polymerase chain reaction (PCR) and the quantitative PCR HCV RNA test were made. Five years after establishing SVR in 2.3% (3/129) of patients, the relapse of HCV infection was registered by qualitative and quantitative PCR HCV RNA assay and all of these patients had additional autoimmune diseases: vasculitis, autoimmune hepatitis, and vasculitis of central nervous system. **Conclusion.** The existence, but low rate of LVR HCV infection was confirmed, dominantly in patients with additional autoimmune diseases. Due to this SVR after therapy by PegIFNα + RBV should be considered as an indicator of successful HCV suppresion, not its complete eradication.

Key words:
hepatitis c; interferon alfa-2b; ribavirin; treatment outcome; recurrence; autoimmune diseases.

Apstrakt

Uvod. Iskustvo u lečenju hepatitis C virus (HCV) infekcije pegilovanim interferonom alfa (PegIFNα) i ribavirinom (RBV) ukazuje na postojanje rizika od kasnog virusološkog relapsa" (*late virological response* – LVR) – ponovna detekcija HCV ribonukleinske kiseline (RNA) u serumu godinama nakon uspostavljanja stabilnog virušološkog odgovora – *sustained virological response* (SVR). Prikazana su tri bolesnika sa LVR lečena PegIFNα i ribavirinom pet godina nakon uspostavljanja SVR. **Prikaz bolesnika.** Analizirano je ukupno 129 bolesnika (38,8% ženskog, 61,2% muškog pola, prosečna starost 37,02 ± 11,99 godina) lečenih od hroničnog HCV PegIFNα + RBV, kod kojih je prošlo najmanje pet godina od uspostavljanja SVR. Pored biohemijskih parametara funkcije jetre, rađen je kvalitativni lančane reakcije polimeraze (PCR) HCV RNA test, odnosno kvantitativni PCR HCV RNA test. Pet godina od uspostavljanja SVR kod tri (2,3%) bolesnika je kvalitativnim i kvantitativnim testom PCR HCV RNA utvrđen relaps HCV infekcije. Sva tri bolesnika imala su i pridružene autoimunske bolesti: vaskulitis, autoimunski hepatitis i vaskulitis centralnog nervnog sistema. **Zaključak.** Potvrđeno je postojanja LVR HCV infekcije prvenstveno kod bolesnika sa pridruženim autoimunskim bolestima. Stoga SVR nakon terapije PegIFNα + RBV treba shvatiti kao pokazatelja uspešne HCV supresije, a ne potpune eradikacije HCV.

Ključne reči:
hepatitis c; interferon alfa-2b; ribavirin; lečenje, ishod; recidiv; autoimunske bolesti.

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Introduction

Hepatitis C viral (HCV) infection is the most common cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC) and the leading cause of liver transplantation worldwide [1, 2]. Therapy with pegylated interferon alfa 2a/2b (PegIFNα) and ribavirin was successful in 50% to 80% of the patients who achieved sustained viral response (SVR), and more than 98% of them were considered cured [3]. However, over a longer follow-up period, the occurrence of a „late virological relapse" (LVR), redetection of HCV ribonucleic acid (RNA) in the serum years after achievement of SVR was recorded [4]. Little is known about the diagnostic criteria, LVR incidence and risk factors of its occurrence.

We presented three patients with LVR, treated with PegIFNα and ribavirin 5 years after the SVR was established.

Case report

We analyzed 129 patients with chronic hepatitis C (CHC) with established SVR within the period of 5 years who had been treated by PegIFNα-2a and ribavirin during 2014 at the Clinic for Infectious Diseases of the Clinical Center of Vojvodina. The patients underwent a qualitative HCV RNA polymerase chain reaction (PCR) test, COBAS AMPLICOR Hepatitis C test, version 2.0 (Roche Diagnostics systems, Basel, Switzerland). In the patients who obtained indeterminate or positive results, transitional viremia was excluded by the quantitative PCR HCV RNA test [COBAS AmpliPrep/COBAS TaqMantest (Roche Diagnostics systems, Basel, Switzerland], where the values of the detected flickers were expressed in the International Units/mL (U/mL), with lower limit of detection of 15 U/mL. Gene sequencing of 5' NTR type-specific PCR or commercial kits (InnoLipa, Innogenetics, Genotyping Linear Array hepatitis C virus test, Roche Diagnostics) were performed for HCV genotyping.

Out of the 129 patients, 50 (38.8%) were females and 79 (61.2%) were males. At the initiation of the therapy, the mean age was 37.02 years [19–66 years; standard deviation (SD) 11.99]. Average estimated duration of the HCV infection was 10 years (95% CI 10.00-14.00). Mean activity of alanine aminotransferase (ALT) before beginning of the therapy amounted to 98.00 U/mL (95% CI 80.00 to 122.00) (normal range 7–56 U/L). Before the treatment, a majority of the patients 100/129 (77.5%), had a high viral load detected (> 400,000 U/mL) and the average value was 1,142,100.00 (95% CI; 999,973.09 to 1,485,084.68 U/mL). Characteristics of CHC are shown in Table 1.

Out of 129 patients included in the study, 5 (3.9%) were treated with recombined interferon alpha 2/2b (IFNα2a/2b) before the combined therapy with PegIFNα 2a and ribavirin was applied. During the treatment, the dose of PegIFNα was corrected in 29 (22.5%) patients (mostly due to neutropenia, or thrombocytopenia), and the dose of ribavirin was corrected in 19 (14.7%) patients (most commonly for anemia). Other side effects of the treatment were noted in 21 (16.3%) patients. Five years after SVR, in 3 (2.3%) patients relapse of the HCV infection was suspected with the qualitative HCV RNA PCR assay, and then confirmed by the quantitative PCR HCV RNA test. Clinical characteristics of CHC, a course of the treatment and the disease course in these 3 patients are given in Table 2.

Table 1

Characteristics of chronic hepatitis C (CHC) in the patients treated with pegylated interferon alfa 2a/2b (PegIFNalfa) and ribavirin who achieved sustained viral response (SVR)

Characteristics of CHC	n	%
HCV Genotype 1	82	63.5
HCV Genotype non 1	47	36.5
Without fibrosis (METAVIR 0)	21	16.3
Fibrosis light to moderate (METAVIR 1 and 2)	85	65.9
Severe fibrosis/cirrhosis (METAVIR 3 and 4)	12	9.3
Steatosis	21	16.3

HCV – hepatitis C virus.

Table 2

Comparison of three patients with detected late relapse of hepatitis C virus (HCV) infection

Variable	Patient No. 1	Patient No. 2	Patient No. 3
Female	yes	yes	no
Age > 60 years	no	no	no
BMI > 30 kg/m^2	no	yes	no
HCV Genotype 1	yes	yes	yes
VL > 400,000 U/mL	yes	no	yes
Severe liver fibrosis (3 and 4)	yes	yes	yes
PegIFN 80%	yes	no	yes
RBV 80%	no	no	yes
Previously treated	no	no	yes

BMI – body mass index; VL – viral load of HCV in the serum;
PegIFN 80% – cumulative dose of pegylated interferon;
(Peg IFN) not lower than 80% of the prescribed dose; RBV 80% – cumulative dose of ribavirin not lower than 80% of the prescribed dose.

Patient 1

A 55-year-old female patient suffered from vasculitis and was in hemodialysis (HD) program for over 3 decades. Six years after achieving SVR with 48 weeks PegIFNα monotherapy of the HCV infection (fibrosis 4, HCV genotype 1), a discrete increase of ALT was detected. The qualitative HCV RNA PCR test was positive, and the quantitative PCR HCV RNA resulted in 280,000 U/mL, genotype 1. Clinical reevaluation did not report any progression of liver cirrhosis. From the moment SVR was achieved, the patient was included in the HD program. Bearing in mind the excellent epidemiological situation in the HD centre, the HCV reinfection was unlikely.

Patient 2

A 57-year-old female patient suffered from "overlap" autoimune hepatitis (AIH)/HCV syndrome [fibrosis 2, with "interface" hepatitis, detected antinuclear antibodies (ANA) 1: 320 and antimitochondrial antibodies (AMA) 1: 320 in serum, HCV genotype 1]. After initial treatment with azathioprine and methylprednisolone, SVR was achieved on 48 week of PegIFNα-2a and ribavirin therapy. Even though the qualitative HCV RNA PCR was repeated annually due to continually increased ALT activity, the HCV viremia was confirmed after 5 years by the quantitative HCV RNA (82,000 U/mL, genotype 1). Clinical reevaluation of liver disease showed progression to liver cirrhosis (CP B). The risk for the HCV reinfection from the moment of achieving SVR to the moment of the HCV infection relapse was not known.

Patient 3

A 44-year-old male patient achieved SVR after 48 weeks of PegIFNα-2a and ribavirin therapy of CHC (fibrosis 3, HCV genotype 1). During the follow-up period, the increased activity of aminotransferases was detected, however, the quantitative HCV RNA test was repeated several times and was negative. Six years after achieving SVR, transverse myelitis was diagnosed. Reevaluation of the liver diseases determined progression to cirrhosis (A CP score, liver fibroelastography 16.4 kPa) and the HCV relapse was confirmed by the quantitative HCV RNA (VL 1,289,000 IU/mL, genotype 1). From the moment of achieving SVR to the moment of HCV infection relapse, the risk factors for HCV reinfection were not found.

Discussion

According to the study of Swain et al. [4], Manns et al. [5] and Formann et al. [6], the LVR rate ranges from 1% to 8%, in the follow-up period of 1 to 12 years after achieving SVR. In our research, the LVR HCV infection was rare; it was identified in 2.3% (3/129) of patients treated and followed for more than 5 years.

It was proved that in spite of SVR, in up to 6% followed-up patients, it was possible to detect HCV RNA in the liver tissue, lymphocytes and monocyte/macrophage cells [7–10]. Those findings led to the new clinical aspects of the HCV infection – secondary occult HCV infection (OHC) [11, 12]. In the liver tissue, mild to moderate disease activity was proved, including the presence of lymphocytic infiltration, hepatocyte necrosis and fibrosis of different degree [13]. OHC may be the explanation of LVR HCV, whereas SVR should be seen as successful HCV suppression rather than complete eradication of HCV after combined therapy with PegIFN and RBV [14]. To cure the patient means to achieve complete eradication of the virus, without presence of HCV RNA in the serum and liver tissue as well as having load of antibodies to the nucleus of HCV (anti-HCV core) reduced [15].

Some studies showed that predisposition for the HCV relapse is related to female gender, older age, high body mass index (BMI), high viral load before treatment, HCV genotype 1, HCV molecular characteristics (especially HCV core region), genetic predisposition of patients (ILB28), previous therapy with IFN and also cumulative dose of administered drugs, ribavirin, as most important [16–18]. Despite the statistical irrelevance, due to a small number of patients in addition to CHC, all three patients had some autoimmune disease in common – the patient No. 1 vasculitis, the patient No. 2 autoimmune hepatitis and the patient No. 3 central nervous system (CNS) vasculitis (Table 2). Cytotoxic T lymphocytes (CTLs) are held as the most responsible for the HCV replication control [19]. In addition to the inevitable antiviral effect, the IFN-based therapy has immunomodulatory effects aimed at recovery and reconstitution of T cell activity [20]. If this effect is achieved with elimination of HCV and if the potency of CTLs is sustained after the end of the therapy, the ability of HCV reactivation is excluded [21]. Patients with late virological response detected in this study had severe, recurrent and fulminant forms of autoimmune disease at times that required concomitant immunosuppressive therapy (corticosteroids in all three cases) and doubtlessly had compromised functional capacities of both humoral and cellular immunity. Although it is clear that our patients had secondary OHC [9], in the second and third case, on several occasions the tested HCV viremia was negative, but with present progression of liver disease. This is consistent with the findings of Radkowski et al. [10] and Pham and Michalak [11] regarding presence of active liver disease in intrahepatic HCV RNA detection, besides achieved SVR.

However, there is a possibility of the re-HCV infection in the presented patients, especially in the patient No. 1. who was on chronic HD. Owing to the stricter blood bank screening rules, widespread use of erythropoiesis-stimulating agents instead of blood transfusions and stronger adherence to infection control practices in dialysis units, there was a reduction in the prevalence of the HCV infection in the HD patient group [22]. The percentage of the anti HCV positive patients dropped from 23.2% in 1999 to 12.7% in 2009 in Serbia [22]. In this particular HD Center our patient was the only anti-HCV positive and during 10 years of follow-up, there was no new HCV infection among the patients on HD. On account of this positive epidemiological situation, we excluded the possibility of the HCV re-infection in the patient

No. 1. Unfortunately, serum samples of the patients before treatment PegIFN were not available and we could not exclude the re-HCV infection by the molecular analysis of HCV sequences [21–23].

Conclusion

Even SVR achieved on IFN based therapy of CHC protocol cannot be considered as an absolute cure, but the rate of its reliability is high. One should be aware of the possibility of late relaps and therefore it is necessary to monitor the patients actively after establishing SVR, especially those with autoimmune disorders within the HCV infection.

The existence of a low rate LVR HCV infection was confirmed predominantly in the patients with additional autoimmune diseases.

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CASE REPORT

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Atypical primary melanoma of the umbilicus – A case report

Atipični primarni melanom pupka

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Abstract

Introduction. The umbilicus (omphalos; the navel; belly button; tummy button) is important in a medical and psychosocial context. Umbilical tumors are rare and can be benign or malignant, primary or secondary. The most common are malignant metastatic tumors, especially The Sister Mary Joseph Nodule, an eponym for the umbilical metastasis of intra-abdominal malignant tumors. Primary melanoma of the umbilicus is very rare, there is few literature data about it and its incidence is not well known. Therefore, the aim of this study was to present a patient with a rare localization of the primary skin melanoma, that was, also, of atypical form, large and involved the whole umbilicus and surrounding skin. **Case Report.** In this report, the patient had nodular red tumor which involved the whole umbilicus. Tumor had rapid growth. In the differential diagnosis, the tumor most by resembled a pyogenic granuloma. After the surgery, the histopathological finding showed the primary nodular skin melanoma (Clark V, Breslow 10 mm, positive for: vimentim S – 100 protein, melan – A and HMB – 45, and negative for EMA, with moderately high proliferative activity). **Conclussion.** According to literature data, this is 27th case of the primary melanoma of the umbilicus since 1916 when it was first reported. By presenting this rare clinical case, we emphasize that any skin lesion that is removed, must be sent to a histopathologic analysis.

Key words:
melanoma; umbilicus; reconstructive surgical procedures; diagnosis; treatment outcome.

Apstrakt

Uvod. Pupak je značajan u medicinskom i psihosocijalnom kontekstu. Tumori pupka su retki i mogu da budu dobroćudni ili zloćudni, primarni ili sekundarni. Najčešći su maligni metastatski tumori, pogotovo Meri Jozef čvor koji je eponim za metastazu malignog tumora nekog od abdominalnih organa. Primarni melanom pupka je veoma redak, malo je dostupnih podataka u literaturi o njemu, a incidencija nije dobro poznata. Zbog toga cilj rada bio je da se prikaže ovaj tumor, uz to u atipičnoj formi, velikih razmera, koji je zahvatao čitav pupak i okolnu kožu. **Prikaz bolesnika.** U radu je prikazan bolesnik sa nodularnim tumorom crvene boje, koji je zahvatio čitav pupak. Tumor je imao brz rast. U diferencijalnoj dijagnozi, ovaj tumor je najviše ličio na piogeni granulom. Posle operacije, patohistološki nalaz ukazao je da se radi o primarnom nodularnom melanomu kože (V stepen po Klarku i manje od 10 mm po Breslovu, pozitivan na vimentim, S – 100 protein, melan – A i HMB – 45, a negativan na EMA, sa umereno povećanom proliferativnom aktivnošću). **Zaključak.** Prema podacima iz literature ovo je 27. bolesnik sa primarnim melanomom pupka od 1916. godine, kada je prvi put opisan. Prikazujući redak klinički slučaj, naglašavamo da svaka promena koja se otkloni sa kože mora da se uputi na patohistološku analizu.

Ključne reči:
melanom; pupak; hirurgija, rekonstruktivna, procedure; dijagnoza; lečenje, ishod.

Introduction

Melanoma is the most malignant tumor of the skin and one of the most malignant tumors at all. Melanoma incidence rate is increasing and lifetime risk of getting melanoma is about 2.5% (1 in 40) for whites. This tumor can appear anywhere in the body [1] and sometimes in an atypical form [2, 3].

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The umbilicus is one of the most significant anatomical and clinical structures of the trunk. Besides the cosmetic importance, the umbilicus is very important in medical praxis. Many diseases are first represented by umbilical pain. The umbilical pathology [4] includes inflammation and infections of the umbilcus, hernias, endometriosis [5], operative scars and tumors. Tumors are of the greatest importance [6–14].

Umbilical tumors can be primary, metastatic, benign and malignant. Like others skin tumors, the most common benign and malignatnt tumors in medicine, there is a great variety of the umbilical tumors. Melanoma is one of them, but very rare. According to literature data there are only 26 reports of the primary umbilical melanoma, but not of this size and morphology. Surgery of the umbilical area is very complex, especially when reconstruction is necessary.

In this regard, the purpose of this paper is to present the unusual case of a rare localization and morphology of skin melanoma.

Case report

Female patient, aged 65 years, was admitted to the Center for Plastic Surgery in the Clinical Centre "Kragujevac" with vegetant umbilical skin tumor. According to the patient's explanation, the tumor existed for about 6 months, had rapid growth and was treated by a dermatologist. The tumor was red, oval, partly with a irregular surface, measuring 38 × 18 mm and it was most similar to a pyogenic granuloma (Figures 1 and 2). The tumor involved the umbilicus and a part of the skin, on the right side. Axillary and groin nodes were not palpable and the chest X-ray was normal. The excision was done, 0.5 cm from the edges of the tumor, deep to the fascia and the tumor with healthy surrounding tissue was sent to a histological examination. The wound was closed by a advancement skin flap. The part of the flap was shaped and sutured by buried stitches, in order to achieve a satisfactory cosmetic result. The postoperative course was normal, and the stitches were removed after ten days (Figure 3).

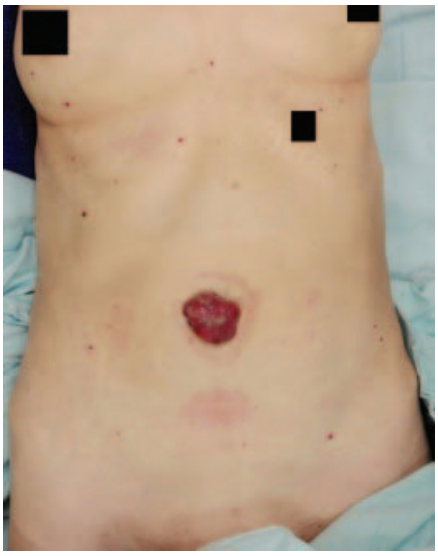

Fig. 1 – Oval, nodular, red, giant tumor of the umbilicus.

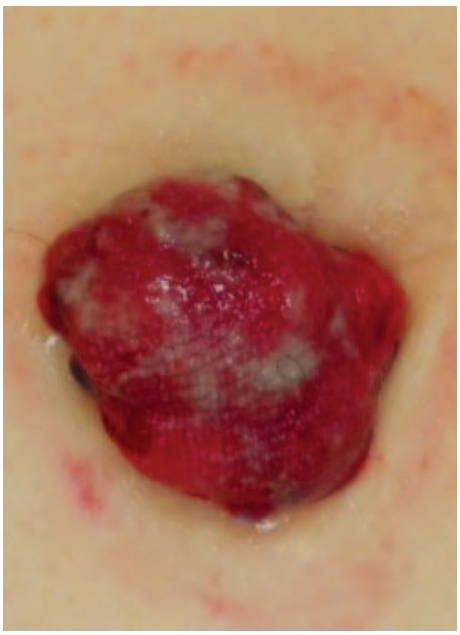

Fig. 2 – The close-up photography of the tumor.

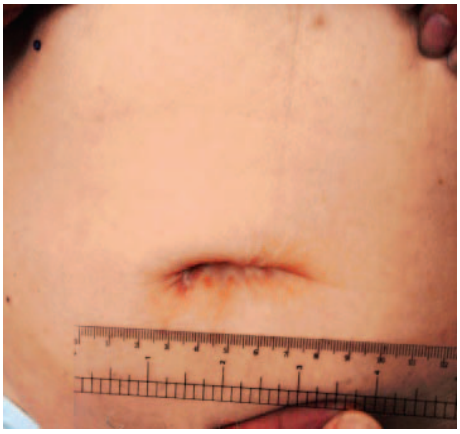

Fig. 3 – The umbilical region, ten days after the surgery.

The histopathological finding was: primary skin melanoma (Figure 4) of a nodular type, with depth of invasion: Clark V, Breslow 10 mm. The spindle-type cancer cells dominated, with no detectable melanin pigmentation. There were superficial ulcerations, larger than 6 mm, invasion of the lymphatic vessels, capillaries and the proliferation of moderate-stromal mononuclear reaction. Tumor cells were positive for vimentim, S - 100 protein, melan - A and HMB - 45, and negative for EMA, with moderately high proliferative activity (about 35% of the cells were Ki-67 positive). Histopathological examination showed that deep and lateral margins were clear. Further course included detailed systemic examinations for melanoma. Stadium of the disease was: pT4b, No, Mo. Lymphoscintigraphy findings (Sentinel lymph node mapping – SLNM) was regular. After the consilium decision, radical excision was done, 2 cm from the scar, and deep to the peritoneum, with primary reconstruction of the umbilical area with local skin flaps (Figure 5). Postoperative course was regular. The histological examination showed clear resection margins. Conclusion of the con-

silium examination was that there is no need for an adjuvant therapy. One month after surgery there were no signs of local recurrence (Figure 5), and there were no palpable axillary and groin nodes, and that was verified by ultrasound examination.

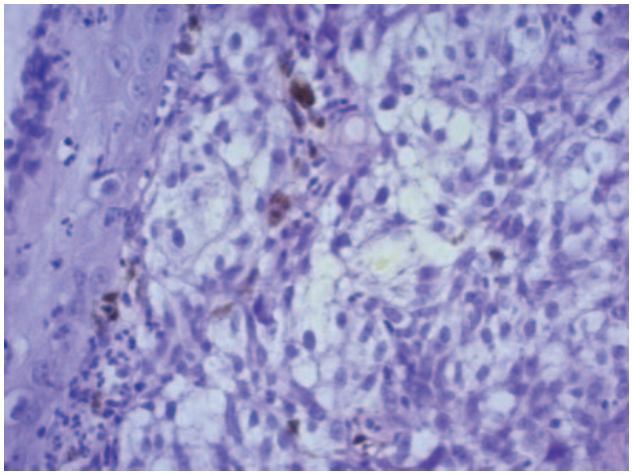

Fig. 4 –On the left, there is the epidermis, underneath the papillary dermis invaded with malignant cells, epitheloid histological spindle-shaped, with focal deposits of the melanin pigment, brown-brown color. Hematoxylin & eosin (H&E) method (high magnification ×400).

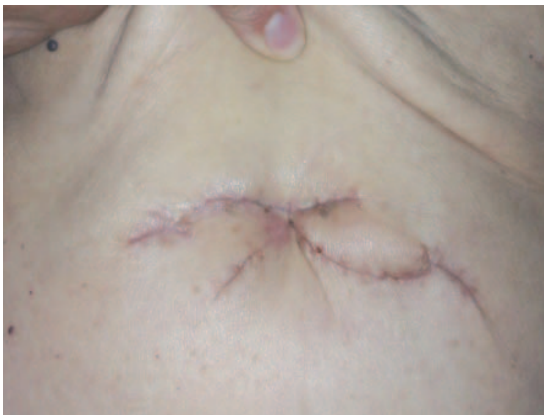

Fig. 5 – One month after radical surgery with reconstruction by skin flaps.

Discussion

Medical importance of the umbilicus is well known. Umbilical pain can be a symptom of more than ten diseases. The umbilicus changes its morphology during the pregnancy. There are various pathological conditions of the umbilicus: granuloma (inflammatory growth after resection of the umbilical cord), infection, hernias, endometriosis, tumors and post-traumatic or postoperative scars, defects and deformities [4, 5].

About 60% of the umbilical tumors are benign [6]. Primary tumors of the umbilicus may have origin from the skin, soft tissues or congenital rests in this region. The most common primary benign tumors are naevi, verrucae, haemangiomas and keratoses [7]. Primary malignant tumors are less frequent (14.59%) than metastatic ones. Skin carcinoma is the most frequent primary malignant tumor. The malignant metastatic tumors are the most common (85.41%) [6–8]. Among them, Sister Mary Joseph Nodule, as an eponym for intra- abdominal metastases in the umbilicus, is the most common (approximately 80%) [8].

It is well known that the melanoma of the skin is one of the most malignant tumors and that can occur in any region of the body. Melanoma arising in the umbilicus, as a primary tumor, is very rare, with unknown worldwide incidence statistics [9–14]. The first description of primary umbilical melanoma was made by Cullen in 1916, who described three patients [9]. There are a few other reports of the primary melanoma of the umbilicus [10–14]. Those reports are about melanoma in the typical form and of smaller size. According to the report of Di Monta et al. [10] in 2015, there were 26 cases of primary umbilical melanoma in medical literature, so that the case we are presenting is 27th.

There are many cases of atypical forms of melanoma, especially according to the color, but there is no available data for the umbilical melanoma. In the patient who is presented here, the tumor was red, growing rapidly, and mostly resembled a pyogenic granuloma [15–17].

There is no strict definition for the giant tumor. It depends on its size according to size of the certain region of the body or organ. Tumor that affects a significant part or the whole of some anatomical structure is a giant. For example, there is the rule that giant hand tumors are those that are greater than 5 cm in diameter [18]. Since the navel is a specific anatomical region, this tumor spread all over the navel and therefore we classified it as giant.

Conclusion

This is 27th case of the primary melanoma of the umbilicus, which is atypical in its color and size. Treatment of the umbilical tumors is excisional biopsy. Radical excision is necessary in a malignant tumor of the umbilicus, which may be complex because of the specific umbilical anatomy when adequate reconstruction is required.

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CASE REPORT

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Unrecognized urinoma caused by infiltrative bladder cancer

Neprepoznati urinom izazvan infiltrativnim karcinomom mokraćne bešike

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Abstract

Introduction. Urinoma develops after disruption of collecting system of urinary tract and urine leak in surrounding tissue. Most common causes of urinoma are blunt or penetrating trauma. Less common causes are iatrogenic injuries or urinary tract obstruction. In this article we presented a rare case of the urinoma caused by infiltrative bladder cancer. **Case report.** Acutely ill, a septic patient with ileus and profound azothaemia was admitted to medical intensive care unit. Native computed tomography revealed moderate ileus, right kidney hydronephrosis, extensive retroperitoneal urinoma and vesical thickening with excluded infiltration of ureteral orifices. Computed tomography guided percutaneous drainage was done. Upon stabilization, patient underwent transurethral bladder tumor electroresection (histopathology report was: infiltrative transitional cell tumor of urinary bladder). Radical cystectomy was done. The patient's recovery was uneventful. **Conclusion.** Urinoma formed due to spontaneous rupture of collecting system based on ureteral obstruction caused by urinary bladder tumor is very rare clinical case scenario. In case of urinoma of unclear etiology invasive bladder cancer should be excluded.

Key words:
urinoma; diagnosis; tomography, x-ray computed; histological techniques; urinary bladder, neoplasms.

Apstrakt

Uvod. Urinom se javlja kao posledica disrupcije kolektorskog sistema urinarnog trakta i curenja urina u okolno tkivo. Najčešći uzroci razvoja urinoma su tupa ili penetrantna trauma. Ređi uzroci pojave urinoma su jatrogene lezije ili opstrukcija urinarnog trakta. Prikazan je redak klinički slučaj urinoma izazvanog infiltrativnim karcinomom mokraćne bešike. **Prikaz bolesnika.** Bolesnik u akutnom stanju, sa znacima sepse, ileusom i izraženom azotemijom, primljen je u jedinicu internističke intenzivne nege. Nativni kompjuterizovani tomografski pregled ukazao je na postojanje srednje izraženog ileusa, hidronefroze sa desne strane, ekstenzivnog retroperitonealnog urinoma i zadebljanja zida mokraćne bešike uz infiltraciju orificijuma uretera. Urađena je perkutana drenaža urinoma vođena kompjuterizovanom tomografijom. Posle poboljšanja opšteg stanja urađena je transuretralna elektroresekcija tumora mokraćne bešike (Patohistološki nalaz glasio je: infiltrativni karcinom prelaznog epitela mokraćne bešike). U daljem postupku lečenja urađena je radikalna cistektomija; postoperativni tok je protekao bez komplikacija. **Zaključak.** Razvoj urinoma usled spontane rupture kolektorskog sistema uzrokovane opstrukcijom uretera infiltrativnim tumorom mokraćne bešike je vrlo retka klinička prezentacija. U slučaju urinoma nejasne etiologije trebalo bi isključiti postojanje infiltrativnog tumora mokraćne bešike.

Ključne reči:
urinom; dijagnoza; tomografija, kompjuterizovana, rendgenska; histološke tehnike; mokraćna bešika, neoplazme.

Introduction

Urinoma develops after disruption of collecting system of urinary tract and urine leak in surrounding tissue. Most common causes of urinoma are blunt or penetrating trauma. Posttraumatic urinomas are well-described complications associated with the nonoperative management of major blunt renal injuries [1]. Less common causes are iatrogenic injuries of urinary tract obstruction. We presented a rare case of the urinoma caused by infiltrative bladder cancer.

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Case report

Urology consulting was called for a patient with ileus and hydronephrosis who was admitted to medical intensive care unit. The patient was a 83-year-old male in acute distress, with clinical signs of moderate ileus (Figure 1).

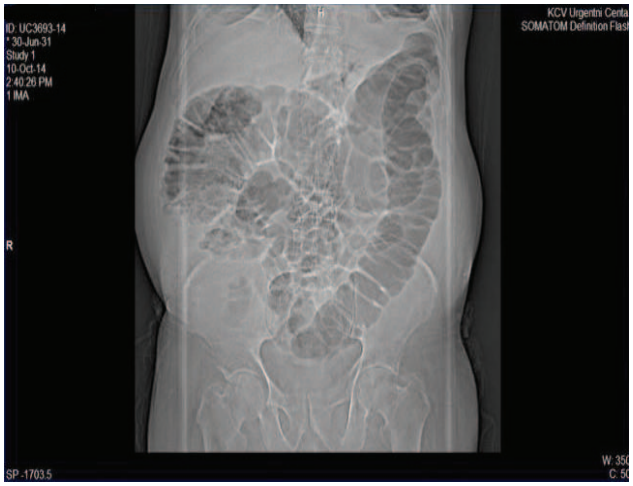

Fig. 1 – A native radiograph of the abdomen shows distended bowels – moderate degree of ileus.

Laboratory findings revealed severe azothaemia [creatinine level of 800 μmol/L [normal range (nr) (60–110 μmol/L)], moderate acidosis [pH of 7.2 (nr 7.35–7.45)], white blood cells (WBC) of 19 × 10^9 (nr 3.5–10.5 × 10^9), findings met criteria for sepsis. Computered tomography (CT) scan findings were consistent with hydronephrosis grade 2 on the right and minimal hydronephrosis on the left side – urine was leaking out trough the tear in the left kidney pelvicalyceal (PC) system (Figure 2).

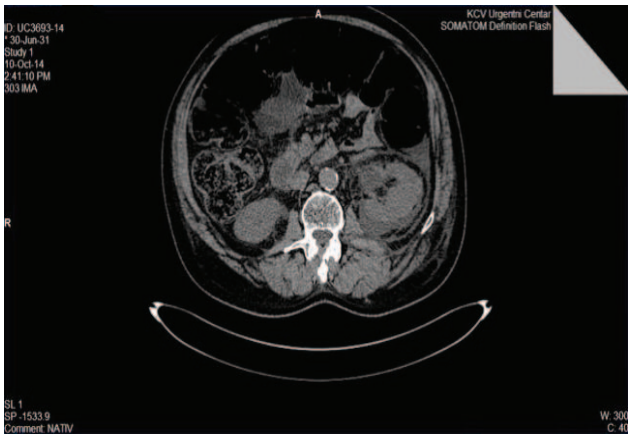

Fig. 2 – Computed tomography (CT) scan finding reveals hydronephrosis grade 2 on the right and minimal hydronephrosis on the left side.

There was obvious retroperitoneal urinoma – predominantly on the left side (Figure 2 and 3).

CT scan of the urinary bladder showed irregularly thickened wall at the trigonal part of the bladder, more prominent on the left side (Figure 4).

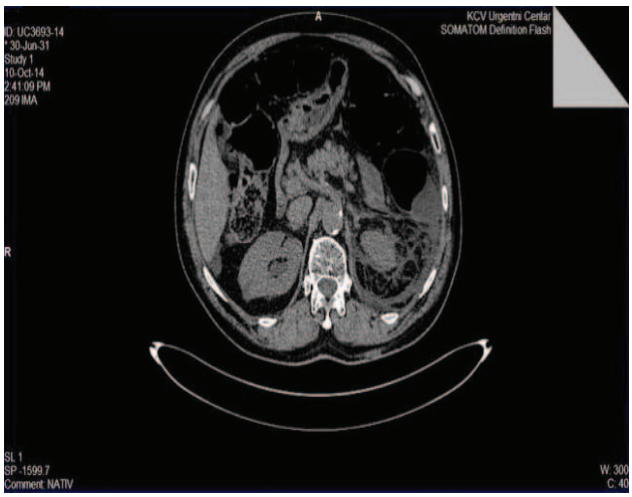

Fig. 3 – Computed tomography (CT) scan finding of retroperitoneal urinoma predominantly of the left side.

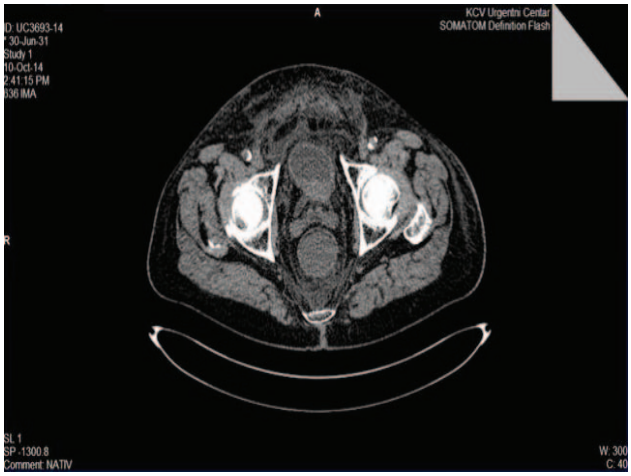

Fig. 4 – Computed tomography (CT) scan of the urinary bladder reveals irregularly thickened wall at the trigonal part of the bladder, more prominent of the left side.

Patient underwent urgent CT guided percutaneous drainage procedure, with drain placement in the left retroperitoneum. Drained fluid was tested and it was confirmed to be urine. Soon after the procedure, the patient felt much better, ileus resolved and laboratory findings slowly normalized. Patient was scheduled for transurethral electroresection (TUR) of suspected urinary bladder tumor. During the TUR procedure tumorous obstruction of both ureteral orificial openings were seen. Histopathology report confirmed infiltrative transitional cell cancer (TCC) of the urinary bladder. After TUR of bladder tumor, follow-up ultrasonography (US) was done and it showed hydronephrosis stage one on the right side (partially unblocked right ureteral orifice by previous TUR bladder tumor), no hydronephrosis of the left kidney and small amount of perirenal fluid on the left side. The patient was scheduled for radical cystectomy. During the surgery, no obvious tear of the left pyelon or ureter were found, both ureters were stented with J-J stents and Bricker's urinary derivation was done. On postoperative day 3, output at previously installed percutaneous drainage dramatically decreased and therefore drain was removed. Postoperative recovery was otherwise unremarkable.

Discussion

By definition, urinoma is a mass of extravasated urine delineated by perirenal fascia within reactively formed fibrous capsule. Urinoma might also manifest as a free fluid [2]. There are three factors necessary for urinoma (caused by urinary tract obstruction) to be developed: a tear in PC system, functional kidney and distal obstruction [3].

Most often, urinoma is caused by a trauma, less common causes are distal ureteral obstruction by calculus, pelvic masses or iatrogenic injuries. The PC system injury and urinary leakage trough the tear is not so rare, but in most of these cases urine leaks undergo spontaneous resolution and formed urinoma develops only in few instances [4].

Usual clinical presentation of urinoma includes: mild to moderate flunk fullness/pressure/pain, atypical abdominal pain, poor appetite, weakness, weight loss. It takes time for urinoma to develop. Urinoma presentation depends on causes, extent, urinoma localization and time window between time of injury of the PC system and time until diagnosis was established. Rarely, if not recognized or left untreated, urinoma may present as an ileus, peritonitis and abscesses and sepsis. In our case delayed establishing of correct diagnosis was crucial for severe clinical presentation.

Method of choice for urinoma diagnosis is CT with radio contrast agents. In some cases, like in this one, where an use of contrast is contraindicated, native (non-contrast) CT should be performed. In addition, an image guided percutaneous needle aspiration drainage (which is both diagnostic and therapeutic) might be done [5].

In most cases, small urinoma would reabsorb spontaneously, and drainage would not be necessary [6]. In some cases of large or persistent urinoma, or in a case of moderate to severe illness (fever, sepsis), first step should be CT or US guided drainage [7]. Further treatment depends on a cause of the urinary obstruction and should be aimed accordingly.

Conclusion

Urinoma formation due to collecting system rupture because of ureteral obstruction caused by urinary bladder tumor is very rare clinical case scenario. In case of urinoma of unclear etiology invasive bladder cancer should be excluded.

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Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u desnom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

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