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The building where the Military Hospital in Valjevo was situated in the World War I (see the article by Aleksandar Nedok and Vladimir Krivošejev pp. 1189–1196).

Zgrada u kojoj je bila smeštena Vojna bolnica u Valjevu tokom Prvog svetskog rata (vidi članak Aleksandra Nedoka i Vladimira Krivošejeva str. 1189–1196).

Dear Authors, Editors, Peer reviewers and Readers of the Vojnosanitetski pregled,
I thank you for your cooperation and support in the last year
and wish you all the best in the coming 2018!

Merry Christmas and Happy New Year!

Cordially,
Prof. Silva Dobrić, PhD
Editor-in-Chief

Poštovani autori, urednici, recenzenti i čitaoci Vojnosanitetskog pregleda,
Uz zahvalnost na saradnji i podršci u protekloj godini,
želim vam sve najbolje u nastupajućoj 2018!
Srećna Nova godina i Božićni praznici!

Srdačno,
Prof. dr Silva Dobrić
glavni i odgovorni urednik

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Poziv za reklamiranje u 2018. godini

U prilici smo da vam ponudimo mogućnost oglašavanja i reklamiranja proizvoda i usluga u časopisu „**Vojnosanitetski pregled**“ (VSP). To je sigurno najbolji vid i najzastupljeniji način upoznavanja eventualnih korisnika sa vašim uslugama i proizvodima.

Časopis „**Vojnosanitetski pregled**“, zvanični organ lekara i farmaceuta Vojske Srbije, naučno-stručnog je karaktera i objavljuje radove iz svih oblasti medicine, stomatologije i farmacije. Radove ravnopravno objavljuju stručnjaci iz vojnih i civilnih ustanova i iz inostranstva. Štampa se na srpskom i engleskom jeziku. Časopis izlazi neprekidno od 1944. godine do sada. Jedini je časopis u zemlji koji izlazi mesečno (12 brojeva), na oko 100 strana A4 formata, a povremeno se objavljuju i tematski dodaci (suplementi). Putem razmene ili pretplate VSP se šalje u 23 zemlje sveta. Radove objavljene u VSP-u indeksiraju: *Science Citation Index Expanded (SCIE)*, *Journal Citation Reports/Science Edition*, *Index Medicus (Medline*), *Excerpta Medica (EMBASE), EBSCO* (preko ove baze VSP je dostupan *on line* od 2002. godine u *pdf* formatu) i *Biomedicina Serbica.*

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Changes in linear and nonlinear measures of RR and QT interval series after beer intake

Promene linearnih i nelinearnih mera nizova RR i QT intervala posle uzimanja piva

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Abstract

Background/Aim. There are only several studies on the acute effect of alcoholic drinks intake on heart rhythm and this phenomenon is still not well understood. We wanted to examine whether linear and nonlinear measures of RR interval and QT interval series could quantify the effect of beer in healthy subjects. **Methods**. Eighteen young volunteers drank 500 mL of beer (21 g of ethanol). Electrocardiogram (ECG) recordings were taken in supine position: 20 minutes before (relaxation) and 60 minutes after drink intake. The RR interval series and the QT interval series were extracted from ECG and we calculated short-term (α_1) and long-term (α_2) scaling exponents and sample entropy (SampEn) for both series; low frequency (LF) and high frequency (HF) spectral components from RR interval series and QT variability (QTV). Blood pressure was measured every 10 minutes. **Results**. It was shown that beer induced changes in variability and correlation properties of these series. Immediate effect of beer intake was detected as a transient increase in the QT variability, heart rate and blood pressure. Delayed effects of beer were shortening of the RR and QT intervals and reduction of the HF spectral component. Beer intake also increased short-term scaling exponent (α_1) of the RR time series and long-term scaling exponent (α_2) of the QT time series. **Conclusion**. Our results suggest that acute effects of beer are reduced parasympathetic control of the heart and changed dynamic complexity of the ventricular repolarization.

Key words:
alcohol drinking; beer; heart rate; electrocardiography; adult.

Apstrakt

Uvod/Cilj. Akutni efekat uzimanja alkoholnih pića na kardiovaskularne ritmove je fenomen koji još uvek nije dovoljno razjašnjen i u literaturi postoji svega nekoliko radova na tu temu. Cilj rada je bio da se ispita da li se linearnim i nelinearnim merama nizova RR i QT intervala može kvantifikovati akutni efekat male količine piva kod zdravih osoba. **Metode**. Osamnaest mladih zdravih muškaraca je pilo po 500 mL piva (21 g etanola). Elektrokardiogram (EKG) je beležen u ležećem položaju: 20 minuta pre (u relaksaciji) i 60 minuta neposredno posle uzimanja pića. Iz digitalizovanog zapisa EKG-a izdvojeni su nizovi RR i QT intervala. Iz oba niza smo izračunali kratkodometni (α_1) i dugo-dometni skalirajući eksponent (α_2), kao i entropiju uzorka (SampEn). Iz nizova RR intervala određene su spektralne komponente niskofrekventnih (LF) i visokofrekventnih (HF) opsega, a iz nizova QT intervala varijabilnost QT intervala (QTV). Krvni pritisak je bio meren svakih 10 minuta. **Rezultati**. Pokazali smo da pivo menja varijabilnost i korelacione osobine ovih nizova. Neposredni efekat uzimanja piva ogleda se u prolaznim povećanjima QT varijabilnosti, srčane frekvence i krvnog pritiska, a produženi u skraćenju dužine RR i QT intervala i smanjenju spektralne komponente HF. Uzimanje piva je takođe dovelo do porasta kratkodometnog skalirajućeg eksponenta (α_1) RR niza i dugodometnog skalirajućeg eksponenta (α_2) QT niza. **Zaključak**. Akutni efekat uzimanja piva je redukovana parasimpatička kontrola srca i izmenjena kompleksnost dinamike ventrikularne repolarizacije.

Ključne reči:
alkohol, pijenje; pivo; srce, frekvencija; elektrokardiografija; odrasle osobe.

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Introduction

In last decade(s) studies appeared that revealed benefitial effect of low doses of alcoholic drink [1, 2]. However, it is still poorly understood in which way social drinking influences cardiovascular system. Spaak et al. [3] showed that accute efect of ethanol on heart rate variability (HRV) was dose-related; one standard alcoholic drink (155 mL) had no influence on heart rate (HR) and HRV measures but two drinks had. Nonlinear measures of HRV have gained recent interest as powerful methods for quantification of integrated cardiac control in various clinical settings [4]. Scaling exponents have been used as relatively simple scale-independent measures which quantified correlations in the output signals of complex biological systems [5]. In the RR interval fluctuations they quantified integrated control of the heart. A breakdown of scaling (changes in scaling exponents) indicated disturbances in the overall control of the heart [6]. The sample entropy (SampEn) is frequently used nonlinear measure which quantifies complexity/regularity of physiological time series [7]. Lorsheyd et al. [8] reported that acute ingestion of alcohol in healthy population could induce prolongation of QTc interval (QT interval corrected for HR) and that the mechanism causing the QT prolongation originated from changes in the duration of ventricular repolarization.

Recently, we have reported the analysis of perturbation of HRV by a low dose of red wine using linear and nonlinear measures [9] which has not been done before [9]. We found that nonlinear properties of RR and QT interval series could be used to differentiate effect of wine and ethanol. Changes in the RR and QT interval series induced by a low dose of red wine were more detectable by methods that quantify the structure of the series than methods that quantify their variability.

The same analytical methodology was used in the present study as in the study on the effect of wine. The aim of the study was to assess acute effect of beer intake on linear and nonlinear measures of RR interval and QT interval series. We wanted to estimate whether these methods of time series analysis are sensitive to reveal small changes induced by a small quantity of beer intake.

Methods

Study population

The study group comprised eighteen men without medical history, aged 26 ± 4 years with body mass index (BMI) = 24 ± 2 kg/m^2. They were apparently healthy, with no history or symptoms of heart disease, hypertension, or diabetes, with normal findings at clinical checks, and with no alcohol, no smoking or drug related problems. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade, and each subject signed an informed consent to the study protocol.

Study protocol

Subjects attended one session in the morning at 10.00 AM when after relaxation in supine position for 30 minutes they drunk 500 mL of beer (21 g of ethanol). The drink was taken within 5 minutes time frame. The subjects were instructed to eat nothing on the study morning and to abstain from caffeine and alcohol since the afternoon of previous day. The beer was made in the Department of Food Technology and Biochemistry at the Faculty of Agriculture, University of Belgrade [10].

Data acquisition and analysis

Electrocardiogram (ECG) was recorded by two-channel ECG type recorder (Rozinn Electronics Inc, USA) in supine position, for 20 minutes before (relaxation) and 60 minutes after drink intake, but not during actual drinking. ECG recordings were digitized using commercial software Wavelab (Steinberg Media Technologies GmbH, Germany) on a personal computer via an analogue-to-digital converter, with a sampling frequency of 1,000 Hz. The R peaks and the RR intervals were determined by Origin (Microcal Software, Inc., Northampton, MA, USA) software. The QT intervals were determined by our own software based on tangent method estimation [11]. According to this method, the end of the T wave was defined as the point where the line from the peak of the T wave to the steepest point of the descending limb of the T wave intersected the isoelectric baseline. Mean RR, mean QT, HRV indices, and QT variability were calculated from non-overlapping segments of 256 points. The number of these 256 points segments was: 5 in period of relaxation and 15 after drink intake. Presented quantities are mean values calculated from all segments and averaged over all subjects. Power spectrum densities and spectral components were calculated as reported previously [12]. QT variability (QTV) was calculated as square of the QT standard deviation. Blood pressure BP and HR were recorded by automatic wrist blood pressure monitor (Geratherm Medical AG, Germany) at the end of relaxation, immediately after the drink intake, and then every 10 minutes until the end of the recordings.

Detrended fluctuation analysis (DFA)

The DFA, modification of the random walk model analysis was used to quantify the fractal-like scaling properties of time series [5]. The root mean-square fluctuations of the integrated and linearly detrended data were calculated in observation windows of varying sizes and then plotted against the size of window on a log–log scale. The power-law behaviour was quantified as the slope of the linear regression line. The slopes, the short-term scaling exponent α_1 and long-term scaling exponent α_2, were calculated over the window size $n < 32$ and $n > 32$ intervals. The values of scaling exponent indicate type of noise: $\alpha \sim 0.5$ (uncorrelated white noise), $\alpha \sim 1$ (correlated 1/f noise) and $\alpha \sim 1.5$ (strongly correlated noise). The scaling exponents were calculated from the whole time series of RR and QT in relaxation and after the drink intake.

Sample entropy

Sample entropy (SampEn) was computed according to the procedure published by Richman and Moorman [7]. The

SampEn quantifies the irregularity of a time series and estimates the conditional probability that two sequences of m consecutive data points, which are similar to each other (within given tolerance *r*), will remain similar when one consecutive point is included. The SampEn algorithm considers two parameters: tolerance level (*r*) and pattern length (*m*). According to the authors' recommendation, we chose a tolerance level of r = 0.15 times standard deviation of the time series and *m* = 2. SampEn was also calculated from the whole time series of RR and QT in relaxation and after the drink intake.

Statistics

Data are presented as mean ± standard error (SE). Due to their skewed distributions, spectral components of RR intervals and variability of the QT intervals were analyzed after natural logarithmic transformation. Scaling exponents and SampEn values were averaged over subjects for relaxation and over subjects and time after beer intake. Paired samples *t*-test was used for all comparisons. A $p < 0.05$ was considered statistically significant. Analyses were performed using the SPSS (Statistical Package for the Social Sciences, Chicago, IL) software release 15.0.

Results

Immediately after the intake, beer induced simultaneous significant peaks of HR and systolic and diastolic blood pressures (SBP and DBP, respectively) (Figure 1A). However, after 10 minutes these quantities returned to initial values or below them. Compared to baseline values, beer induced decrease of DBP and increase of HR in the last 10 minutes of recordings. In addition, in the last 10 minutes there was a steady decrease in the QT intervals (Figure 1B). However, beer induced different behavior of the QT and RR variability (QTV and spectral components LF and HF). QTV had a transient peak after the intake, while HF was reduced significantly (Figure 1C). Beer intake increased short-term scaling exponent (α_1) of the RR time series and long-term scaling exponent (α_2) of the QT time series (Table 1). We examined above results by determining sample entropy as an independent measure, also derived from nonlinear dynamics. Beer intake decreased SampEn of both, the RR and QT time series compared with relaxation value but not significantly (Table 1).

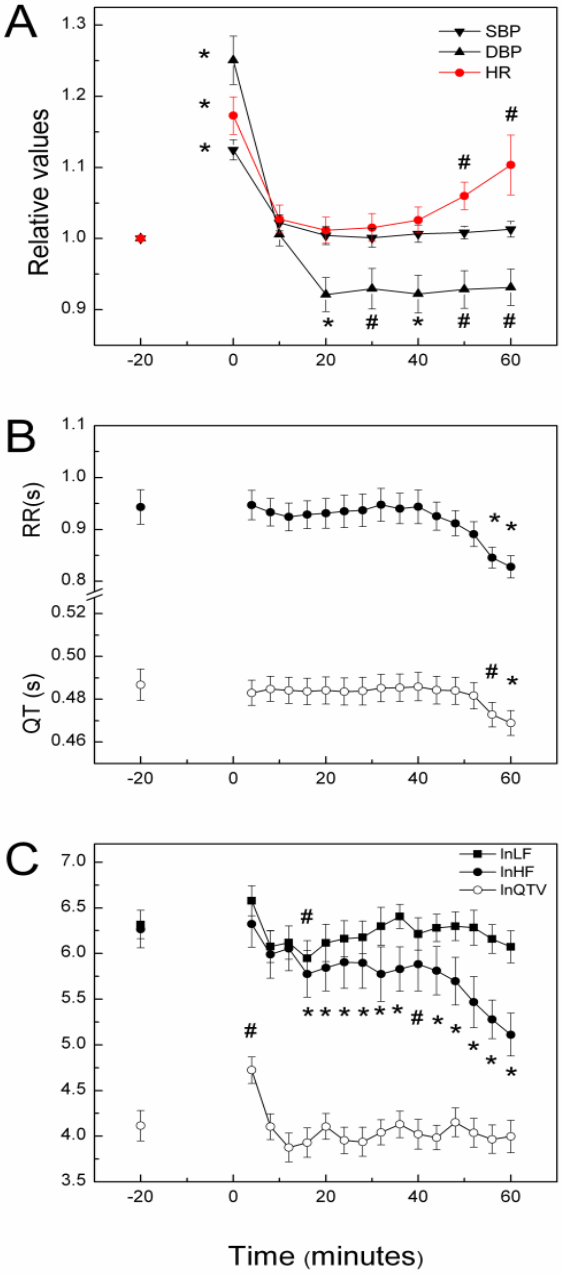

Fig. 1 – Time course of relative values: A) of heart rate (HR) and systolic and diastolic blood pressures (SBP and DBP); B) RR and QT; C) logarithm of spectral components of low frequency (LF) and high frequency (HF) and QT variabililty (QTV) in ms^2.
Mean values ± standard error (SE); $^*p < 0.01$, $^\#p < 0.05$ post drink period *vs* relaxation (-20 minutes).

Table 1

Nonlinear measures of RR and QT interval time series

Variable	RR		*p*	QT		*p*
	Relaxation	Beer		Relaxation	Beer	
α_1	0.76 ± 0.04	0.85 ± 0.04	0.01	0.62 ± 0.02	0.60 ± 0.02	0.38
α_2	0.84 ± 0.03	0.88 ± 0.03	0.19	0.88 ± 0.04	0.95 ± 0.03	0.04
SampEn	1.5 ± 0.1	1.4 ± 0.1	0.54	2.20 ± 0.08	2.13 ± 0.06	0.47

Values are expressed as mean ± standard error (SE).
***p* – significance of statistical comparison of post drink period with relaxation. α_1 – short term scaling exponent; α_2 – long-term scaling exponent; SampEn – sample entropy.**

Disscusion

In this study, the acute effect of beer was quantified for the first time by linear and nonlinear measures of RR and QT interval series. We found a similar shortening of RR and QT intervals between 50 and 60 minutes after beer intake. However, beer had different effect on variability and structure properties of these series.

Increase of HR immediately and about 50 minutes after drinking beer probably had origin in different physiological mechanisms. The first increase is well known as direct pressor effect due to direct acute vasoconstrictor effect of alcohol induced by shift in intracellular calcium [13, 14]. We also observed this effect as increase in blood pressure. The latter one corresponds to reduced HF, i.e. reduction of parasympathetic control of the heart, which has been found after intake of ethanol and red wine [3].

QT variability was significantly increased after beer intake and returned to initial values. Exact mechanisms which generate and modulate QTV are unknown. It is probably influenced by both, the intrinsic adaptation of the action potential duration and the autonomic nervous system activity. Cardiac norepinephrine spillover, the direct index of cardiac sympathetic activity, is related to QTV in patients with hypertension [15], but not in patients with panic disorder and depression [16] , or in normal conditions [17]. Our results point out that the structure of the QT interval series was different from that of the RR series in relaxation and that beer intake had different effects on these two series. Values of scaling exponents were lower and sample entropy was higher for the QT series during resting state. Lewis and Short [18] suggested that the greater complexity in the modulation of the QT interval compared with the RR interval might be explained with regard to differential autonomic nervous system (ANS) modulation of the atrial and ventricular myocardium. SampEn of QT reflects the ANS modulation of both the atrial and ventricular myocardium [18]. We found that beer induced a significant increase in short-term scaling, α_1, exponennt of RR series, and long-term scaling exponennt, α_2, of QT series. It seems that beer induced changes in short-term regulatory mechanisms of RR series and long-term regulatory mechanisms of more complex QT series (higher SampEn). Both changes, are probably caused by transient reduction of several control mechanisms of the heart or transient domination of vasomotor control.

Immediate effect of beer on the BP is a significant jump and return to the baseline values. However, in the interval 20-60 minutes after beer intake the DBP was significantly reduced. As diastolic pressure is determined mainly by cardiac output and peripheral vascular resistance, it might be that they both are influenced by beer.

The major limitation of our study is relatively low number of subjects introduced in the study. However, according to our knowledge this is the first study that investigated the interaction of bear intake and changes in linear and nonlinear measures of RR and QT interval series in humans. Having in mind that population which consume bear every day is very big, the investigation of proarithymic effect of this beverage is very important for cardiovascular science.

Conclusion

The beer has immediate and delayed effect on the whole cardiovascular system which can be followed directly through changes of BP and HR. However, our study shows that delayed, fine and complex effect of beer could be quantified by properties of the RR and QT interval series. The results indicate reduction of parasympathetic control of the heart and changed dynamic of QT intervals.

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Sociodemographic characteristics as suicide risk factors in Belgrade, the capital of Serbia

Socio-demografske karakteristike kao faktori rizika od samoubistva u Beogradu, Srbija

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Abstract

Background/Aim. Suicide and self-inflicted injuries are one of the leading causes of injury-related deaths worldwide. The aim of this work was to investigate sociodemographic characteristics as a risk factor for suicide. The investigation covered the period from 1997 to 2011 on the territory of the capital of Serbia, the city of Belgrade. **Methods**. The data was taken from the index books of suicides committed in the city of Belgrade, held at the Institute of Informatics and Statistics. Statistical analysis was done by using the crude specific rate. The variability of the rate was estimated by computing a confidence interval. **Results**. The average suicide rate in the observed period was 9.88. The suicide rate has shown a regular decline until 2005, and since then, a mild growth was observed (the highest in 2000, the lowest in 2004). The results of our study pointed out the following sociodemographic profile as a risk factor for suicide: male with uncompleted elementary school who lives in a suburban community, aged 66 and over. Suicides were usually realized in the area of residence during the day, in late spring, and hanging was the most frequent method of suicide. **Conclusion.** Preventive public health measures should be implemented focusing on typical socio-demographic profile. Prospective studies should include more variables in order to identify more risk factors for suicide and suicidal behavior. To achieve this goal, a serious national strategy for recording suicide and suicide prevention would have to be developed.

Key words:
suicide; serbia; socioeconomic factors; demography; risk factors.

Apstrakt

Uvod/Cilj. Širom sveta samoubistvo i samopovređivanje jedni su od vodećih uzroka smrti. Cilj ovog rada je bio da se ispitaju sociodemografske karakteristike kao faktori rizika od samoubistva. Istraživanje je obuhvatilo period od 1997. do 2011. godine i teritoriju Beograda, glavnog grada Srbije. **Metode**. Podaci su uzeti iz sveski registra samoubistva Instituta za informatiku i statistiku grada Beograda. Statističkom analizom obuhvaćena je stopa samoubistava (učestalost u odnosu na 100,000 stanovnika), a njen varijabilitet procenjen je na osnovu izračunatog intervala poverenja. **Rezultati**. Prosečna stopa samoubistava u posmatranom periodu bila je 9.88. Stopa samoubistava pokazuje pravilan pad do 2005. godine, i od tada blag rast (najviša stopa bila je 2000. godine, a najniža 2004. godine). Rezultati našeg istraživanja ukazali su na sledeći sociodemografski profil kao faktor rizika za samoubistvo: muškarac sa nedovršenom osnovnom školom koji živi u predgrađu, starosti 66 godina i više. Samoubistva su se najčešće dešavala u oblasti stanovanja tokom dana, u kasno proleće, a vešanje je bio češći način samoubistva. **Zaključak**. U okviru preventivnih mera trebalo bi usmeriti posebnu pažnju na dobijeni sociodemografski profil potencijalnih samoubica. Buduće studije sa više varijabli suzile bi, a ujedno i obogatile sociodemografski profil karakterističan za samoubistvo i samoubilačko ponašanje. Da bi se ovo postiglo potrebno je da se napravi i razvije obiljna nacionalna strategija prevencije i registracije suicida.

Ključne reči:
samoubistvo; srbija; socioekonomski faktori; demografija; faktori rizika.

Introduction

Suicide and self-inflicted injuries are one of the leading causes of injury-related deaths worldwide. There are an estimated 10–20 million attempted suicides each year [1, 2]. A 2002 report by the World Health Organization (WHO) states that nearly a million people take their own lives every year which is more than those killed in war [3]. WHO figures show that suicide takes place somewhere in the world every 40 seconds. In the last 45 years suicide rates have increased

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by 60% worldwide. Suicide is among the three leading causes of death among people aged 15–44 in some countries, and the second leading cause of death in the 10–24 years age group. Mortality from suicide constitutes a significant public health problem. Data from the USA indicates that reported suicide is almost 40% higher than homicide. However, much more public attention in that country focuses on homicide than on suicide [4]. Keeping in mind the aforementioned data, an important task for researchers and public health officials is to seek effective intervention strategies for suicide prevention. Nordentoft [5] describes two preventive interventions: universal, directed toward the entire population, and selective, geared toward individuals who are at greater risk to suicidal behavior. Suicidal risk factors can be estimated from different points of view, as suicide is a complex phenomenon involving psychological, social, biological, cultural and environmental factors.

Belgrade is the capital of Serbia with 1,576 124 citizens, 747,854 males and 828,270 females, according to the 2002 census. There are 17 municipalities in the city.

The aim of this work was to investigate the sociodemographic characteristics as a selective risk factor for suicide. Our investigation covered the period from 1997 to 2011 (except 2009) on the territory of the capital of Serbia, the city of Belgrade. Data was only available for this period. There was no statistical data available about this, and all information included were unprocessed data.

Methods

The data was taken from the index books of suicides committed in the city of Belgrade, held at the Institute of Informatics and Statistics. The information was limited, and included the recording of nine variables: name, sex, time and date of death, date of birth, place of residence (district and address), city area (central/suburban) and municipality of the suicide, education, profession and cause of death [method of suicide including International Statistical Classification od Diseases – 10th revision (ICD-10 code)]. For statistical analysis we used a crude specific rate, as it is easily computed based on the number of suicides per 100,000 members of the population. The variability of the rate was estimated by computing a confidence interval.

Results

In the observed fifteen-year period 2,181 people took their own lives in Belgrade. Annual suicide rates from 1997 until 2011, as well as the average suicide rate for that period are shown in Table 1.

The average suicide rate in the observed period was 9.88. The highest suicide rate was recorded in the year 2000 while the lowest one in 2004. The suicide rate showed a regular decline until 2005, and since then - a mild growth. The suicide rate in the last year of the period observed (2011) was almost the same as in the period ten years before (2002).

The distribution of the average suicide rate by gender showed that for the observed fifteen year period, the average male and female suicide rates were 14.20, and 5.97, respectively. Data analysis pointed out that the average male to female suicide rate ratio was 2.41.

The highest suicide rate was in late spring (May and June), and the lowest one in winter (December and February), which is shown in (Figure 1) below.

It was appropriate to classify persons who committed suicide into five age groups with an age interval of 15 years. The first group consisted of persons younger than 20, and the last group of persons older than 65. The age structure of people who committed suicide is shown in Figure 2 .

Data analysis pointed out that the average rate for a specific age group started at 1.02 (in the age group under 20) and gradually increases up to 21.02 (in the age group over 66).

For the purpose of this study we used four educational categories (based on years of completed school): 1- less than eight years of education, 2- eight years of education, 3- twelve years of education and 4- sixteen or more years of education. The average fifteen-year suicide rates by educational structure of persons who commited suicide are shown in Table 2.

Table 1

Annual suicide rate (1997–2011)

Year of committed suicide	Rates per 100,000 inhabitans	95% confidence interval for rates	
		upper limit	lower limit
1997	12.69	14.45	10.93
1998	11.42	13.09	9.75
1999	12.37	14.11	10.64
2000	14.40	16.28	12.53
2001	11.48	13.16	9.81
2002	10.85	12.48	9.22
2003	7.42	8.77	6.08
2004	6.79	8.08	5.50
2005	6.85	8.14	5.56
2006	7.74	9.11	6.37
2007	7.80	9.18	6.42
2008	9.07	10.56	7.59
2010	9.33	10.83	7.82
2011	10.15	11.72	8.58
Average	9.88	11.44	8.33

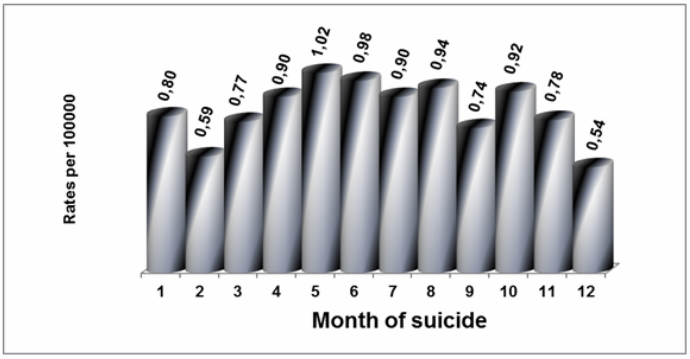

Fig. 1 – Month of suicide – average suicide rate (1997–2011).

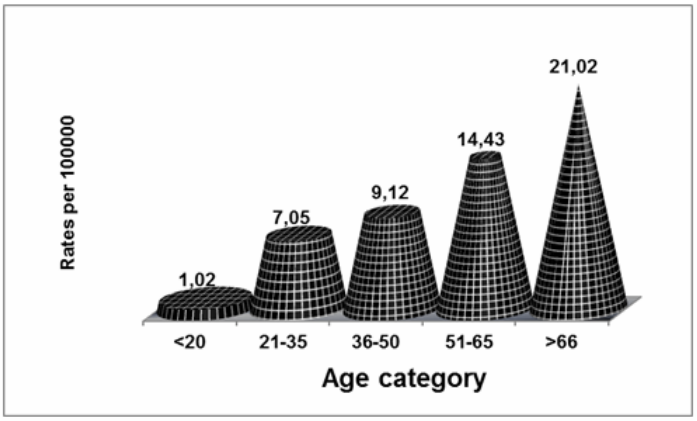

Fig. 2 – Age of suicide – average suicide rate (1997–2011).

Table 2

Educational structure – average suicide rate (1997–2011)

Educational structure (years of school completed)	Rate per 100,000 inhabitants	95% confidence interval for rates	
		upper limit	lower limit
< 8	0.158	0.271	0.045
8	0.078	0.108	0.048
12	0.077	0.098	0.056
≥ 16	0.034	0.056	0.013

The greatest number of Belgrade inhabitants who committed suicide had completed secondary school, but the highest suicide rate (0.158) was in the lowest educational group (less than eight years of education).

Table 3 shows the average fifteen-year suicide rate related to the area in which the subjects lived, as well as to the area where suicide occured (central city area or suburban municipality).

The average fifteen-year suicide rate was higher in suburban municipalities.

The highest number of suicides occurred in the most populated municipalities of the city. The highest number of suicides occurred on the territory of the central city municipality, Savski venac, but the average fifteen year suicide rate was higher in suburban municipalities.

Considering the time of suicide (day, evening, morning), the average fifteen-year suicide rate was the highest during the day (3.72), and the lowest during the night and early morning (1.76).

Table 3

Area of residence and suicide rate (1997–2011)

Area of residence/suicide	Rate per 100,000 inhabitants	95% confidence interval	
		upper limit	lower limit
Residence			
central city area	8.73	10.37	7.10
suburban area	14.41	18.59	10.23
Suicide			
central city area	8.94	10.59	7.29
suburban area	13.64	17.71	9.57

Considering the methods of suicide, hanging, drowning and suffocation (ICD-10 code: X 70) were the most frequently chosen ones – 30.2%. Other frequent methods in order of frequency were: suicide by unspecified means (ICD-10 code: X 84) – 26.5%, jumping from a high place (ICD-10 code: X 80) – 4.4% and firearms (ICD-10 code: X 74) – 4.4%.

Discussion

Last years have been a period of great changes, as well as significant and serious social turmoil when the people of Serbia have experienced the consequences of economic blockade, the NATO intervention, social transformation and economic crisis. The people have lived, and still do, in a state of prolonged stress which has become their reality, a part of everyday life [6]. All of these may become the reason for suicide.

In the observed fifteen-year period 2,181 people committed suicide. The average suicide rate since 1997 until 2011 was 9.88. The highest suicide rate was in 2000 (14.40), which is not surprising if we bear in mind the NATO intervention in the preceding year. Suicide rates showed a regular decline till 2004, and a mild growth since then. Comparing suicide rates in Belgrade and Serbia, we can see that the year 2000 was the one with the highest suicide rate, both in the whole country and its capital. However, the trend from that time was different: in Serbia suicide rates showed a regular decline [7, 8], and mild growth in Belgrade. It is not easy to interpret this data because analysis of these trends demands knowledge and availability of various different variables which were not available to us. The average fifteen-year suicide rate in Belgrade (9.88) was below the suicide rate for the world as a whole (11.6), and for Serbia (18.8) [9].

In the observed fifteen-year period 1,487 males and 694 females committed suicide and this is just under twice as many male suicides as there were female ones. Keeping in mind that by the 2002 census, Belgrade had rather a greater number of female than male citizens; the male to female suicide ratio is 2 to 1. The male average suicide rate was 14.20 and the female average suicide rate is 5.97. Data analysis pointed out that the male to female average suicidal rate ratio was 2.41:1. It is similar to male/female average suicidal rate ratio in Serbia [10] and in the capital of neighbor country Montenegro [11]. Data from literature shows that there is a relatively constant predominance of male suicide rates over female ones: 3.2 : 1 in 1951, 3.6 : 1 in 1995 and 3.9 : 1 in 2002 [12, 13]. There is only one exception (China), where suicide rates in females are consistently higher than suicide rates in males [14]. Comparing male to female suicide rates in Belgrade with those in the world, one can see that male to female suicide rates in Belgrade are lower than in the rest of the world.

The highest suicide rate is expected in the spring and late autumn. The idea that suicide is more common during the winter holidays (including Christmas in the northern hemisphere) is actually a myth generally reinforced by media coverage associating suicide with the holiday season. A study examining the relationship between suicide attempts and major public holidays in Europe [15] reported that there were fewer suicide attempts before Christmas and 40% more attempts after Christmas than expected. The lower suicide rate in February (0.59) in our study can be explained by the fact that February is the shortest month of the year. The smallest suicide rate in December (0.54), may be the consequence of strong family relations in our society (family gatherings during the holidays). The USA National Center for Health Statistics found suicide drop during winter months and peak during spring and early summer. A report from 20 Organization for Economic Cooperation and Development (OECD) countries [16], investigating the hypothesis that sunshine exposure may trigger suicidal behavior, suggested that suicides followed a seasonal pattern with a dominant peak during the month in which daylight was longest. Our results are similar to the above mentioned: the highest suicide rate was in late spring - May (1.02) and June (0.98).

Suicide risk has been historically considered to increase with age, in older men having been identified as the group of highest risk [17]. This was so in our investigation: the average rate for a specific age group started at 1.02 (in the age group under 20) and gradually increased up to 21.02 (in the age group over 66). The youngest citizen who committed suicide was 13 years old, and the oldest one was 101.

The greatest number of Belgrade citizens who committed suicide had completed secondary school. Twelve year education is not a risk factor for suicide because the population with secondary school education is numerically high in the city (669,927). The highest suicide rate (0.158) was in the lowest educational category (less than eight year education).

The municipalities in which the greatest number of suicides was recorded are also the most densely populated municipalities in the city. Suburban municipalities had a higher suicide rate than central city areas, considering both the area of residence (14.41) and the area of suicide (13.64). It is interesting that the highest (absolute) number of suicides was seen in a small central city municipality, Savski venac. This may be because the big central city hospital, where people who attempt or commit suicide are admitted, is situated in this area (thus the hospital might be registered as a place of death).

When considering the time of suicide (day, evening, morning), the average fifteen-year suicide rate was the highest during the day. The fact that the highest suicide rate (3.72) was registered during the day might not be accurate, as it may refer to the time when the body was found.

This study found that suicide by hanging was the most predominant method of suicide in Belgrade (30.2%) in the period 1997–2011. A study of suicide methods in 16 countries participating in the "European Alliance Against Depression" [18] project had the same result – hanging, as the most frequent method of suicide. Although hanging as a simple method of suicide is the most common one in many countries worldwide [19], there is a considerable international variability. A study of suicide methods in a large number of cases in Japan and the United States [20] revealed that Japan had a very high proportion of hanging (70.4% of males and 35.6% of females), while this proportion was much lower in the United States (18.2% of males and 16.2% of females). Jumping from a high place and using firearms take third and fourth

place as suicide methods both in Belgrade (this study) and in Europe [18].

Conclusion

The aim of this work was to investigate the sociodemographic characteristics as a selective risk factor for suicide. The results of our study point out the following sociodemographic profile as a risk factor for suicide: a male, aged 66 and over, uncompleted elementary school, living in a suburban community, committing suicide in the area of residence during the day in late spring by hanging as the most frequent method of suicide.

It is easy for a well-organized country to measure mortality, including injury mortality. However, diagnosing suicide also includes determining the component of intent, which makes it more difficult to obtain unequivocal statistical data. There is also a need for wide standardization of routine suicide data. In our investigation we answered the question related to the sociodemographic risk factors that provoke suicide. Available data was limited. Prospective studies should include more variables, such as the presence of mental or somatic illness, origin, marital status etc. which means that this data should be recorded. To achieve this, a serious national strategy for recording suicide rate and suicide prevention should be developed.

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Risk factors for recurrent otitis media with effusion

Faktori rizika od nastanka rekurentnog sekretornog otitisa

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Abstract

Background/Aim. Otitis media with effusion (OME) is a common disease among the children aged from 6 months to 4 years, but recurrences are common after the extraction of ventilation tubes. The aim of the study was to determine the risk factors for recurrent OME after extraction of ventilation tubes. **Methods.** The research was a prospective study with 305 children aged 0 to 10 years with OME. Forty three (14%) of them had recurrent OME. We analyzed the factors that could lead to the redevelopment of the disease after extrusion of the tubes. **Results.** It was found that the majority of children with recurrent disease was between the ages of 5 to 7 years (56%) and had allergy in significantly higher rate than children without recurrence. In most cases (37.7%), the retention time of ventilating tube was above 10 months and the recurrent disease was diagnosed in 46.5% cases within a period of 10 to 12 months after extrusion of tubes. **Conclusion**. Children with OME and after ventilation tube extrusion need to be followed up for 1 year after the removal of tubes. It is necessary to inform the parents that the disease can recur. Children in the kindergarten, in preschool age and with a respiratory allergy had higher possibility for recurrent OME.

Key words:
otitis media with effusion; risk factors; child, preschool; recurrence; serbia.

Apstrakt

Uvod/Cilj. Rekurentni sekretorni otitis je često oboljenje dece uzrasta od šest meseci do četiri godine, a relapsi se najčešće javljaju nakon ekstrakcije ventilacionih cevčica. Cilj istraživanja je bio da se utvrde faktori rizika za rekurentni sekretorni otitis nakon ekstrakcije ventilacionih cevčica. **Metode.** Istraživanje je bila prospektivna studija sa 305 dece sa sekretornim otitisom uzrasta od 0 do 10 godina, od kojih je 43 (14%) imalo rekurentni sekretorni otitis. Analizirano je koji faktori mogu dovesti do ponovnog razvoja bolesti nakon ispadanja ventilacione cevčice. **Rezultati.** Utvrđeno je da je većina dece sa recidivom bolesti bila uzrasta od pet do sedam godina (56%) i sa pojavom alergija na znatno višoj stopi nego deca bez recidiva. U većini slučajeva (37.7%) vreme zadržavanja ventilacionih cevčica je trajalo više od 10 meseci, a rekurentna bolest je dijagnostikovana u 46.5% slučajeva u periodu od 10 do 12 meseci nakon ekstrakcije cevčice. **Zaključak.** Decu sa sekretornim otitsom i posle ispadanja ventilacinih cečica treba pratiti godinu dana. Neophodno je informisati roditelje da se bolest može ponovo javiti. Deca u vrtiću, u predškolskom uzrastu i sa respiratornim alergijama imaju veću mogućnost za pojavu rekurentnog sekretornog otitisa.

Ključne reči:
otitis medija, serozni; faktori rizika; deca, predškolska; recidiv; srbija.

Introduction

Otitis media with effusion (OME) is a very common disease in children and those aged between 6 months and 4 years are the most threatened [1]. Many episodes resolve spontaneously within 3 months, but in about 30% to 40% of children OME recurs [2].

The treatment modality includes watchful waiting, medical treatment and surgical intervention [1]. Ventilation tube insertion is one of the most frequently performed treatments for OME and about 20% of children who underwent ventilation tube insertion required additional insertion within 2 years since their first procedure [3]. It is necessary to identify a group of children with high risk of recurrent OME due to choosing efficient treatment policies.

The aim of this study was to determine risk factors for recurrent OME after ventilation tube extrusion.

Methods

A prospective study was performed in one-year period (March 2012 to March 2013) in the tertiary referral centre and included 305 children with OME treated by the same

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senior surgeon. The study excluded all children with chronic or systemic diseases, such as children with craniofacial anomalies. Ethical Board of the Institute for Health Protection of Mother and Child of Serbia in Belgrade improved this study and signed informed consent was obtained from parents. Diagnosis of OME was made by otomicroscopy and tympanometry. During otomicroscopic examination, presence of fluid or air bubbles behind ear drum and any retraction suggested OME. Also, type B tympanogram was considered positive for OME. All patients underwent skin prick tests for common perennial and seasonal allergens: dust, feathers, tobacco, grass pollen, tree pollen, weed pollen, ragweed, dust mites, cat hair, dog hair. The results were evaluated after 10 minutes and reaction upper than 3 mm were considered positive. Children were in conservative treatement with an antihistaminic (either with allergic negative reaction) and a secretolytic for peroral usage 2 months prior surgery. Also, they used hypertonic saline solution and intranasal steroids (older than 6 years old). Children with 3-months-lasted OME underwent surgical treatment. There was an initial ventilation tube insertion with adenoidectomy in 262 patients due to OME and additional ventilation tube insertion in 43 children due to recurrent OME. Surgeon used ventilation tube type Shepard (Spiggle and Theis, Germany) for initial insertion and type Good for prolonged ventilation in patients with recurrent OME. All children with recurrent OME had adenoidectomy and ventilation tube insertion as an initial treatment of OME in previous history. Their data were collected from questionnaire answered by parents and history of illness and included age, gender, economic status (low, high), ear disease in another member of family, out of home day care, presence of frequent middle ear and upper respiratory infections before initial tube insertion, ages of the initial ventilation tube insertion, the time of extrusion of tubes, respiratory allergy status and the time of onset the recurrent OME. Twenty-two children of 43 (53.5%) with recurrent OME had a single tube insertion in previous history and rest of them had two ventilation tube insertions.

Statistical analysis included a descriptive statistics and χ^2 test using SPSS for Windows.

Results

In one year period 305 children (181 male and 124 female, 59% and 41%, respectively) had surgery due to OME. They were divided into 3 age groups: below 2 years (54 children = 17.7%), between 3 and 6 years (201 children = 66%), between 7 and 10 years (50 children = 16.3%). Forty three of them were with recurrent OME for a total of 81 ears (38 cases with bilateral recurrent OME). The overall prevalence rate of recurrent OME was 14%; 16% between the age 2 and 4; 56% between 5 and 7; 28% above 8 years. A significant statistical relation was reported between age and recurrent OME ($p < 0.05$) with peak in age group between 5 and 7. It was found that 56% of children with recurrent OME had a respiratory allergy. Children had an allergic rhinitis. It was statistically significant when compared with children without recurrent disease who had allergy in 23% cases ($p < 0.001$). Children with bronchial asthma were excluded from the study.

Distribution of children with OME in dependence of the retention time of ventilating tube after the first implantation and the time of diagnosis of recurrent OME is shown in Table 1. There was no statistically significant association between these times ($p = 0.306$) i.e. recurrence rate was not dependent on ventilating tube retention time.

The factors identified in children with recurrent OME are shown in Table 2. Children with a recurrent OME were dominantly from kindergarten (70%).

In the group of children with recurrent OME, 22 (53.5%) of them had only one ventilation tube insertion and

Table 1

Distribution of children with otitis media with effusion (OME) in dependence on retention of ventilating tube and time of diagnosis of recurrent OME

Parameters	0–3 months n (%)	4–6 months n (%)	7–10 months n (%)	above 10 months n (%)	Total n (%)
Retention time of ventilating tube	1 (2.3)	16 (37)	10 (23)	16 (37.7)	43 (100)
The time of diagnosis of recurrent OME	6 (14)	14 (32.5)	3 (7)	20 (46.5)	43 (100)

n (%) – number (percentage) of children.

Table 2

Risk factors for recurrent otitis media with effusion

Parameters	Number (%) of children	
	yes	no
History of frequent AOM	24 (56)	19 (44)
History of frequent URTI	21 (49)	22 (51)
Family history of ear disease	10 (23)	33 (78)
Kindergarten	30 (70)	13 (30)
Low economic status of the family	1 (2.3)	42 (97.7)
Craniofacial abnormalities	2 (4.6)	41 (95.4)

AOM – acute otitis media; URTI – upper respiratory tract infections.

21 (46.5%) had two ventilation tube insertion. Table 3 demonstrates the comparison between various characteristics of children who had a single ventilation tube insertion *vs* those who had more than one tube insertion. Children with recurrent ventilation tube insertion had significantly more allergy than children who had only a single ventilation tube intervention ($p < 0.05$).

moval of a chronic infection of adenoids. Children with recurrent OME not necessarily need to have readenoidectomy, if carried out during the first operation. Iwaki et al. [6] found that there was no correlation between adenoidectomy and the recurrence rate of OME. However, some studies showed that adenoidectomy reduced the need for additional tubal insertion [3,7].

Table 3

The comparison between various characteristics of children with recurrent otitis media with effusion (OME) who have undergone single ventilation tube (VT) insertion *vs* those who have undergone two VT insertions in previous history

Characteristics	One VT (n = 22; 53.5%)	Two VT (n = 21; 46.5%)	*p*
Allergy			
yes	9	17	0.01*
no	13	4	
Age of the first VT surgery (years)			
0–2	7	7	
3–6	15	14	0.09
7–10	0	0	ns
older than 10	0	0	
Retention time of the last ventilating tube (months)			
0–3	0	1	
4–6	7	10	0.12
7–10	4	6	ns
above 11	11	4	
History of frequent AOM			
yes	11	11	0.75
no	11	10	ns
History of URTI			
yes	10	11	0.53
no	12	10	ns
Family history of ear disease			
yes	2	8	0.03
no	20	13	ns
Kindergarten			
yes	15	12	0.33
no	7	9	ns
Low economic status of the family			
yes	3	1	0.55
no	19	20	ns

AOM – acute otitis media; URTI – upper respiratory tract infection; * – significant difference; NS – non significant difference.

Discussion

The literature presents OME prevalence in a bimodal distribution, with peak incidence at 1 to 2 years of age and again at 5–7 years [4]. In this study, 66% of children with OME were between 2 and 6 years old. OME is characterized by a high rate of spontaneous recovery but also by a high rate of recurrence [5]. The reccurence rate of OME in children was found to be higher at ages from 2 to 5 years than from 7 to 8 years [4]. About 20% of children need additional tube insertion due to recurrent OME [3]. In our study, the overall prevalence rate of reccurent OME in children who need additional ventilation tube insertion was 14%, and the most of them (56%) were between 5 and 7 years old.

All children with OME underwent adenoidectomy with ventilation tube insertion as the first surgery treatment. The rationale for adenoidectomy in the treatment of OME was reduction of nasal obstruction, improved Eustachian tube function and re-

Clinical and experimental studies showed that respiratory allergies promote adenotonsillar hypertrophy as well as inflammatory alternations in the mucous membranes of middle ear and Eustachian tube and can thus promote the formation and presence of OME [8].

In our study, children with allergic rhinitis had more possibilities for OME. Frequently, symptoms of allergic rhinitis were treated by parents themselves or neglected. Untreated allergic rhinitis caused mucosal edema and occlusion of Eustachian tubes with effusion in the middle ear with prolonged duration.

In our study, group of children with recurrent OME had a significantly higher rate of allergy than children with no recurrence. Kwon et al. [9] found that allergic rhinitis was more frequent among pediatric patients with than without OME and the rates of other allergic diseases were not significant. The epidemiological data of Martines et al. [10] supported the existence of a relationship between OME and atopic disease

and suggested that allergy was an important risk factor for OME among the higher age group.

There was no correlation between the retention time of ventilating tube after the first implantation and the time of diagnosis of recurrent OME. In all cases of initial insertion, the surgeon used Shepard grommets and Good tubes for secondary insertion. Yaman et al. [11] showed that recurrence rate was 20.7% in ears after extrusion of Shepard tubes. They concluded that recurrence rates were the highest in the group with extrusion time of tube of less than six months and suggested that recurrence rate would be reduced when the tubes stayed in place for a long time. Talmon et al. [12] used T-tubes for treatment patients with OME and tubes were retained for 20 months. Reinsertion was performed only in 4.9% of ears due to recurrent OME.

In our study, the history of frequent AOM in children with recurrent OME was almost in distribution half and half (56% positive *vs* 44% negative). Similarly, the history of frequent upper respiratory tract infection (URTI) was in the same distribution (49% positive *vs* 51% negative).

Our results showed a statistically significant high number of children with recurrent OME who were in the kindergarten and with high economic status of the family. Children who attend day care are more often exposed to ear infections and respiratory infections, which may be the reason why the recurrent OME is more common in this group of patients. In some studies, it has been reported that the higher socioeconomic status is, the higher otitis media prevalence is [4, 13].

Conclusion

Careful study of the related risk factors for recurrent OME is recommended for better understanding prognostic indicators and avoiding treatment failure. Parents must know that the disease can recur and that patients need to be follow-up for another year after the removal of tympanostomy tubes. Children in the kindergarten and with respiratory allergy are in the group with higher possibility for recurrent OME and need at least one-year-follow-up period after extrusion of tubes.

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Relation between grades of intervertebral disc degeneration and occupational activities of patients with lumbar disc herniation

Povezanost stepena degeneracije intervertebralnih diskusa i radnih aktivnosti kod bolesnika sa lumbalnom diskus hernijom

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Abstract

Background/Aim. Intervertebral disc degeneration (IDD) occurs as consequence of combined effects of genetic, age-related, environmental and occupational factors. Lumbar disc herniation (LDH) develops mostly due to IDD. The aim of this study was to investigate whether the frequency of LDH is higher at the level of the most pronounced IDD, and whether a category of physical workload influences higher IDD on level L3-L4, L4-L5 and L5-S1 separately. **Methods**. The research included 60 patients with permanent employment, hospitalized due to LDH. A grade of IDD was assessed by lumbosacral preoperative magnetic resonance imaging (MRI), according to Pfirmann's MRI classification system. Occupational factors were determined by a specific questionnaire. **Results.** Out of the 60 patients participating in the study, 33.3% had jobs with easy workload, 23% had moderate workload, while 43% had heavy workload. Herniated discs were found at level L3-L4 in 8.3%, at level L4-L5 in 46.7% and at level L5-S1 in 45% patients. The symptomatic discs at level L5-S1 showed statistically significant frequency of degenerative changes of grades IV and V. Binary logistic regression results showed that the strongest predictor of IDD grade for examined levels was physical workload. Positive association of physical workload and IDD grade was observed in all cases. Higher grades of IDD are more likely for patients with both higher TE and heavier physical workload (OR 2.011) at level L3-L4. At levels L4-L5 and L5-S1 higher degree of IDD was more likely for females with heavier physical workload (OR 1.978 and 2.433 respectively). **Conclusion**. Symptomatic discs show higher frequency of higher grades of IDD but herniation does not occur solely at the disc of the most prominent degenerative changes. The results suggest importance of inter-influence of physical workload and the years of employment and the inter-influence of physical workload and gender, on degeneration of lumbar discs.

Key words:
lumbosacral region; inervertebral disc degeneration; occupational exposure; intervertebral disc displacement.

Apstrakt

Uvod/Cilj. Degeneracija intervertebralnog diskusa (DID) nastaje kao posledica udruženog dejstva genetskih, starosnih faktora kao i faktora iz životne i radne sredine. Lumbalna diskus hernija (LDH) je najčešće posledica DID. Cilj rada bio je da se istraži da li je učestalost LDH viša na nivou najizraženijih degenerativnih promena intervertebralnog diskusa i da li kategorija fizičkog opterećenja na radnom mestu ima uticaj na stepen DID na nivou L3-L4, L4-L5 i L5-S1 pojedinačno. **Metode.** Istraživanjem je obuhvaćeno 60 bolesnika u stalnom radnom odnosu, hospitalizovanih zbog LDH. Stepen DID utvrđen je na osnovu preoperativnog nalaza pregleda lumbosakralne kičme magnetnom rezonancom (MRI) prema Firmanovom MRI sistemu klasifikacije. Profesionalni faktori su određeni pomoću specifičnog upitnika. **Rezultati.** Od 60 bolesnika koji su učestvovali u istraživanju, 33.3% obavljalo je lak fizički rad, 23% srednje težak, a 43% bolesnika težak fizički rad. Hernijacija diskusa na nivou L3-L4 nađena je kod 8,3%, na nivou L4-L5 kod 46,7%, a na nivou L5-S1 kod 45% bolesnika. Simptomatski diskusi na nivou L5-S1, su pokazali statistički značajno veću učestalost degenerativnih promena IV i V stepena. Rezultati binarne logističke regresije pokazali su da je fizičko opterećenje najjači prediktor stepena DID na svim ispitivanim nivoima. Pozitivna veza između fizičkog opterećenja i stepena

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DID je uočena u svim slučajevima. Viši stepen DID je verovatniji kod bolesnika sa dužim radnim stažom i teškim fizičkim opterećenjem (OR 2.011) na nivou L3-L4. Na nivoima L4-L5 i L5-S1 viši stepen DID je verovatniji kod žena koje obavljaju težak fizički rad (OR 1,978 i 2,433 respektivno). **Zaključak**. Simptomatski diskusi imaju veću učestalost degenerativnih promena višeg stepena, ali se hernijacija ne dešava isključivo na nivou diskusa sa najizraženijim degenerativnim promenama. Rezultati ukazuju na značaj interaktivnog uticaja kategorije fizičkog opterećenja i dužine radnog staža kao i interakcije kategorije fizičkog opterećenja i pola, na degeneraciju lumbalnih diskusa.

Ključne reči:
lumbosakralni predeo; diskus degeneracija; profesionalna izloženost; hernija diskusa.

Introduction

The degenerative process of intervertebral discs (IVD) includes many biochemical and pathoanatomical alterations, leading to changes in its biomechanical characteristics. It is the result of combined effects of genetic, age, mechanical, chemical, and autoimmune factors [1]. Degeneration of IVD is clinically significant if its pathogenesis, directly or indirectly, causes the low back pain (LBP), and in cases of lumbar disc herniation (LDH), leads to the compression of neural and vascular elements in the surrounding tissue [2]. Low back pain occurs more often when combined with the existence of degenerative alterations in IVD [3]. However, intervertebral disc degeneration (IDD) in itself does not need to be the cause of pain [1].

Low back pain is usually related to the factors from occupational environment, such as exposure to vibrations, nonphysiological position of a body during the work (extreme forward bending, trunk twisting), carrying and lifting heavy weights [3–5]. Therefore, the LBP is regarded as an occupation related disease [6]. Low back pain is caused by pathological changes in lumbar spine and surrounding tissue, commonly due to IDD [1, 2, 7].

Although the effect of physical workload on the occurrence of the LBP has been extensively investigated, only a few quantitative studies have examined the morphological changes visualized via magnetic resonance imaging (MRI) in relation to occupational variables [8].

The aim of this study was to investigate whether the frequency of LDH is higher at the level of the most pronounced IDD, and whether a category of physical workload in the patient's working position influences higher IDD at the most common level L3-L4, L4-L5 and L5-S1, separately.

To the best of our knowledge, this is the first research about relation between physical workload and the grade of IDD changes in patients with LDH for each level separately, and similar studies has not been published at the national level.

Methods

This study included 60 patients with permanent employment who were hospitalized for operative treatment due to the LDH on one level, marked as symptomatic level. Study was conducted on the Clinic of Neurosurgery of the Clinical Centre of Vojvodina in Novi Sad, Serbia, in the period between January 2013 and December 2014. The study excluded patients with spinal trauma consequences, spinal deformities, associated rheumatic and degenerative conditions of musculoskeletal system, and metabolic disorders with manifestations on the locomotor system, oncological diseases, infective and inflammatory diseases of the spinal column.

Lumbosacral preoperative MRI was analyzed. The standard protocol included the imaging of spinal segments between Th10 and S5, with T1-weighted (T1W) and T2-weighted (T2W) sequences in sagittal projections, T2W sequence in axial projections of the lower three levels of the lumbar segment parallel to the intervertebral disc, as well as Short Tau Inversion Recovery (STIR) sequence in coronar projections. Additional axial projections also included levels where suspect protrusions; radiculopathies and spinal stenosis were detected on sagittal projections. If needed, more T2W sequences in sagittal projections were taken with the obliteration of fat tissue signal. The images were processed on an MRI scanner and obtained in a digital form. On the basis of this examination, the grade of IDD was assessed (levels L3-L4, L4-L5 and L5-S1), according to Pfirmann's magnetic MRI classification system [9].

Assessment of the IDD was performed independently by a single radiologist, blind for the patients' demographic, occupational and clinical characteristics. Using self-evaluation questionnaires in the preoperative evaluation we collected the following basic demographic and occupational data: gender, age, number of years of employment in total, number of years of service in the workplace where patients were currently employed. By measuring the weight and height of participants we calculated body mass index (BMI). Demands of the physical load at work place of participants were determined by self-assessment, using "Workload Assessment Questionnary" (Table 1). This questionnaire was specially designed for the purpose of assessing physical activity in the workplace. As a part of the questionnaire, participants provided information on the length of work activities in relation to the eight-hour day, expressed as: 0% absence of load, < 30% of the eight-hour working time, > 30% of the eight-hour working time. Work activities that we assessed were related to: static load, dynamic load, sitting, standing, lifting and carrying loads, the presence or absence of whole body vibration, bending and rotation of the trunk. Based on data collected from this questionnaire, an independent specialist in occupational medicine, licensed to perform risk assessments in the workplace, blind for clinical status, neurological status of participants, and their MRI findings, determined the category of physical workloads of each participant, according to the recomended methodology for "Grading System for Physical Workload" (Table 2). For statistical analysis we grouped a category of hard and very hard work as the same group.

Table 1

Workload Assessment Questionnary of the Institute of Occupational Medicine "Dr Dragomir Karajović", Belgrade, Serbia, designed for the purpose of assessing physical activity in the workplace.

Physical workload	0%	< 30%	Above 30%
Dynamic work			
Static work			
Sitting			
Standing			
Walking			
Forced position			
bending			
trunk twisting			
Lifting and carrying weights			
The amount of weight (kg)			

Table 2

Grading System for Physical Workload of the Institute of Occupational Medicine "Dr Dragomir Karajović", Belgrade, Serbia, for determination of the category of physical workloads

Category	Description of workload
I – Easy	Sitting type of work with limited walking and standing (0%); mostly easy manual work (using arms and hands), without forced body position, without lifting and carrying weights and without static work.
II – Medium	Alternation of sitting, standing and walking (< 30%) including carrying and lifting light and medium heavy weights (women 5 kg and men 12 kg) without forced body position and with little static work.
III – Hard	Alternation of mostly standing and walking including lifting and carrying heavy weights (women 5–10 kg and men 12–25 kg), occasionally (up to 8%) forced body position and static work.
IV – Very hard	Alternation of mostly standing and walking; whole body work with constant lifting (> 30%), (women over 10 kg and men over 25 kg), forced body position and very often (> 30%) static work.

Summary statistics was presented as a mean ± SD for metric variables and as percentages for categorical variables. Analysis of disk degeneration was based on the following classification of the patients: the first group consists of patients whose grade of IDD was II or III, while the second one consists of patients with grade IV or V which is considered as a higher grade of IDD. Differences between groups were tested using χ^2 test and binomial test. The tested proportion in binomial test was 0.5. Crosstab procedure and χ^2 test were used to examine differences in characteristics between groups for nominal variables. Binary logistic regression models were built to discover which factors were affecting grades of degeneration on levels L3-L4, L4-L5 and L5-S1 including symptomatic and asymptomatic levels. The dependent variables for all three levels were coded using the same classification of the patients according to their grade of the IDD mentioned above. All models were built on the basis of carefully chosen independent variables which can be divided into two categories: basic characteristics of patients (gender, age, BMI) and the patient's occupational characteristics [total years of employment (TE), years on the actual working position (AWP), sitting, standing, walking, presence of vibrations at work place, nonphysiological position of a body during the work (extreme forward bending, trunk twisting), carrying and lifting of heavy weight and variable that describe the patient's workload]. Models were presented with the regression coefficients (B), standard errors (S.E), *p* values (Sig.) and odd ratios (OR) for sets of statistically significant variables. The level of significance in all tests was set to ($p < 0.05$). All calculations were performed using standard statistical packages (R CRAN and SPSS, version 20).

Results

Thirty-one male and 29 female patients aged between 35 and 60 years (average age was 46.22 ± 7.71) participated in the study. Average employment period for the actual professional position was 16.47 ± 8.66 years (ranging between 5 and 38 years) while the total period of employment was on average 21.10 years (ranging between 5 and 38 years). As for the physical workload, 33.3% of the patients had jobs with easy workload, 23% had moderate workload, while 43% had heavy workload. No statistically significant difference was found between different categories of the physical workload ($\chi^2 = 3600$, df = 2, $p = 0.165$).

Herniated discs were found at level L3-L4 in 8.3% patients, at level L4-L5 in 46.7% and at level L5-S1 in 45% patients. Frequency of LDH on level L3-L4 is statistically significant lower comparing with other two examined levels. ($\chi^2 = 16.900$, df = 2, $p = 0.000$).

The differences in frequencies of IDD grades (Figure 1) on symptomatic discs were not statistically significant (χ^2 = 2.341, df = 2, p = 0.310) for each level. A majority of the patients had higher grade of IDD. However, the difference in number of patients with grades II and III and patients with grades IV and V of IDD was only statistically significant at level L5-S1 (test. prop. = 0.5, exact sig. = 0.000).

Contrary to the symptomatic discs, on asymptomatic discs at level L3-L4 (Figure 2), the Binomial test showed statistically significant difference between frequency of patients with IDD changes grades II and III and patients with grades IV and V (test. prop. = 0.5, exact sig. = 0.000).

The patients were more likely to have higher grade of IDD, if they had longer TE, AWP and sitting during working time. (Table 3) Precisely, patients with higher TE have 1.371 higher chances to have higher grade of IDD in comparison to the patient with lower TE. When it comes to AWP, the chance for the patients with higher AWP to have higher grade of IDD was 1.147. Furthermore, patients who were sitting more during working time were 1.873 times likely to have higher grade of IDD. The patients with both higher TE and heavier physical workload are 2.011 times likely to have higher grade of IDD.

For level L4-L5, the patients are more likely to have higher grade of IDD if their TE was higher (Table 4). The patients with higher TE were 0.512 times likely to have higher grade of IDD. Physical workload in interaction with gender and TE increases chances for higher grade of IDD. Females with heavier physical workload were 1.978 likely to have higher grade of IDD. Moreover, patients with both higher TE and heavier physical workload are 1.151 times likely to have higher grade of IDD.

The results for level L5-S1 indicated that patients with the presence of vibrations at work place were likely to have higher grade of IDD (Table 5). Physical workload and lifting and carrying, but both in interaction with gender were likely to have higher grade of IDD. Females with heavier physical workload were 2.433 times likely to have higher grade of IDD, while females who lift and carry more than 30% were 2.502 times likely to have higher grade of IDD.

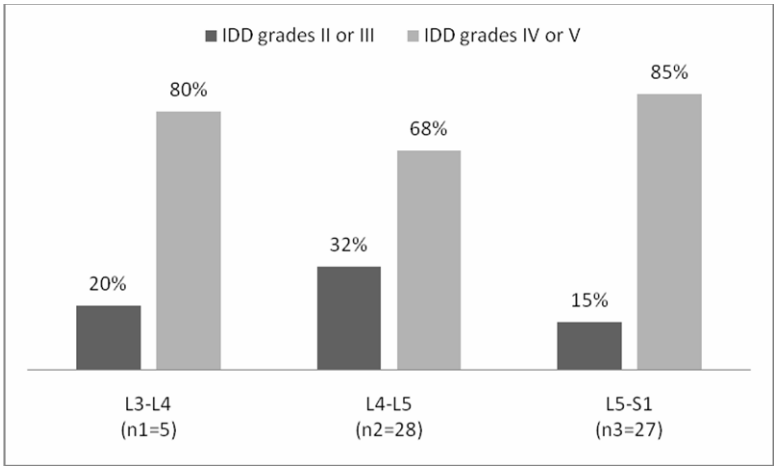

Fig. 1 – The frequencies of intervertebral discs degeneration (IDD) grade II and III (dark gray column) and grade IV and V (light gray column) according to Pfirmann's magnetic resonance imaging (MRI) classification system, for symptomatic discs (n = 60) in 60 patients, at level L3-L4, L4-L5, L5-S1.

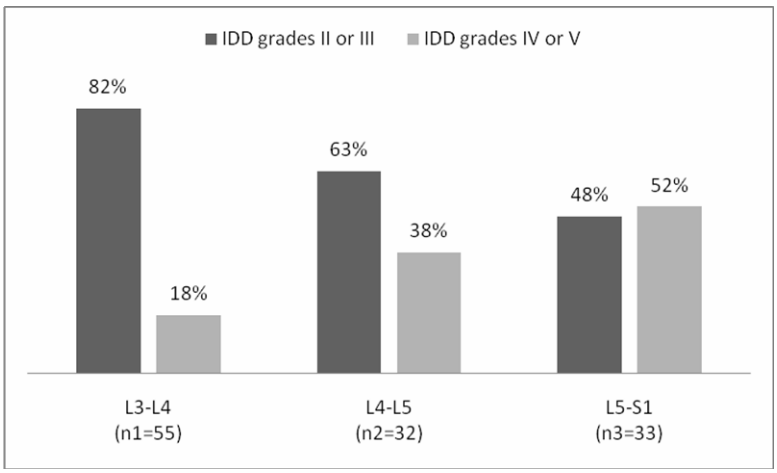

Fig. 2 – The frequencies of intervertebral disc degeneration (IDD) grade II and III (dark gray column) and grade IV and V (light gray column) according to Pfirmann's magnetic resonance imaging (MRI) classification system, for asymptomatic discs (n = 120) in 60 patients, at level L3-L4, L4-L5, L5-S1.

Table 3

Statistically significant results of binary logistic regression analysis that represent relation between grades of intervertebral disk degeneration (IDD) and patients occupational characteristics for level L3-L4

Occupational characteristics	B	SE	Sig.	OR
TE	0.316	0.107	0.045	1.372
AWP	0.137	0.001	0.020	1.147
Sitting	0.628	0.038	0.004	1.873
TE by physical workload	0.698	0.004	0.020	2.012

TE – total years of employment; AWP – years on the actual working position.
SE – standard error; OR – odds ratio.

Table 4

Statistically significant results of binary logistic regression analysis that represent relation between grades of intervertebral disk degeneration (IDD) and basic characteristics of patients and the patients occupational characteristics for level L4-L5

Occupational characteristics	B	SE	Sig.	OR
TE	0.234	0.547	0.040	0.512
Physical workload by gender (1)*	0.682	0.418	0.046	1.978
TE by physical workload	0.141	0.783	0.020	1.151

TE- total years of employment; SE – standard error; OR – odds ratio; *reference category for gender was male (0), where gender (1) denotes females.
SE – standard error; OR – odds ratio.

Table 5

Statistically significant results of binary logistic regression analysis that represent relation between grades of intervertebral disk degeneration and basic characteristics of patients and the patients occupational characteristics for level L5-S1

Occupational characteristics	B	SE	Sig.	OR
Vibration (1)	-0.669	0.339	0.033	0.009
Physical workload by gender (1)*	0.889	0.543	0.027	2.433
Lifting and carrying by gender (1)*	0.917	0.594	0.035	2.502

***reference category for gender was male (0), where gender (1) denoted females; SE – standard error; OR – odds ratio.**

Discussion

Lumbar disc herniation is the most common cause of surgical treatment of active working population [10]. Most commonly, it occurs between 40 and 50 years of age [1] with a predominance of intervertebral discs herniation at the L4-L5 and L5-S1 level [11], as it was shown in our study.

Degenerative changes on symptomatic intervertebral disc

Degeneration of intervertebral discs usually preceded LDH [12]. Studies that have examined the degenerative diseases of IVD show that the herniated disc is not mandatory degeneratively changed [13]. Modic et al. [14] and Jacob et al. [15] showed that in 60% of cases disc was degenerated, and in 8% of cases degeneration did not preceded herniation. Our research confirmed that the herniated discs showed higher degree of degeneration (IV and V) on all three analyzed levels, in 68–85% cases. Herniated discs at level L4-L5 in 32% cases showed lower degree of degeneration. However, significantly higher frequency of higher degree degenerative changes was revealed only at the herniated disc at level L5-S1. Cross-sectional study by Vardhan et al. [16] also showed that in the lumbar region maximum degenerative changes (Modic type end plate degenerative change, disc contour abnormalities and disc inflammation and infiltration) were seen at L5-S1 level. This points out possible interactions of the anatomical, biomechanical factors at L5-S1 level with other factors that lead to faster progression of degenerative changes, as well as that pathogenetic mechanisms of disc herniation and degeneration of intervertebral discs are somewhat different.

Degenerative changes at asymptomatic level

Asymptomatic discs have a lower incidence of higher degree degenerative changes compared to symptomatic discs at all three analyzed levels. Asymptomatic discs at the L3-L4 in 82% of cases have a lower degree of degenerative changes (grade II and III), while the discs on other two analyzed levels represent degenerative changes even lower and higher grades. The fact that the discs at the L4-S1 levels are more degenerated than at the L1-L4 level indicates that there are other unresolved variables that play a role in the pathogenesis of disc degeneration, and that they affect the lower levels of the lumbar segment more than the upper ones [1].

Is the physical load associated with the degeneration of intervertebral discs?

The potential impact of physical activity as the etiologic cause of the IVD degeneration is still being discussed. Several experimental sudies suggest that compressive forces can

lead to structural changes in IVD (decreased disc thickness) as well as to changes in the IVD metabolism [17–19].

Results of German multicenter case control study support a clear dose-response relation between cumulative lumbar load and lumbar disc herniation as well as symptomatic lumbar disc narrowing [8]. However, Elfering et al. [20] in their prospective MRI study failed to detect any association between exposure to heavy physical load and the development of degeneration of intervertebral discs. Another group of authors who investigated the morphological changes visualized by MRI examination also found that exposure to physical load was not necessarily associated with the presence of degeneration and narrowing of the disc, but a higher degree to such an exposure was directly associated with a higher degree of degeneration [21]. Based on this association of professional factors and degeneration of intervertebral discs, in some countries as Germany, France, and Denmark, it was decided to include lumbar disc diseases in the list of occupational diseases [8].

Our study demonstrated a significant interaction effect of the category of heavy physical loads and other factors, on the occurrence of degenerative changes at a higher degree at all three levels. For the level of L3-L4 and L4-L5 model of binary logistic regression showed association of hard physical labor and the years of exposure to such work which coincides with the study of Hung et al. [22], which has proven connection between degeneration of intervertebral discs and cumulative workload.

A study conducted in the group of women gave results that lumbar disc degeneration are not associated with the job type and characteristic physical loadings [23]. Our results revealed the significant influence of hard physical labor in women on the occurrence of higher grade of IDD at the level L4-L5 and particularly at level L5-S1. Precisely, our results indicate that women with heavier physical workload have more chances to have higher grade of IDD than men with the same workload on both levels L4-L5 and L5-S1. Additionally, women who lift and carry more than 30% during working time have more chances to have higher grade of IDD than men on level L5-S1.

However, studies that investigated the cumulative load of the lumbar spine in women found a positive relation between the dose of physical activity and the occurrence of LDH [22].

A cross-sectional study, however, showed a significant association between performing sedentary activities in females and the number of degenerative intervertebral discs changed at the level of L5-S1 [24].

Current studies indicate that lumbar intradiscal pressure in sitting is unlikely to pose a threat to non-degenerate discs, and sitting is no worse than standing for disc degeneration or low back pain incidence. If sitting is a greater threat for development of low back pain than standing, the mechanism is unlikely to raise lumbar intradiscal pressure [25].

In our group of patients we found an association between prolonged sitting for over 30% working hours and a higher degree of degenerative changes at the level L3-L4. Namely, patients who are sitting more during working time have increased chances to have higher grades of IDD than patients who sit less.

In our study, the effect of whole body vibrations on discs at L5-S1 level proved to be significant because it gave higher degree of degenerative changes, compared to those in subjects subjects who were not exposed to general vibrations in the workplace. The association between whole body vibration and degeneration of IVD has confirmed in a study carried out earlier [26]. Vibrations at or near the spine's intrinsic resonance frequency cause abnormal pressure-dependent fluid shifts in the intervertebral disc. The combination of certain postures of the spine with vibrations in the resonance range leads to increased energy consumption, probably because of the 2.5-fold increase in axial stress, that experimental studies have shown, to arise with vibrations in this range [2].

Potential limit, or the specificity of our study in the research of degenerative changes in the discs of lumbar region is that our study group represents only those with diagnosed LDH without including subjects who do not have LDH. Being unable to precisely measure the dose of physical activity in interaction with other occupational hazards during work time and their relations with degenerative changes of IVD, adds a strong suspicion of a causal association.

Conclusion

Symptomatic discs show higher frequency of higher grades of IDD, but herniation does not occur solely at the disc with the most prominent degenerative changes. The results suggest the importance of inter-influence of physical workload and the years of employment, and the inter-influence of physical workload and gender, as well as on degeneration of the lumbar discs.

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Analysis of occlusal characteristics of identical homozygous twins

Analiza okluzalnih karakteristika jednojajčanih blizanaca

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Abstract

Background/Aim. Functionally stable occlusion is characterized by optimal and orthopedic stable position of the condyle, harmonious contact ratio of upper and lower teeth in the final occlusal position of the mandible, optimal relationship of dental arches at the eccentric movement of the lower jaw and stable interocclusal space in physiological position. As there are no ideal order of teeth and absolute intermaxilar harmony nor individually acceptable standards or prototype of functionally optimal occlusion, the aim of this study was to analyze occlusal characteristics of identical twins. **Methods**. This clinical-epidemiological and functional study involved 30 pairs of identical twins, both males and females, aged 20 to 40 years. The main criterion for the selection of participants was preserved function of orofacial system, as a precondition for application of the Peer Assessment Rating (PAR) index, which is the most reliable tool for diagnosing malocclusion and evaluating occlusal parameters: the distance between the contact points of agonists of front segments of the dental arches, side occlusion of the sagittal, vertical and transversal position, overjet, anterior crossbite, deep and open bite, overbite of incisors and relationship of middle dental arches. **Results**. Using *t*-test for independent samples, no significant difference in the values of PAR index, according to gender, was established. The average difference of 0.833 between the twin groups (for male twins: $\bar{x}$ = 7.97, SD = 6.625; for female twins: $\bar{x}$ = 7.13, SD = 5.606) was not statistically significant [t (58) = 0.526; p = 0.601; 95% confidence interval: -2.339–4.005]. **Conclusion**. The lack of significant differences in occlusal PAR index analysis in both the same and different twin groups, implies the dominance of hereditary factors.

Key words:
twins; sex factors; denal occlusion; malocclusion.

Apstrakt

Uvod/Cilj. Funkcionalno stabilnu okluziju karakterišu optimalan i ortopedski stabilan položaj kondila, harmoničan kontaktni odnos gornjih i donjih zuba u završnom okluzionom položaju mandibule, optimalna relacija pri ekscentričnim kretnjama donje vilice i stabilan interokluzioni prostor u položaju fiziološkog mirovanja. Pošto ne postoje idealni poredak zuba i apsolutni međuvilični sklad, kao ni individualno prihvatljivi standardi optimalne okluzije, cilj rada bio je analiza okluzalnih obeležja jednojajčanih blizanaca. **Metode**. Kliničko-epidemiološkim i funkcionalnim ispitivanjem obuhvaćeno je 30 parova jednojajčanih blizanaca, podjednake polne zastupljenosti, starosti od 20 do 40 godina. Osnovni kriterijum za izbor ispitanika bila je očuvana funkcija orofacijalnog sistema, kao preduslov primene *Peer Assessment Rating* (PAR) indeksa kojim se najpouzdanije dijagnostikuju malokluzije i ocenjuju okluzalni parametri: rastojanje između kontaktnih tačaka agonista prednjih segmenata zubnih nizova, bočna okluzija u sagitalnom, vertikalnom i transverzalnom položaju, incizalni stepenik, prednji ukršten zagrižaj, dubok i otvoren zagrižaj, vertikalni preklop sekutića i odnos sredina zubnih nizova. **Rezultati**. Primenom *t*-testa za nezavisne uzorke nije utvrđena statistički značajna razlika u vrednostima PAR indeksa u zavisnosti od pola. Prosečna razlika od 0,833 između blizanačkih grupa (za blizance muškog pola: $\bar{x}$ = 7,97, SD = 6,625; za blizance ženskog pola: $\bar{x}$ = 7,13, SD = 5,606) nije bila statistički značajna [t (58) = 0,526, p = 0,601; 95% interval poverenja -2,339–4,005]. **Zaključak**. Izostanak značajnih razlika pri okluzalnoj analizi PAR indeksa, kod istih i različitih blizanačkih grupa, tumači se dominacijom naslednih faktora.

Ključne reči:
blizanci; pol, faktor; zubi, okluzija; malokluzija.

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Introduction

Occlusion is the direct contact of the upper and lower dental arch at rest and during the various functions of the orofacial system. It indicates the anatomical order of teeth in rows, their relationships to other structures of orofacial system and jaw relationships in the sagittal and frontal plane [1–4]. In the contemporary interpretations there is a prevalent physiological concept of efficient basic functions of the orofacial system, without sensation, pain or discomfort and without damage of the temporomandibular joint, orofacial muscles and the occlusal complex [3, 4].

Evaluation of the occlusal complex is an integral part of every functional analysis of the orofacial system. Despite the extreme variability, not only regarding the shape and position of individual teeth, but also their relationships, the category "physiological occlusion" refers more to the harmonious function, than to the ideal anatomical relationships in the orofacial system [5]. In these cases, if the condyles are in their physiologically optimal and most stable position, the teeth of upper and lower jaws should be in the most stable position, and providing a greater number of simultaneous contacts with the forces directed and transmitted along the axial axis of the tooth, which have a stimulating effect, unlike the compression force that results in bone resorption and the inclination of the teeth. This means that the horizontal forces in the protrusion and laterotrusion are harmful to the lateral teeth, while the intercanine sector is more distant from areas in which vectors of muscles' forces operate [6].

However, the ideal order of teeth and absolute intermaxillar harmony do not exist, nor individually acceptable standards or prototype of functionally optimal occlusion.

Monozygotic (MZ) twins are defined as the miracle of foetal biology, a medical reproductive challenge, the closest and most enduring biological connection [5]. Also, twins are the epilogue of the most complex, most diverse and the most challenging natural phenomenon during phylogenetic-ontogenetic processes that transform one cell, zygote with initial weight of 0.005 mg, into a multicellular adult, with about 12.5 trillion cells [7, 8].

Occlusal characteristics are the basis of any functional analysis of the orofacial system.

In this study we individually analyzed members of the same pair (intrapaired) and members of different pairs (interpaired) in order to evaluate the stability of the temporomandibular joints, the contact relationship of the upper and lower teeth in the final occlusal position of the mandible, optimal relations with eccentric movements of the lower jaw and stability of interocclusal space in physiological position of the mandible. Thus, the aim of this study was to analyse and evaluate the occlusal characteristics of MZ twins.

Methods

Clinical-epidemiological and functional testing was conducted in a sample of 30 pairs of twins, both males and females, aged 20 to 40 years. The main criteria for the selection of subjects were preserved function of the orofacial system (mainly intact dental arch); preserved comfortable relationship and harmony of dental arches, regardless of the third molars; anatomically correct position and arrangement of the teeth in the jaw, adapted to receive a compression force; mainly preserved number of natural antagonists; stable contact ratio in intercuspal position (ICP) of mandible; absence of deflective contacts, occlusal trauma and periodontal disease on the retruded contact position (RCP)–ICP way; presence of interocclusal free space between the upper and lower dental arches in physiological position; difference between mandibular central position and ICP equal to or less than 2 mm (disocclusion of posterior teeth in protrusion of the mandible, disocclusion of teeth on the nonworking side, the mandible laterotrusion, presence of maximum of three fillings, physiologic function of the masticatory muscles, examinees without previous orthodontic or prosthetic treatment; domination of the first class of Angle's occlusion.

These criteria were the indications for functional analysis and a prerequisite for the application of the Peer Assessment Rating (PAR) index, as most reliable tool for diagnosing malocclusion and evaluating occlusal parameters: the distance between the contact points of anterior segment of the dental arches agonists, lateral occlusion of the sagittal, vertical and transversal projections, overjet, anterior crossbite, deep and open bite, vertical overlap of incisors and relationship between the middle of the dental series [9].

The distance between the agonists' contact points

The distance between agonists' contact points, in frontal segments of the dental series was determined by compass. The defined measurements were expressed in millimetres of the ruler, and the obtained data, depending on the numerical amount of 0–1; 1.1–2; 2.1–4; 4.1–8, and more than 8 mm, were quantitatively evaluated with 0, 1, 2, 3, 4 and 5 points.

After scoring, occlusal anomalies from the group of irregular spacing of an individual or a group of teeth and impacted teeth were multiplied by a corresponding intraclass correlation coefficient of 1 (Table 1).

Table1

Evaluation of the distance between the contact point agonists of frontal segments of the dental arches, by the Peer Assessment Rating (PAR) index

Extent of expression (mm)	Points	Coefficient
0–1	0	
1.1–2	1	
2.1–4	2	1
4.1–8	3	
> 8	4	
Impacted teeth	5	

Lateral occlusion of the sagittal, vertical and transversal planes

In this grading system of occlusal characteristics in systematization of the PAR index, the accepted rule of scoring *in situ* and *in loco* was, to measure expression of 0, 1, 2, 3 and 4. The resulting points were multiplied with intraclass correlation coefficient of 1 (Table 2).

Table 2

Evaluation of lateral occlusions in the sagittal, vertical and transversal position by the Peer Assessment Rating (PAR) index

Position	Extent of expression	Points	Coefficient
Sagittal	Good intercuspation	0	
	Less than1/2 class to full intercuspation	1	1
	½ classes on any tooth	2	
Vertical	No open bite	0	1
	Open bite at least two teeth larger than 2 mm	1	
Transversal	No cross bite	0	
	The tendency to bite crossed	1	
	One tooth in crossed bite	2	1
	More teeth crossed bite	3	
	More teeth in the buccal misses	4	

Overjet

The overjet was valorised depending on the overjet expression – horizontal steps or sagittal spacing of incisal edge of the maxillary central incisors from the buccal surfaces of the lower namesakes. The resulting scores were multiplied by the intraclass coefficient of correlation of 6 (Table 3).

Anterior cross-bite (negative incisal overjet)

Anteriorly positioned crossbite is the occlusal characteristic which was validated in 5 variables and categories of the PAR index, for which, respecting prominency, the score range of 0-4 was applied, and then multiplied with intraclass coefficient of correlation of 6 (Table 3).

Overbite

Overbite in indexed PAR system has 4 gradations which were validated in the range from 0 to 3 points, and then multiplied by intraclass coefficient of correlation of 2 (Table 3).

Open bite

This occlusal characteristic in the vertical position, was classified in 5 variables, ranging from 0 to 4 points. It was multiplied than by infraclass coefficient of correlation of 2 (Table 3).

The relationship of the midlines of the dental arches

For this occlusal variable, which is defined by the contact point between central incisors, corresponding to the sur-

Table 3

Evaluation of overjet, anterior crossbite, overbite, open bite and midlines of dental arches relations by the Peer Assessment Rating (PAR) index

Occlusal characteristics	Extent of expression	Points	Coefficient
Overjet (mm)	0–3	0	
	3.1–5	1	6
	5.1–7	2	
	7.1–9	3	
	> 9	4	
Anterior crossbite	No anterior cross bite	0	
	Contact lenses edges of one or more incisors	1	
	One incisor in reverse occlusion	2	6
	Two incisors in reverse occlusion	3	
	More than two incisors in reverse occlusion	4	
Over bite	Coverage of the lower incisorsis less than1/3	0	
	Coverage of the lower incisors from1/3 to 2/3	1	2
	Coverage of the lower incisorsis greater than 2/3	2	
	Complete coverage of the lower incisors	3	
Open bite, (mm)	Does it	0	
	< = 1	1	
	1.1–2	2	2
	2.1–4	3	
	> 4	4	
Relationship of the jaw midlines	Midline series coincide	0	
	Differ between ¼ and ½ of the width of the lower incisors	1	4
	Deviate more than half of the width of the lower incisors	2	

faces of medial raphe in the upper jaw and mental spine for the lower jaw, the specific rule was applied. This rule determines all of the cases where midlines of dental arches are shifted, in which the numerical value must be added to contralateral side of measured disposition, and reduced from correspondent value on the side measured.

For example: if the participant of this study had shift of the middle part of dental arches of 2 mm towards the right side, than this measured numerical value was deducted of the value which was measured for lateral movement on the right side, and additionally this amount was added to measured lateral movement to the left side.

Numerical scores in the PAR system were accompanied by the measure of expression as shown in Table 3.

Score was multiplied by intraclass coefficient of correlation of 4.

All of the examinations were performed in the dental office, in the physiological chair, with artificial lighting. We used a standard accessories, mandatory for specialist's dental examination with obligatory wearing polyethylene gloves.

Instruments for visual inspection, palpation of muscles, determining potential anomalies of temporomandibular joint (TMJ) and dental relationships consisted of distracter, probe and the dental mirror.

Statistics

All results were statistically analyzed in the program Statistical Package for Social Sciences (SPSS), version 11.5 (Chicago, IL). The reliability of the PAR index, whose data were of continuous type, was expressed as the root square of mean error. In that way, the potential risks of accidental errors (measurement error) were eliminated as well as the subjectivity in the evaluation. Statistically significant differences in the evaluation of the two measurements, the differences between the sexes, as well as deviations from the standard values were tested by t-test or non-parametric alternative the Mann-Whitney test. Value of $p < 0.05$ was considered to be statistically significant.

Results

Occlusal characteristics and possible discrepancies of selected MZ twins were diagnosed using the PAR index.

Tables 4–6 show distribution of participants by variables involved in calculation of the PAR occlusal index in twin samples, for which the distance between the contact points of agonist of front segments of the dental arches (a measure of expresiveness), were evaluated, as well as occlusal relationship in sagittal, vertical and transversal reference planes, measures of overjet expression, the modalities of expression of the crossbite and variations in overbite, open bite and modalities of dental arche midlines.

By evaluating modalities of sagittal measure of occlusal relationships expressiveness, in the anteroposterior direction, following results were obtained: good intercuspation in 27 (45%) of twins, half of the class on any tooth in 20 (33.3%), and less than ½ of class II to the full intercuspation were observed in 10 (16.7%) participants.

By analyzing the distribution of identical twins, according to the modalities of vertical dimension expressiveness, in both groups were 58 (96.7%) subjects, with no open bite,

Table 4

Distribution of the respondents according to the modalities of expression of the distance between the contact point agonists of frontal segments of the dental arches

Distance* (mm)	Identical tweens, n (%)
0–1	46 (76.7)
1.1–2	13 (21.7)
2.1–4	1 (1.7)
Total	60 (100.0)

***The distance between the contact points agonists of frontal segments of the dentition (a measure of expression).**

Table 5

Interpairs comparison of the Peer Assessment Rating (PAR) index, for both twin groups

Gender	Number of respondents	Mean value	Standard deviation	Standard error difference
Male	30	7.97	6.34	6.625
Female	30	7.13	5.89	5.606

Table 6

Comparison of the results of the Peer Assessment Rating (PAR) index for the male and for female twins pairs

Gender	Number of respondents	Mean value	Standard deviation
Male	15	6.33	5.58
Male	15	9.61	7.34
Female	15	7.63	7.15
Female	15	6.67	3.65

while testing the occlusal relationship in transversal position revealed no crossbite in 55 (91.7%) respondents. By assessing the distribution of respondents according to the expression of overjet, in 47 twins sagittal amount of 0–3 mm (78.3%) was prevailing. Also, evaluating the distribution of participants, according to the expression of frontal crossbite among identical twins, no anterior crossbite or negative overjet were found in 58 (96.7%) patients.

By analysing overbite, it was found that in 44 (73.3%) of MZ twins coverage of the lower incisors was less than 1/3, while the jaw midlines deviated from ¼ to ½ of the width of the lower incisors, in more than a half of twins [32 (53,3%)].

Using *t*-test there were no significant differences between the values of twins PAR indices, with respect to the gender.

The averaged difference of 0.833 between the two groups of twins (for a group of male twins: arithmetic mean ($\bar{x}$) = 7.97, standard deviation (SD) = 6.625; for a group of female twins: $\bar{x}$ = 7.13, SD = 5.606) indicated that there were no statistically significant differences at the level α = 5%, [t (58) = 0.526, p = 0.601. 95% confidence interval -2.339–4.005].

Also, intrapaired evaluation and analysis of the PAR index, by using paired *t*-test of dependent samples between pairs of male twins revealed that there was no statistically significant difference within this subgroup.

The average difference in the PAR index of -3.267 between pairs of male twins (for the first pair: $\bar{x}$ = 6.33; SD = 5.589; for the second one: $\bar{x}$ = 9.61; SD = 7.347) was not statistically significant at the level α = 5 % [t (14) = -1.55, p = 0.143; 0.95% interval of confidence -7.787–1.254].

Also, neither intrapaired evaluation, nor the analysis of the PAR index (*t*-test of paired samples) revealed any statistically significant differences within sample of female twins.

The average difference in the PAR index of 0.933 between these two groups of twins (the first group: $\bar{x}$ = 7.6; SD = 7.159; the second group: $\bar{x}$ = 6.67; SD = 3.658) was not statistically significant at the level α = 5% [t (14) = 0.753, p = 0.464 (two-sided); 0.95% interval of confidence -1.726–3.593].

Discussion

In this study we evaluated the occlusal characteristics of the PAR index, and registered occlusion in the sagittal, vertical and transversal position in MZ twins. There were determined overjet, deep (overbite) or open bite and positioned midlines of the dental arches.

Among the variables, the PAR index was evaluated not only by the most critical malocclusion factors, but optimal occlusion factors which determine the diagnostic status and priority of therapeutic treatment. It is important because there is a need to check and verify the validity and reliability of the index [10, 11] and determine the relations between normative and subjective need for treatment [12, 13]. Because of that, this index in contemporary practice is widely used to compare the results and determine their accordance [14–16].

By testing, evaluating and analysing the distance between the contact points of agonists of front segments of the tooth lines, the lowest limit of their expressions of 0–1 mm was obtained in 46 (76.7%) twins. By evaluating modalities of sagittal measure of expressiveness of occlusal relationships similar results were obtained: a good intercuspation in 45% partcipants, half of the class on any tooth in 20 (33.3%) participants, corresponding to the results from the literature on interjaw-maxillo-mandibular relationship [17] in which discussing on dysfunctional disorders, it has been stressed that the occlusal interference causes an excitation of the masticatory muscles, as opposed to the physiological occlusal contacts that have an inhibitory influence [18].

Also, with regards to the role and importance of a deep bite, there is no agreement. Sonnesen et al. [19] claimed that the temporomandibular joint disorders are more common in subjects with a deep bite and significant psychological score. On the contrary Gesch [20] believes that the distal occlusion and deep bite equally contribute to the protection of occlusal disharmony.

The incidence of the open bite in our twin sample is equivalent to the analogous studies in the general population [21]. The analysis of the distribution of respondents according to the modalities of vertical dimension expression, showed that 3.3% of participants had an open bite, as quantified by the ratio of 2:58 of respondents, faced with the possibility of occlusal discrepancies.

By evaluating occlusal relationships in the transversal plane, similar results were obtained. As many as 55 of 60 (91.7%) twin respondents had no anterior crossbite or negative overjet, the least frequent malocclusion in a randomized sample from the general population, as a potential etiologic factor of dysfunctional disorders. By analysing the distribution of respondents according to the modalities of overjet expression it was shown prevailing sagittal amount of 0–3 mm was (78.3%), similar to earlier published studies [17, 18, 21]. Their results suggest interaction of only the extreme values of the horizontal and vertical overlaps with occlusal dysfunctions.

In accordance with the literature data, participants of our study did not have front crossbite [58 (96.7%) of MZ twins]. It resembles the analogous studies that favor a unilateral bite as a significant etiological factor of interjaw insufficiency. Most unilateral crossbites are classified as forced bites, which in the intercuspal position generate temporary contacts and forcibly turns the mandible, although some studies deny this role [22].

Empirical differences were confirmed in the vertical relationship. By evaluating the variation of overbite and comparing the results, it was established a domination of coverage of the lower incisors less than ⅓ in 44 twins. Also, by analysing the distribution of respondents according to the modalities of midline series expression, it was found that the jaw midlines deviated from ¼ to ½ of the width of the lower incisors in more than half of respondents [32 (53.3%)], which is in accordance with the results of similar studies [17–19], but without a bimaxillary repercussion. The results of discrepancies in the midline series are equivalent with findings of Pullinger and Seligman [23] as the latest evidence of a statistically insignificant role of this parameter if there is no mandibular dislocation.

However, in the literature, there are disagreements about needs, the role and significance of occlusal characteristics of the PAR index [16, 24]. There are contrasting arguments, emphasizing that the existence of high and medium correlation among the studied parameters does not mean their approval [25, 26].

The results obtained in this study, despite significant association, point to the fact that adherence to stereotypes when registering occlusal contacts is incompatible with the possibility of variations, and that it is practically unacceptable to generalize occlusal modalities with no tolerance of alternatives in occlusal relationships.

Modern aspects imperatively impose similarities and differences of occlusal relationships, but also inflict the nature as the most successful creator of harmonious relations and orofacial harmony.

Conclusion

By statistical analysis of the occlusal characteristics of the PAR index, for the distance between the contact points of agonists in the frontal segments of the dental lines, occlusal relationship in sagittal, vertical and transversal positions, values overjet, variations overbite, midline dental series, modalities of crossbite and open bite no significant differences between couples of the same and different twin groups were found.

The lack of significant difference in occlusal analysis of the PAR index, in the same and different twin groups, implies the dominance of hereditary factors. The few differences are attributed to the influence of environment or lifestyle, trauma, tooth decay, early loss of deciduous and permanent teeth, or bad habits maintaining in tooth hygiene and oral parafunctions.

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Relationship between IL-1β production and endodontic status of human periapical lesions

Povezanost stvaranja IL-1β i karakteristika humanih periapikalnih lezija

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Abstract

Background/Aim. Apical periodontitis is mainly caused by bacterial infection within the root canal and periapical bone destruction which are prominent features of this lesion. The aim of this study was to determine the quantity of interleukin-1β in the tissues of periapical lesions and to analyze its relationships with: lesion size, previous treatments and pathohistological finding of involved teeth. **Methods.** Periapical tissues were obtained from patients undergoing periapical surgery. Out of all 80 cases included in the study, 24 had no previous endodontic treatment (open lesions), 37 were with endodontic failure (closed lesion) and in 15 cases root canal retreatment was performed few months before the surgery. By excluding four samples, the total of 76 samples, consisted of periapical lesions and the apical part of the tooth root, was collected. Each periapical tissue sample was divided into two equal parts. The one half of each lesion was used for quantification of interleukin-1β in tissue homogenates by the enzyme-linked immunosorbent assay (ELISA) method. The other part of each lesion was used for histopathological evaluation. **Results.** For each of the tissue homogenates, the quantity of interleukin-1β was measured, and it ranged from 0.6 pg/mg up to 74 pg/mg. There was no significant difference between the symptomatology and amount of interleukin-1β. Statistical data analysis showed a moderate correlation between lesion size and interleukin-1β measured values. The highest levels of interleukin-1β corresponded with chronic lesions in the stages of acute exacerbation and granulomas in early developing stages. Persistant granulomas, scar tissues, non-inflamed cysts and teeth with recently finished endodontic treatments showed a significantly lower level of interleukin-1β. **Conclusion.** The study results suggest that the differences in quantity of interleukin-1β correlate to lesion progression and phases of development.

Key words:
periapical periodontitis; interleukin-1alpha; interleukin-1beta; disease progression.

Apstrakt

Uvod/Cilj. Periapikalni periodontitis najčešće je prouzrokovan bakterijskom infekcijom unetom putem kanala zubnog korena i periapikalnom destrukcijom kosti koje predstavlja glavno obeležje ovih lezija. Cilj ovog istraživanja bio je da se odredi količina interleukina-1β u uzorcima periapikalnih lezija i da se analizira njihova povezanost sa: veličinom lezija, postojanjem prethodnih endodontskih tretmana i patohistološkim nalazima. **Metode.** Ispitivani su uzorci periapikalnih lezija sakupljeni tokom periapikalnih operativnih intervencija. Od svih 80 bolesnika uključenih u studiju, 24 su pripadali bolesnicima koji nisu podvrgnuti endodontskom tretmanu pre apikotomije (otvorene lezije), 37 su tretirani kao endodontski neuspeh (zatvorene lezije), a kod 15 bolesnika endodontski retreatman je sproveden nekoliko meseci pre oralno-hirurške intervencije; četiri uzorka su isključena iz studije. Svaki uzorak koji se sastojao od periapikalne lezije i vrha korena zuba podeljen je na dva jednaka dela. Prva polovina svakog uzorka korišćena je za kvantifikaciju interleukina-1β u tkivnom homogenatu metodom *enzyme-linked immunosorbent assay* (ELISA). Druga polovina uzorka korišćena je za histopatološku analizu. **Rezultati.** U svakom tkivnom homogenatu izmerena je količina interleukina-1β, čije vrednosti su se kretale od 0,6 do 74 pg/mg. Naši rezultati nisu potvrdili značajnu razliku između simptomatomatičnih i asimptomatičnih slučajeva; dokazano je prisustvo umerene korelacije između veličine lezija i vrednosti interleukina-1β. Najviše vrednosti interleukina-1β potvrđene su u fazama akutnog pogoršanja hroničnih lezija i kod granuloma u ranim razvojnim fazama. Kod starih granuloma, ožiljačnih tkiva, zuba sa nedavno završenim endodontskim retretmanom i neinflamiranih cista, vrednosti interleukina-1β bile su značajno niže. **Zaključak.** Rezultati istraživanja pokazuju da su razlike u količini interleukina-1β u korelaciji sa progresijom lezija i fazama njihovog razvoja.

Ključne reči:
periodontitis, periapikalni; interleukin-1alfa; interleukin-1beta; bolest, progresija.

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Introduction

Apical periodontitis is mainly caused by bacterial infection within the root canal and periapical bone destruction which are prominent features of this lesion [1, 2]. One of the main signs of periapical lesion is bone resorption [3]. The impact of apical periodontitis on the general health can be observed from two aspects: as an unwanted and harmful effect from the response of the organism, and on the other hand, as a positive bone tissue reaction by creating tampon zone around the process and releasing the defense constituents [4]. Bone resorption serves as a main indicator through which the phases of propagation of periapical lesion can be followed up. Contrary, the radiological signs of bone apposition may be used in assessment of the effectiveness of non-surgical endodontic treatment of periapical lesion [5].

Many biologically active molecules such as: prostaglandins, lipopolysacharide (LPS) complex, bacterial endotoxins and inflammatory cytokines: interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), tumor necrosis factor-beta (TNF-β), interleukin-6 (IL-6) and interleukin-11 (IL-11) have been shown to possess the capacity to activate bone resorption [6]. These molecules were previously referred as osteoclast-activated factors [6].

The crucial elements in the proinflammatory cascade, IL-1 and TNF-α are activated as a response to an infection or immunologic insults [6]. Interleukin-1 (IL-1) can be found in two forms: IL-1α and IL-1β. Although both forms have similar biological activities, it is believed that IL-1β is much more efficient in stimulating bone resorption and has a role in functions of a broader spectrum [7]. Apart from the stimulation of bone resorption, IL-1β also hinders bone formation [8, 9]. This is a very important fact when the resorption is already manifested as persistant chronic periapical lesion, because continually low level of cytokine production by the inflammatory cells may be insufficient to trigger additional bone resorption, and further on to prevent the formation of reparative bone tissue [8, 9].

However, IL-1β has been shown to be the most potent stimulus of bone resorption [6]. A variety of cells possess the capacity to produce IL-1β including: fibroblasts, endothelial cells, B-cells and macrophages, representing its major source [6]. IL-1β has been detected in gingival tissue of patients with periodontitis [10, 11], in gingival crevicular fluid after application of orthodontic forces [12], in periradicular lesions, contrary to non-inflamed pulpal tissues [13]. IL-1β is the prevaling form of IL-1 found in human periapical lesions and their exudates [14–16]. It is believed that IL-1 stimulates the proliferation of fibroblasts and induces the formation of granuloma [17].

The aim of this study was to determine quantity of IL-1β in the tissues of periapical lesions, and knowing that its local production demonstrates the activity of human periapical periodontitis, to further analyze its relationship with: lesion size, previous treatments and pathohistological findings of selected teeth.

Methods

Eighty patients of both sexes, aged from 18 to 55 (38 ± 12) years, with diagnosed persistence of periapical lesions, were selected for this study. They were in good general health and seven days prior to examination did not receive any antibiotic therapy. The patients have been asked for anamnestic data, clinical examinations were performed, as well as radiographic analysis. Radiographs were used to evaluate quality of previous endodontic treatments. The assessment of radiographic size of the periapical lesion was performed by measuring two maximum diameters of radiotransparent zone with a caliper (accuracy of 0.05 mm), and expressed in mm^2. The study was approved by the local Ethics Committee (No 09-1849/2).

During clinical examination the special attention was given to the presence of subjective symptoms: pain, and/or objective symptoms such as sensitivity to percussion or palpation, present swelling and possible drainage through the sinus tract. Based on clinical data and history of the disease, lesions were categorized either as asymptomatic or symptomatic lesions.

According to endodontic status, the teeth were divided into four study groups: 1) the group I: 25 samples of periapical lesions obtained from teeth with infected root canals communicating with the oral cavity; 2) the group IIa: 25 samples of periapical lesions obtained from teeth with obviously unsuccessfully endondontic treatment at least two years prior to examination with failure; 3) the group IIb: 15 samples of periapical lesions obtained from teeth with persisting process during a period of two years after endodontic treatment, although, based on radiographic examination the endodontic treatment was successful; 4) the group III: 15 samples of periapical lesions obtained from teeth after the biomechanical root canal instrumentation which lasted for three months and proceed with the final obturation.

For further investigation, in the course of oral surgery procedures, 80 tissue samples were obtained. Periapical tissues samples were collected from patients undergoing periapical surgery at the Department of Oral Surgery at the Faculty of Dentistry in Skopje. The sample consisted of the periapical lesion tissue and apical part of the tooth root. Each periapical tissue sample was divided into two equal parts.

One half of each lesion was used for quantification of IL-1β in tissue samples. The samples were embedded in 0.5 mL neutral buffer with added protease-inhibitors and preservatives (PBS, pH = 7.2). Each piece was frozen and stored at -70 ºC. The concentrations of IL-1β were determined using the enyme-linked immunosorbent assay (ELISA) method (h-Interleukin-1 ELISA, Roche Molecular Biochemicals, Switzerland), according to the manufacturer's instructions. The detection limit of IL-1β was 2 pg/mL.

The other part of each lesion was immediately fixed in 10% formalin; parafin sections were stained with haematoxylin-eosin and processed for histopathologic analysis using light microscopy (Leitz dialux 20, Ernst Leitz Wetzlar). Based on the histopathological evaluation, stages of granulomas were classified as follows: early (presence of numerous capillary blood vessels embedded in edematous connective tissue infiltrated with a variety of inflammatory cells), mature (chronic granulation tissue distributed in four zones around the apex of the tooth), late (high amount of col-

lagen deposits with increased number of fibrocytes and reduced inflammatory infiltrate) and recovering (visible precipitated collagen with significantly reduced cellularity and scattered macrophages and plasma cells infiltrate). Periapical chronic diffuse inflammation implied chronically inflamed granulation tissue with the absence of fibrous tissue and diffuse infiltration of the surrounding tissue. Healthy pulp tissue from five impacted third molars served as a negative control.

The data were analyzed using SPSS/Win programs (version 17.0). Kolmogorov-Smirnov test and Shapiro-Wilk's W test were used to identify the distribution of variables. All data were expressed in mean values ± standard deviations ($\bar{x} \pm SD$) and percentage. The correlations between the observed parameters were detected using the Pearson correlation and Wilcoxon Rank Sum W test. The significant differences among the groups were tested using Student *t*-test, χ^2 test and Median Test. The difference in the testing was considered significant at the level of $p < 0.05$.

Results

The procedure of determining IL-1β in the tissue homogenates was successfully performed on 76 samples (it was impossible to determine IL-1β in four cases due to the insufficient quantity of tissue that was at disposal). Its quantity varied from very low values up to 74 pg/mg of protein. No IL-1β was detected in control pulp tissue samples.

Concentrations of IL-1β in the samples correlated with the size of lesion, clinical symptoms, previous endodontic status and confirmed histological findings. Table 1 presents the relationship between the size of the lesions and concentration of IL-1β in the samples. There was a tendency of smaller lesions to contain a lower quantity of IL-1β, but still smaller lesions with high quantity of IL-1β and large lesions with a minimal amount of the IL-1β were detected.

Linear correlation was determined between the measured values of IL-1β and the size of the lesions, by applying the Pearson's correlation test (Figure 1). The examined relationship between these values demonstrated moderate positive correlation ($r = 0.22$; $p < 0.05$).

The quantity of IL-1β in periapical lesions with respect to the examined groups is presented in Figure 2. It can be noticed that the values of IL-1β of closed lesions (IIa and IIb group) were higher compared to the group I, likewise the III group (cases of recently completed therapy). However, the analysis performed concerning the quantity of IL-1β, showed that there were no significant differences among the investigated groups (Median Test; $p = 0.239$). The differences

Table 1

Relationship between interleukin-1 beta (IL-1β) production and radiographic size of the lesions

IL-1β (pg/mL)	Radiographic size of lesions (mm^2)				
	small (< 4.99) n (%)	medium (5–14.99) n (%)	large (15–25) n (%)	extra large (> 25) n (%)	Total n (%)
< 2.99	3 (37.5)	11 (28.2)	3 (18.75)	1 (7.7)	18 (23.7)
3–5.99	1 (12.5)	10 (25.6)	7 (43.75)	4 (30.8)	22 (28.9)
6–8.99	1 (12.5)	3 (7.7)	4 (25)	2 (15.5)	10 (13.2)
9–11.99	0 (0)	4 (10.1)	0 (0)	0 (0)	4 (5.3)
12–15.99	3 (37.5)	5 (13.0)	0 (0)	1 (7.7)	9 (11.8)
15–17.99	0 (0)	3 (7.7)	0 (0)	0 (0)	3 (3.9)
> 18	0 (0)	3 (7.7)	2 (12.5)	5 (38.5)	10 (13.2)
Total	8	39	16	13	76

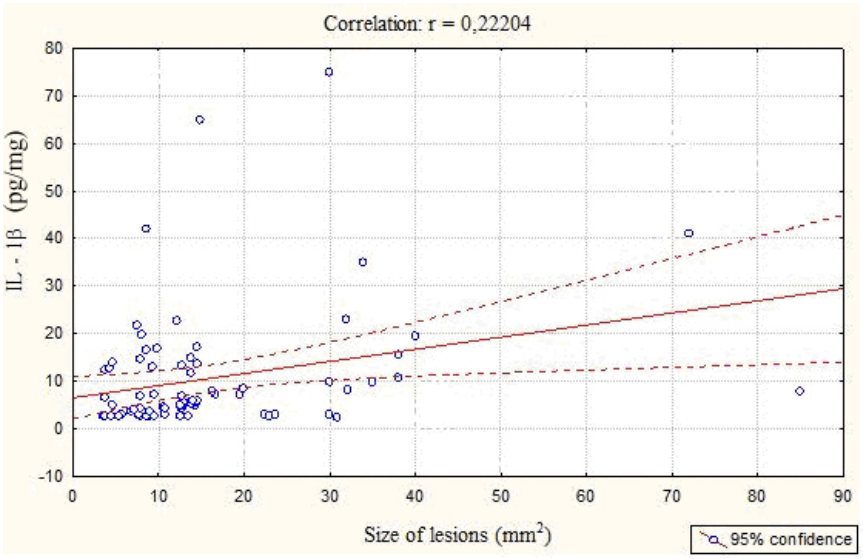

Fig. 1 – The correlation between the sizes of the periapical lesions and the quantity of interleukin-1β (IL-1β).

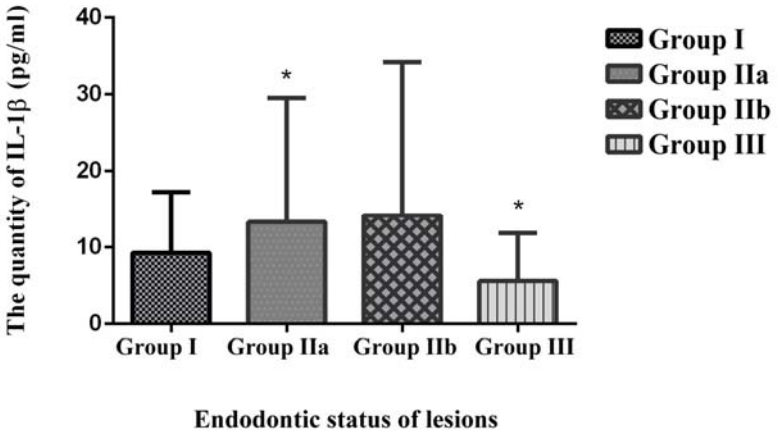

Fig. 2 – The quantity of interleukin-1 beta (IL-1β) in periapical lesions
[Asterisks indicate statistically significant difference among investigated groups ($p < 0.05$)]
group I – lesions obtained from teeth with infected root canals communicating with the oral cavity; group IIa – lesions obtained from teeth with obviously unsuccessfully endondontic treatment at least two years prior to examination with failure; group IIb – lesions obtained from teeth with persisting process during a period of two years after endodontic treatment, although, based on radiographic examination the endodontic treatment was successful; group III – lesions obtained from teeth after the biomechanical root canal instrumentation which lasted for three months and proceed with the final obturation.

between all the groups (comparing each group with all the others) were analyzed using the Student *t*-test.

Symptomatic lesions were the most frequent (52%) in the group I. In the group II cases with symptomatic and asymptomatic lesions were approximately equal. In the group III, the most cases were asymptomatic (93.3%). Kruskal-Wallis analysis showed significant differences among the investigated groups ($\chi^2 = 14.4$; $p = 0.0024$).

The correlation between the symptomaticity of the lesion with the quantity of IL-1β was examined by Wilcoxons Rank Sum W test which proved that there were no significant differences between the existence of the symptoms and the quantity of IL-1β in the samples (W = 1,601; $p = 0{,}0666$).

The differences between the quantities of IL-1β in samples with different patohistological diagnosis are shown in Table 2. The highest values of IL-1β were detected in early granulomas, while the quantity of IL-1β amounted 21.48 pg/mg, followed by 19.06 pg/mg, for the cysts. The lowest quantities of IL-1β were detected in late granulomas, scars and recovering granulomas. The analysis with the χ^2-test pointed to the fact that there was a very high significant difference between the pathohistological diagnosis and the quantity of IL-1β ($\chi^2 = 34{,}185$; $p = 0.000$).

Furthermore, the analyses of IL-1β among the groups significantly differed. The analysis performed by the *t*-test showed that there were statistically significant differences in the noted values of IL-1β between: the diffuse inflammation and mature granuloma ($p = 0.0021$), the early and mature granuloma ($p = 0.000$), abscess and the cyst ($p = 0.0175$), as well between the abscess and mature granuloma ($p = 0.0004$). No significant differences were confirmed in the quantity of IL-1β between: the diffuse inflammation and early granuloma, the diffuse inflammation and abscess, or between the cysts and mature granuloma ($p > 0.05$).

Discussion

Considering the fact that the local production of IL-1β in human periapical lesions reflects the activity of the disease [18] was the crucial reason for conducting this investigation.

Table 2

The quantity of interleukin-1 beta (IL-1β) in lesions with different histopathological manifestation

Histopathological manifestation	n	IL-1β (pg/mg), $\bar{x} \pm$ SD
Diffuse inflammation	7	12.43 ± 6.60
Granuloma		
early	9	21.48 ± 13.50
mature	25	4.90 ± 4.40
late	7	3.84 ± 2.34
recovering	4	2.36 ± 1.20
Scar	4	1.82 ± 1.02
Abscess	13	6.48 ± 4.80
Cyst	11	19.06 ± 16.91

$\bar{x}$ – mean value; SD – standard deviation.

In addition, IL-1β level in the samples may reflect disease severity [18], opposite to transforming growth factor-β [19].

The ratio between the size of the lesion and the quantity of IL-1β showed a moderate positive correlation in our study ($p < 0.05$), meaning that higher quantities of IL-1β were detected in bigger lesions. However, smaller lesions containing large quantities of IL-1β and large lesions with barely measurable quantities of IL-1β were also detected. It remains unresolved whether there is another factor which could have possible influence on this ratio. In Fouad's [20] research, the number of cells containing IL-1β were proportional to the size of the lesion. Higher levels of IL-1β and endotoxin contents were also related to the larger size of the radiolucent area [21].

The distribution of IL-1β in the samples belonging to the groups with the different endodontic status showed that closed lesions (groups IIa and IIb) had highest measured values, especially in cases where the endodontic treatment was unsuccessful. These values were lower regarding the open lesions. The most logical explanation concerning this result could lay in the possible natural drainage in these clinical cases. Matsuo et al. [16] examined the quantity of IL-1β in the periapical exudates during the endodontic treatment and showed that there was a possibility of its decreasing by the drainage through the root canal.

The decreasing tendency of IL-1β values was observed following the treatment of chronic inflammation, endodontic treatment of root canal [16], or periodontal treatment [22]. This finding supports our results, showing the lowest values of IL-1β in the group with recent endodontic treatments. These results are also in accordance with those of Tavares et al. [23].

Symptomatology did not have significant effect on the quantity of IL-1β in the lesion, although there was a tendency to isolate a higher quantity of IL-1β in the samples obtained from the symptomatic cases. Lim et al. [15] and Matsuo et al. [16] showed the same results, i.e. these authors could not find any significant difference between levels IL-1β in the samples of periapical lesions with different clinical findings. The different results were published by Gazivoda et al. [24] and Jakovljevic et al. [25], demonstrating that IL-1β levels were significantly higher in specimens obtained from symptomatic lesions compared to asymptomatic ones.

It has been known that the diffuse chronic inflammation belongs to the group of active forms of periapical lesions with significant activity of the granulation tissue [26]. On the other hand, it is proved that the macrophages are predominant immunocompetent cells during the development of the early granulomas [27]. The early granuloma is characterized by increased cellularity and presence of higher number of monocytes and groups of foamy macrophages [27]. Since these cells are the most productive source of interleukins, increasing quantity of IL-1β was expected result. In late granulomas, the number of macrophages decline, as does, in fact, the total number of inflammatory cells [27]. Wang and Stashenko [17] clearly showed that even in the non-inflamed tissue around the apex, the minimal resorption could be observed. This is probably the reason why a certain minimal quantity of IL-1β was detected, even in scars, as shown in our study (1.82 pg/mg).

The results of Jakovljevic et al. [25] showed that the concentration of IL-1β was significantly higher in radicular cysts compared to periapical granulomas. These results are not in accordance with the results of our study, meaning that higher content of IL-1β was registered especially in early granulomas, but not in cysts which had a slightly higher content of IL-1β compared to granulomas. Possible explanation for acquiring such results could be that the cysts included in our investigation were in a relatively asymptomatic phase with very little chronic infiltration into their walls. The literature is not abound with such data, and only few studies have compared the levels of pro-inflammatory cytokines between periapical granulomas and radicular cysts [28–30]. According to Hoenig et al. [28], human radicular cystic tissue contained between 0.823 pg/mg and 18.026 pg/mg of IL-1β. These authors concluded that these findings may be extremely relevant in cystic growth and episodes of alveolar bone resorption around the cystic lesion [28]. Also, according to the results of Meghji et al. [29] IL-1β was only found in the samples of inflammatory radicular cysts. Except two abscesses in our study sample, which were typically chronical, all the others were phoenix-abscesses with recent exacerbations. In the course of these exacerbations a change in the cell populations is expected to occur, involving massive infiltration of polymorphonuclears and lymphocytes, which are clearly separated from the rest of the tissues [30]. Johannessen et al. [31] have shown that macrophage during exacerbations often form major aggregates near the apex, unlike their diffuse placement in chronic lesions.

Conclusion

The highest levels of IL-1β corresponded to chronic lesions in the stages of acute exacerbation and granulomas in early developing stages. Persistent granulomas, scar tissues, non-inflamed cysts and teeth with recently completed endodontic treatments showed a significantly lower levels of IL-1β. The study results suggest that the differences in quantity in IL-1β may be an indicator for lesion progression and its phases of development.

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Epidemiological characteristics of brucellosis in Vojvodina, Serbia, 2000–2014

Epidemiološke karakteristike bruceloze u Vojvodini, Srbija, 2000–2014

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Abstract

Background/Aim. Despite the fact that brucellosis occurs sporadically in the epidemic form, this disease is still one of the world's most widespread zoonoses. **Methods.** Data from the register of infectious diseases of the Institute of Public Health of Vojvodina and Scientific Veterinary Institute in Novi Sad were used in this study. Using descriptive statistics, data were analyzed for the period 2000 to 2014. **Results.** In the observed period in Vojvodina 102 cases of brucellosis were registered with different frequency of notification cases by districts of province. Most frequent modes of transmission of brucellosis were consumption of contaminated food (especially sheep cheese) or direct contact with domestic animals. In 70.2% of the patients, occupational exposures to the agent or direct daily contact with animals were noted. The dominant source of infection in the urban area was food, whereas a direct contact with sick animals was dominant mode of transmission in the rural area. Overall, 14 epidemic outbreaks of brucella were registered with direct contact as dominant mode of transmission. The predominant age-range of patients with brucella confirmed infections was 30–59 years (50.0 male %), and 2.5 times more males than females were affected. Seasonal distribution was highest during spring, with 50.0% of all confirmed cases. During the period 2004–2005, prevalence of serum positive patients in the South Bačka District coincided with the highest number of laboratory confirmed serum samples among animals. **Conclusion.** Although the incidence of brucellosis shows a declining trend, education and improving of surveillance of disease of all relevant institutions seems as necessary for better recognition and notification of the disease.

Key words:
brucellosis; serbia; disease outbreaks; disease transmission, infectious; infection control.

Apstrakt

Uvod/Cilj. Uprkos činjenici da se bruceloza sporadično registruje u epidemijskoj formi, ona i dalje predstavlja jednu od najraširenijih zoonoza u svetu. **Metode.** Podaci za istraživanje dobijeni su iz registra infektivnih bolesti Instituta za javno zdravlje Vojvodine i Veterinarskog instituta u Novom Sadu. Upotrebom deskriptivne statistike, analizirani su podaci za period 2000–2014. godina. **Rezultati.** U posmatranom periodu u Vojvodini je registrovano 102 slučaja bruceloze sa različitom učestalošću registrovanih slučajeva u odnosu na okruge pokrajine. Najčešći putevi širenja bruceloze su bili konzumiranje inkriminisane hrane (posebno ovčiji sir) ili direktni kontakt sa domaćim životinjama. Profesionalna izloženost agensu ili direktan dnevni kontakt sa životinjama naveden je kod 70,2% bolesnika. Dominantan izvor infekcije u gradskim naseljima bila je hrana, dok je u ruralnim delovima direktan kontakt sa bolesnim životinjama bio najčešći put prenosa infekcije. Registrovano je 14 epidemija bruceloze usled direktnog kontakta kao dominantnog puta širenja infekcije. Najveće učešće među potvrđenim slučajevima bruceloze bilo je u uzrastu 30–59 godina (50%), a oboljenje je 2,5 puta češće registrovano kod muškarca nego kod žena. Od ukupnog broja obolelih, 50% slučajeva oboljenja registrovano je tokom proleća. Tokom 2004. i 2005. godine, prevalencija pozitivnih seruma među bolesnicima u Južnobačkom okrugu koincidirala je sa najvećim brojem laboratorijski potvrđenih pozitivnih seruma među životinjama. **Zaključak.** Iako je trend bruceloze u opadanju, čini se da su edukacija i unapređenje nadzora nad oboljenjem svih relevantnih institucija neophodni za njegovo bolje prepoznavanje i registraciju.

Ključne reči:
bruceloza; srbija; epidemije; infekcija, putevi širenja; infekcija, kontrola.

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Introduction

Brucellosis is a disease that originates from the anthropozoonosis group. It is primarily an occupational disease that predominantly affects people who are employed on farms, work with infected animals, veterinarians and slaughterhouse workers. The disease occurs sporadically in the epidemic form. The most common source of the infection is aborted material of the infected animals and their secretions. Brucellosis is transmitted by direct skin contact or mucous of the infected animals, by animal's offspring, amniotic fluid, and their urine and through inhalation of aerosols indoors where animals stay. Alimentary mode of transmission due to consumption of dairy products of raw milk is a predominant mode of spreading the infection in general population and most often comes from raising animals that are not registered [1].

The causative agents of the disease are bacteria of the genus *Brucella*. Types of *Brucella* that cause human disease are: *B. melitensis* – primary hosts are sheep and goats; *B. abortus* – primary hosts are cattle; *B. suis*, biotype 3, the primary host is the pig, and *B. canis* – primary host is the dog [2].

Nonspecific or atypical course of the disease in humans makes confirmation of diagnosis difficult. The clinical features are usually dominated by fever, headache, sweating, chills, arthralgia, depression, weight loss with pain throughout the body. Duration of the disease depends on the timely recognition of the disease diagnosis and adequate treatment, which can last several days, up to a year, or even longer [3].

The clinical manifestations of the disease in sheep are abortions, retention of placenta, orchitis, epididymitis and changes on the joints. The abortion in sheep, being the most common clinical manifestation of the disease, usually occurs once and is followed by autosterilization for a period from 6 months to one year. However, a certain number of animals remains carriers which retain the infection in the herd. Unlike sheep, in which spontaneous healing is observed relatively often, goats remain infectious throughout life. Brucellosis can spread in the herd during the two periods – the mating period and the lambing period. The diagnosis of disease among animals is made on the basis of epizootic data, clinical presentation, pathomorphological findings, epidemiological investigations and laboratory tests [4].

Considering that this disease in humans is often underdiagnosed or underestimated, and that in animals the form is usually non-clinical infection, the true frequency and distribution of brucellosis are unknown neither in humans nor in animals [5].

The aim of this study was to determine the epidemiological characteristics of brucellosis in the Autonomus Province (AP) of Vojvodina, Serbia, and epizootiological characteristics in the South Bačka District in the period of 15 years, from 2000 to 2014.

Methods

The AP of Vojvodina is located in the northern part of Serbia and is divided into 7 districts and 44 municipalities. As a source of data we used the database from the register of infectious diseases of the Centre for Disease Control and Prevention of the Institute for Public Health (IPH) of Vojvodina, data of laboratory results of the Centre for Microbiology of the IPH and data of the Scientific Veterinary Institute in Novi Sad.

Information about individual cases of brucellosis in humans and data about patients in epidemics were analyzed on the basis of the notifications about infectious diseases and epidemiological data obtained by interviewing patients, on the data of the official monthly and annual reports of the IPH of Vojvodina in the period from 2000 to 2014. The incidence rate of the disease was presented as the ratio of the number of patients and the whole population according to the census as the denominator for 100,000 inhabitants.

More than two linked cases of the disease were considered as a potential epidemic.

Microbiological investigation

In the Centre for Microbiology of the IPH, the cases of the disease were confirmed by serology tests: SAT (standard agglutination test) Wright or BMAT (*Brucella micro agglutination test*).

According to the Ordinance on establishing measures for the early detection, diagnosis, prevention and suppression of spread, as well as eradication of infectious diseases of cattle, the method of implementation, and the manner of determining the status of farms free of brucellosis in all cattle, sheep, goats and pigs, all animals which were tested positive on brucellosis were stamped out. Laboratory diagnosis of brucellosis during this period was carried out by the methods of the Rose Bengal test, complement fixation, indirect ELISA and complement ELISA test as a confirmatory test. All samples were examined during the regular annual review and animal health protection monitoring was applied as ordered by the program of measures for each year on the territory of the Republic of Serbia. Samples of animals from all the registered farms as well as of animals in private ownership were taken. All animal samples were tested in the Scientific Veterinary Institute, Novi Sad.

The data were analyzed chronologically, demographically and topographically for the observed period by descriptive epidemiological study. We used the basic statistical indicators, general and specific incidence rates.

Results

In the observed period in Vojvodina, 102 cases of brucellosis were registered. The highest incidence rate of brucellosis was reached in 2004 when 35 cases of brucellosis with incidence rate of 1.8 cases per 100,000 populations (Figure 1) were registered.

Most patients in the territory of Vojvodina (80.4%; 82/102) were registered in the territory of the South Banat and the South Bačka District. The registered incidence rates were less than 0.2/100,000 inhabitants in the North Banat and the West Banat District. Cases of brucellosis were registered on the whole territory of the province (Figure 2) with exception of the North Bačka District.

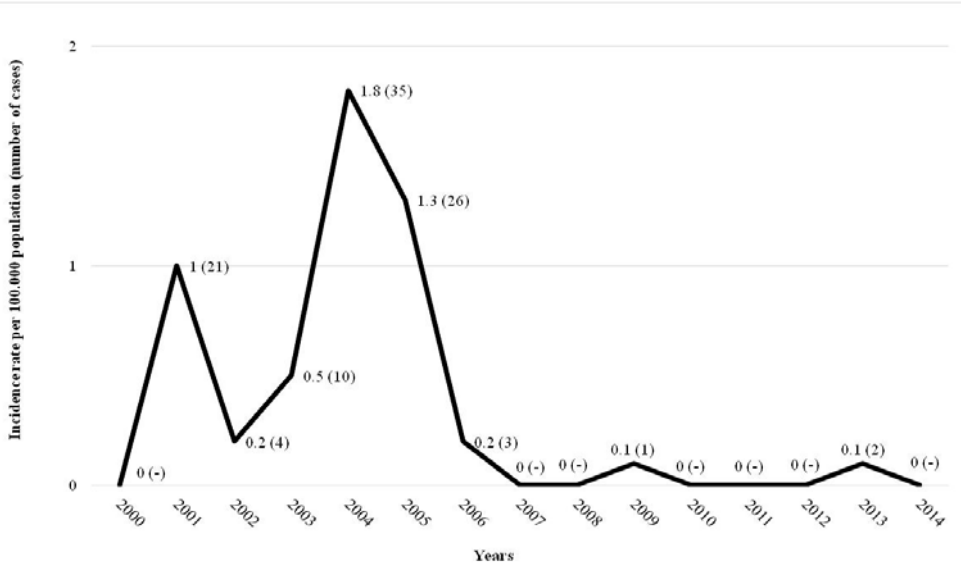

Fig. 1 – Incidence rates and number of cases of brucellosis in Vojvodina, 2000–2014.

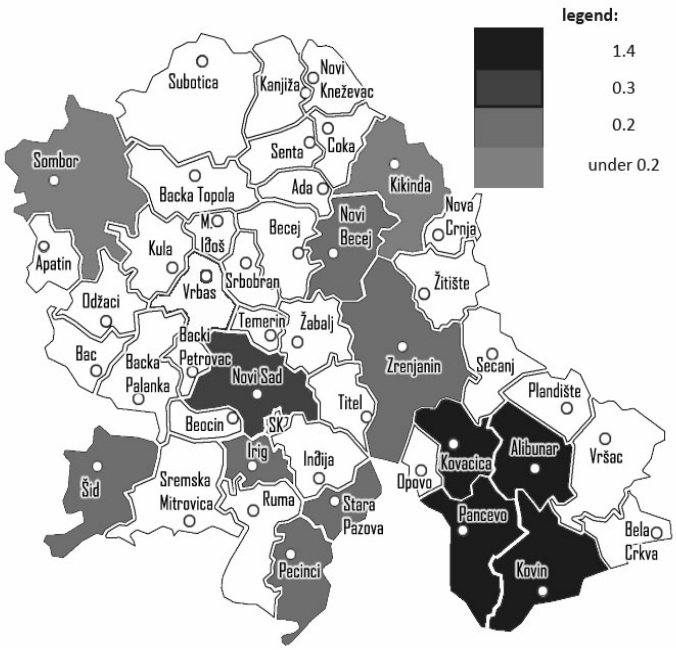

Fig. 2 – Incidence rate of brucellosis per 100,000 populations by districts in Vojvodina, 2000–2014.

Observed by modes of brucellosis transmission in the Province, distribution of brucellosis in two districts (South Bačka and South Banat) showed larger differences. On the territory of the South Bačka District the majority of 22 cases registered, which reached 68% of the patients, the spread of infection was caused by consuming dairy products homemade, purchased at city markets. On the territory of the South Banat Districts, out of 59 registered cases, food was pointed out by 19% of affected individuals as a possible way of spreading the disease. Most of the patients from the territory of the South Banat Districts (45% of patients) were professionally exposed to direct contact with animals, and 31% of the patients faced an alimentary risk by being with animals.

Out of the total number of patients (44 patients), 43.1% indicated daily direct contact with domestic animals in the maximum incubation period as the only possible way of spreading the infection. The consumption of thermally untreated domestic dairy products was represented in 27.5% of patients. Both possible modes of transmission were indicated in 22 patients (21.6%), whereas for 8 patients (7.8%), the mode of spreading infection remained unknown (Table 1).

In 74% of the cases, the sheep cheese was recognized as the food from domestic production which exposed patients consumed in the maximum incubation period. Cow's milk, cheese and raw milk was specified in 10–16% of patients as potential sources of infection.

Table 1

Probably modes of brucellosis transmission in Vojvodina by districts, 2000–2014.

Mode of transmission	South Bačka n (%)	Srem n (%)	West Bačka n (%)	North Bačka n (%)	Central Banat n (%)	North Banat n (%)	South Banat n (%)
Contact	3 (14.0)	5 (45.0)	0 (-)	0 (-)	8 (100.0)	1 (100.0)	27 (45.0)
Incriminated food	15 (68.0)	2 (18.0)	0 (-)	0 (-)	0 (-)	0 (-)	11 (18.6)
Contact and food	1 (0.9)	3 (27.0)	0 (-)	0 (-)	0 (-)	0 (-)	18 (31.0)
Unknown	3 (2.9)	1 (9.0)	1 (100.0)	0 (-)	0 (-)	0 (-)	3 (5.0)
Total	22 (100.0)	11 (100.0)	1 (100.0)	0 (0)	8 (100.0)	1 (100.0)	59 (100.0)

n – number of human brucellosis cases.

Data concerning the occupation of patients were defined for 94 patients. Occupational exposures to the agent or direct daily contact with animals were determined in 70.2% (66/94) persons. Infection among males was about three times more often registered than among females. The most common occupation appeared to be agriculturist, and there was 34.9% registered cases (Table 2).

In the observed period for the Province territory, 73% (74/102) of brucellosis cases were covered in 14 epidemic outbreaks of brucellosis. During 10 epidemic outbreaks of the infection spread, 57 people were infected by direct contact with diseased animals. The alimentary mode of transmission was present in 4 outbreaks when 17 patients were infected, while individual cases of the disease were registered in 28 infected individuals (Table 3).

The largest number of patients (50%) belonged to the working age category of population (30–59 years). Registered 7.7% of infected patients were up to 19 years old. Males were 2.5 times more affected than females (Figure 3).

Half of the total number of the infected patients were

Table 2

Occupation and sex distribution of brucellosis cases in Vojvodina, 2000–2014.

Occupation	Male (n = 49)	Female (n = 17)	Total (n = 66)
	n (%)	n (%)	n (%)
Agriculturist	20 (40.8)	3 (17.6)	23 (34.9)
Rearer	11 (22.4)	1 (5.9)	12 (18.2)
Housewife	0 (-)	11 (64.7)	11 (16.7)
Veterinary Institute professionals	6 (12.2)	1 (5.9)	7 (10.6)
Cattleman	3 (6.1)	0 (-)	3 (4.5)
Stockbreeder	2 (4.1)	0 (-)	2 (3.0)
Agricultural cooperatives workers	1 (2.2)	0 (-)	1 (1.5)
Miscellaneous (student, painter, locksmith, unemployed, senior)	6 (12.2)	1 (5.9)	7 (10.6)

Table 3

Reported brucellosis case in outbreaks by modes of transmission in Vojvodina, 2000–2014.

Mode of transmission	Number of outbreaks (n)	Number (%) of cases
Direct contact	10	57 (77.0)
Others modes	4	17 (23.0)
Total	14	74 (100.0)

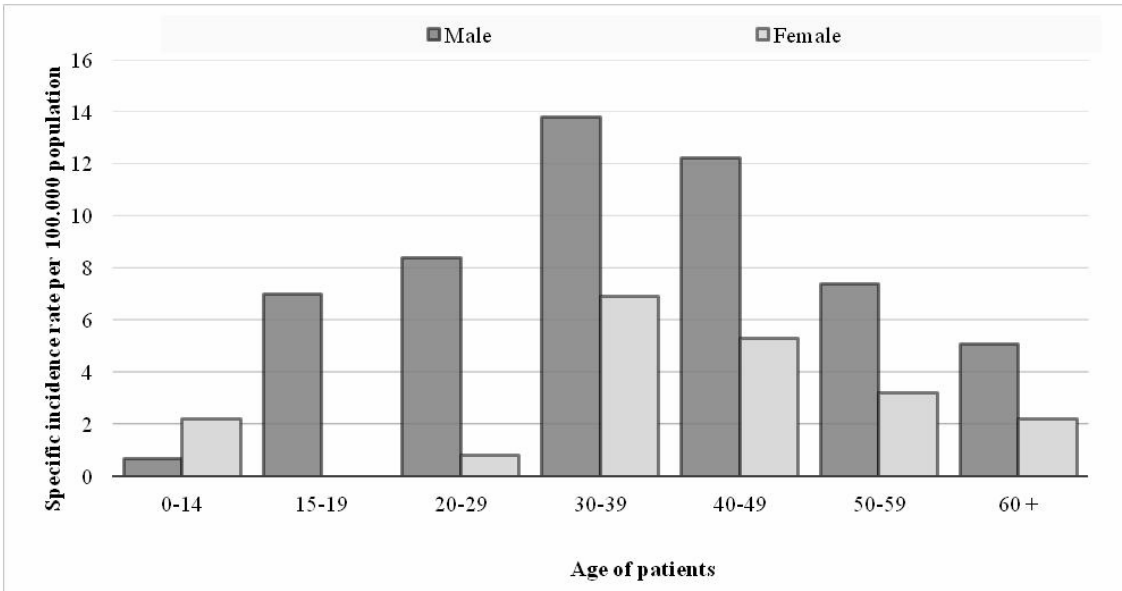

Fig. 3 – Average specific incidence rate of brucellosis cases by age and gender in Vojvodina, 2000–2014.

registered in the period from April to June. The smallest number of infected patients (5.8%) were registered in the period from October to December (Figure 4).

The epizootic characteristics of brucellosis in the South Backa District

In the period from 2001 to 2014, in the Centre for microbiology IPH 993 sera of patients from the South Bačka District, were tested and the disease was confirmed in 33 (3.3%) cases.

The highest percentage of positive results (91%) was recorded in 2004 and 2005, when the highest number of the infected patients were registered in the territory of Vojvodina. The last laboratory-confirmed case of brucellosis in Vojvodina was recorded in 2011. However, yearly in the IPH laboratory processing, an average of 66 serum samples of patients were analyzed due to suspected differential diagnosis of brucellosis in the South Bačka District.

In order to timely detect infected animals and proceed with their elimination, regular checkups and inspections of cattle, sheep, pigs, horses and dogs, in the period from 2001 to 2014 in the South Bačka District were performed and a total of 1,487,225 serum samples were analyzed, out of which only 1,498 (0.1%) were positive for brucellosis. In the period from 2003 to 2007 seropositive reports were registered in different animal species. In 2004 and 2005, there were animals with clinical symptoms, as well as abortions in females. The highest number of positive findings for brucellosis from sera of sheep and goats, were up to 1,316. The positive findings were detected in the serum of 168 pigs (153 in 2014), 13 cows and one in a horse serum sample (Table 4).

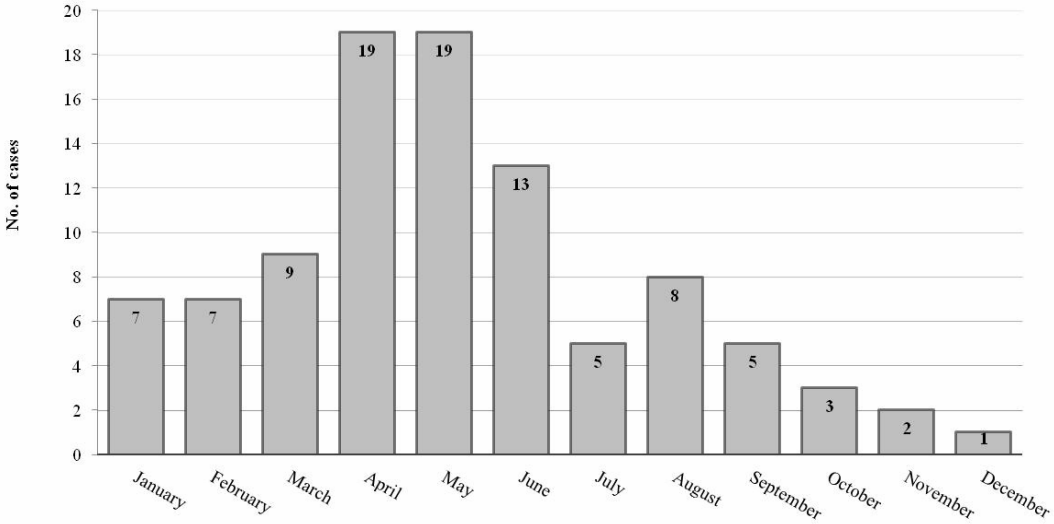

Fig. 4 – Seasonal distribution of brucellosis in Vojvodina, 2000–2014.

Table 4

The number of serology tested and serology positive for brucesellosis humans and animals in the South Bačka District, 2001–2014.

Years	Cows		Sheep and goats		Pigs		Horses		Dogs		Humans	
	T	P	T	P	T	P	T	P	T	P	T	P
2001	26,730	0	786	0	1,1917	0	296	0	0	0	5	0
2002	34,408	0	1,194	0	1,1826	0	332	0	3	0	9	0
2003	34,331	0	50,233	293	8,740	0	48	0	0	0	1	0
2004	37,619	3	91,052	449	11,241	4	202	0	3	0	148	10
2005	31,236	9	90,056	549	8,369	11	226	1	2	0	215	20
2006	9,160	0	56,224	20	3,593	0	0	0	0	0	96	0
2007	54,902	0	64,230	5	4,863	0	24	0	6	0	94	0
2008	58,015	0	108,369	0	11,736	0	4	0	2	0	86	0
2009	40,389	0	49,286	0	10,993	0	1	0	19	0	89	2
2010	37,891	0	67,475	0	3,301	0	3	0	11	0	52	0
2011	42,954	0	74,762	0	4,708	0	3	0	30	0	50	1
2012	40,229	0	71,090	0	2,586	0	0	0	7	0	39	0
2013	38,631	1	68,737	0	1,934	0	12	0	80	0	53	0
2014	39,611	0	67,162	0	3,133	153[a]	179	0	30	0	56	0
Total	526,106	13	860,656	1316	98,940	168	1,330	1	193	0	993	33

T – Tested; P – Serology positive; [a]Ecologically reared animals were caught and tested that year.

Discussion

Brucellosis is a disease that is widespread throughout the world, especially in countries with developed livestock breeding. According to the World Health Organization (WHO), about 500,000 people in the world are affected annually, including 20,000 cases registered in Europe. With different frequency, the disease is registered both in Europe and in the United States of America (USA). High-risk areas are the Mediterranean countries (Portugal, Spain, southern part of France, Italy, Greece, Turkey and North Africa), South and Central America, Eastern Europe, Asia, Africa, the Caribbean and the Middle East [6, 7].

A new risk area for developing human brucellosis is the region of Central Asia due to the deterioration of the epidemiological situation in Syria [7].

Epidemiological surveillance of brucellosis in Vojvodina has been implemented from 1954. By the year 1970, in Vojvodina, individual cases of the infection were registered – in total there were 40 registered patients. The highest incidence was registered in 1965 with the incidence rate of 0.9 per 100,000 inhabitants. In the period from 1971 to 1999, despite the unfavorable epidemiological situation in the region, on the territory of the Province, brucellosis was not registered and was considered to be eliminated in Vojvodina. After three decades without any registered cases, in 1999 in the South Banat District brucellosis was registered in three infected patients, and epidemiological research determined that the infection came from imported animals (sheep) [1].

In the period from 2004 to 2007 a large epizootic and epidemic of brucellosis in Bosnia and Herzegovina (B & H) were registered. In the same period on the territory of Vojvodina the highest number (35) of patients were recorded with the incidence rate of 1.8/100,000 population in 2004 [8].

The epidemiological situation in the region is similar to the situation in Vojvodina, except in Slovenia, where brucellosis has been eradicated. According to the data from epidemiological service of the Republic of Slovenia, in the period from 1948 to 2013, there were 93 cases of human brucellosis evidenced. From the mid-fifties, only imported cases of the infection in humans were reported; they traveled through the countries where brucellosis was still endemic [8].

In 2006, the incidence of the infection in Serbia was 0.1/100,000 inhabitants, which was lower than in the EU countries recorded that year (0.2/100,000), while in Vojvodina, because of the epidemic spread of brucellosis, the registered incidence was identical to the average incidence of human brucellosis in the European Union [9].

On the territory of the European Union, during 2012, 328 confirmed cases of brucellosis in human population were registered (0.1/100,000), which was 2.4% less than in 2011. The highest number of reported cases was from the territory of Greece (1.1/100,000) and Portugal (0.4/100,000), while the incidence of the disease was the same in Sweden, Spain, Norway and Austria (0.1/100,000) [10, 11]. Together with Bulgaria, Serbia recorded the lowest rate of brucellosis incidence in the Balkans [12]. Differences in the distribution of brucellosis incidence among districts of the Province can be interpreted as the result of the exchange of livestock fund among these regions (e.g. South Bačka and South Banat) and in neighboring countries, especially B & H.

In relation to the seasonal distribution of the infection, in endemic countries the highest numbers of patients are registered in June and July [13–15]. On the territory of countries where the disease is not endemic, seasonality does not show variation in reporting. On the territory of Germany, the highest number of cases are usually reported in August and September, when German citizens, originally from Turkey, return from vacations and bring with them incriminating foods in the form of dairy products (unpasteurized goat cheese) originating from Turkey [16]. In central Greece, where brucellosis is an occupational disease, most cases are reported during March and May [17].

The results of our study show that the greatest number of patients was registered in April and May. During these months the lambing and kidding sheep and goats are done, as well as intensive milking, sheep shearing and other activities in which humans are in contact with potentially infected animals.

In countries where preventive measures are carried out in order to prevent the spread of brucellosis through food, brucellosis is primarily an occupational disease and mostly affects male patients between 20 and 45 years of age. The incidence of infection in children is higher in countries where the common way of spreading infection is through thermally unprocessed food products of animal origin, which is typical for nomadic society [6].

The results of our investigation showed that half of the total number of patients belonged to the economically active population. The largest number of cases was reported in the age group of 30 to 59 years of age.

During the epidemic spread of brucellosis on the Greek island of Thasos in 2008, where 98 people were infected, alimentary mode of infection spreading was dominant and locally produced cheeses were labeled as incriminated foods [18].

In the observed period, in Vojvodina, alimentary mode of transmission was detected in every fourth patient, with the largest number of patients infected after consumption of incriminated foods during outbreaks in South Bačka District, the City of Novi Sad. The largest number of infected patients in this part of the Province can be explained by the fact that Novi Sad is the largest city in the Province with a developed network of services in the form of market supply of milk and milk products from different parts of Vojvodina.

The different way of production process and the preparation of the cheese in domestic conditions may contribute to increasing concentrate of *Brucella* spp, which in this type of foods can last up to several months. Since the beginning of the 1990s it was well known that alimentary mode of transmitting is more important in cities, where the infection is registered among patients who consumed dairy products from the previously untreated milk from domestic production; these incriminating groceries were bought at city markets [2].

Frequent distribution of these types of food from endemic countries may jeopardize the process of elimination and eradication of the infection in the territories of developed countries. In Germany, human brucellosis was registered as an

occupational infection, but by entering different incriminating store, brucellosis is still registered in the general population [16].

In the area of Vojvodina sheep cheese was registered as the most common food that provokes the infection in individuals. It is known that milk products of sheep and goats *B. melitensis* bring higher risk to individuals who would not otherwise be professionally exposed to these animals, but they consume their meat and milk [19, 20].

In our study, the contact mode of infection spreading, as the only possible route of the infection transmission, was registered among 43.1% of the registered cases. Observed by districts of the province, this contact mode of transmission was usually detected in the South Banat District, where a total of 59 patients were registered with known epidemiological data, while 46% of patient's data on contact with the animals were obtained.

Number of epidemic contact in the Province is higher in relation to the rest of the Republic of Serbia. This is explained by the existence of a more developed sector of animal husbandry and greater opportunities of exposure to potentially infected animals [12]. During the observed period, out of 74 patients, 57 affected individuals, had a direct in contact with diseased animals, while 17 patients were registered in the outbreaks with alimentary modes of transmission.

Gender-specific distribution of the infection is different from country to country. In Greece, brucellosis is an occupational disease and is diagnosed three times more in males [18, 21].

In the area of Germany after 2000, there were no gender-specific differences (54% male: 46% female), [16] unlike Uganda where the majority of infected patients were females, who were in daily contact with animals [22].

In our study, 70% of the affected people (farmers, workers flocks, workers in the Veterinary Institute, homemakers on farms, shepherds and cattle breeders) had occupational exposure to animals. In relation to gender specificity, men were 2.5 times more likely to be infected in comparison to females. These data can be explained by the fact that professional exposure and exposure in households are more characteristic for men than women.

In support to the favorable, declining trend of brucellosis in Vojvodina, goes the fact that over the past five years only one case of human brucellosis was detected, and that in the last 9 years no outbreaks of brucellosis in Vojvodina were registered. This favorable epidemiological situation is also the result of previously taken measures to remove the animals that were positive for brucellosis.

Besides on the territory of the Province, from 2005 the declining trend of the brucellosis incidence was also registered in the territory of the South Bačka District, both in humans and animals. During 2014, brucellosis was detected in pigs that represented a population of domestic animals who lived in the pasture released (i.e. an environmentally friendly way of growing animals). These pig populations are often in contact with wild reservoirs of brucellosis infection. This method of raising animals complicates monitoring and implementation of diagnostic procedures, as well as regular annual sampling.

In recent decades, in the EU countries, various programs were implemented for cattle, sheep, goats and pigs to control, suppress, eliminate and eradicate brucellosis with disputable success. The brucellosis incidence in animals varies among different countries of Europe, and in some cases, there are variations among different regions of the same country. North European countries have the status free of brucellosis in cattle (*Brucella abortus*), sheep and goat brucellosis (*Brucella melitensis*). In the countries of Southern Europe, the brucellosis incidence in animals, particularly in sheep and goats, is similar to that in humans, where there are still registered cases of infection with *B. melitensis*. As a result of contact with wild pigs, cases of brucellosis occasionally registered are among pigs that are pasture released (*Brucella suis* biovar 2) [23].

Conclusion

Data from the IPH of Vojvodina on the number of tested people on the territory of the South Bačka District indicate the continuity in the number of people who were being tested, which is in favor of prudence in the differential diagnosis of patients with atypical clinical course suspected of brucellosis.

Due to the long incubation period and nonspecific clinical presentation of the disease, the restrictions in establishing the definitive diagnosis of brucellosis are evident, and for the majority of patients it is not possible to determine the infection reservoir.

Although the incidence of brucellosis shows a declining trend, education and improving of surveillance of disease by all relevant institutions seems to be necessary for better recognition and notification of the disease.

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Subclavian steal syndrome – surgical or endovascular treatment

Sindrom krađe krvi potključne arterije – hirurško ili endovaskularno lečenje

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Abstract

Background/Aim. A phenomenon of subclavian steal is caused by occlusion or stenosis of the proximal subclavian artery with subsequent retrograde filling of the subclavian artery via the ipsilateral vertebral artery. The aim of this research was to compare surgical method [carotid-subclavian bypass grafts (CSBG)] and endovascular methods [percutaneous transluminal angioplasty (PTA) and stenting of subclavian artery] from the aspect of immediate and long-term results. **Methods.** Thirty patients [16 (53.33%) males], of average age between 60.1 ± 8.25 years were treated with CSBG and compared with a group of forty patients [18 (45%) males], of the average age between 57.75 ± 6.15 years treated by PTA and stenting of subclavian artery. Immediate and long-term results were determined clinically and confirmed by Doppler pressures and duplex ultrasound/angiography. All patients were followed-up after 1, 6 and 12 months post-procedure, and annually thereafter. **Results.** The average follow-up for both groups was 22.37 ± 11.95 months. There were 2 (6.67%) procedural complications in the CSBG group (transient ischemic attack in 2 patients) and 3 (7.5%) ones in the PTA/stent group (dissection and distal embolization in one patient and puncture site hematoma in one patient). Systolic blood pressure difference between the two brachial arteries in CSBG group was: 42.6 ± 14.5 mmHg *vs* 4.75 ± 12.94 mmHg ($p < 0.05$). In the PTA/stent group it was: 41.2 ± 15.35 mmHg *vs* 3.58 ± 5.83 mmHg ($p < 0.05$). Long-term success was 93.33% in the CSBG group and 92.5% in the PTA/stent group ($p > 0.05$). **Conclusions.** Both, the CSBG and PTA/stenting of subclavian artery are safe, efficacious and durable procedures. They have similar immediate and long-term results. PTA and stenting are the methods of choice for high grade stenosis, near total occlusions and segment occlusions of subclavian artery. CSBG is indicated in case of diffuse occlusive lesions and when the PTA and stenting do not succeed or cause complications.

Key words:
subclavian artery; subclavian steal syndrome; angioplasty; stents; blood vessel prosthesis; endovascular procedures; vascular procedures, operative.

Apstrakt

Uvod/Cilj. Sindrom "krađe" krvi potključne arterije uzrokovan je okluzijom ili stenozom proksimalnog segmenta potključne arterije sa njenim posledičnim retrogradnim punjenjem putem ipsilateralne vertebralne arterije. Cilj ovog rada bio je poređenje hirurške [karotido-supklavijalni bajpas (KSBP)] i endovaskularne metode [perkutana translumenska angioplas-tika (PTA) i stenting] sa aspekta ranih i udaljenih rezultata. **Metode.** Trideset bolesnika [16 (53,33%) muškaraca, prosečne starosti 60,1 ± 8,25 godina tretirano je KSBP i poređeno sa grupom od 40 bolesnika [18 (45%) muškaraca], prosečne starosti 57,75 ± 6,15 godina koji su tretirani PTA i stentingom potključne arterije. Rani i udaljeni rezultati su verifikovani razlikom u segmentnim pritiscima ruku, kliničkim i ultrasonografskim/angiograf-skim pregledom. Svi bolesnici su praćeni nakon mesec dana, 6 meseci i 12 meseci, a jednom godišnje nakon toga. **Rezultati.** Prosečni period praćenja iznosio je 22,37 ± 11,95 meseci. Utvrđene su 2 (6,67%) periproceduralne komplikacije u KSPB grupi (tranzitorni ishemijski atak kod 2 bolesnika) i 3 (7,5%) u PTA i stenting grupi (disekcija i distalna embolizacija kod jednog bolesnika, i hematom na mestu punkcije takođe kod jednog bolesnika). U KSPB grupi razlika u sistolnim pritiscima dve brahijalne arterije iznosila je: 42,6 ± 14,5 *vs* 4,75 ± 12,94 mmHg ($p < 0,05$), a u PTA i stenting grupi: 41,2 ± 15,35 *vs* 3,58 ± 5,83 mmHg ($p < 0,05$). Udaljeni uspeh u bypass grupi bio je 93,33%, a u PTA i stenting grupi 92,5% ($p = 0,83$). **Zaključak.** Obe metode, KSBP i PTA i stenting potključne arterije su bezbedne, efikasne i trajne. Imaju slične rane i udaljene rezultate. Tehnika PTA i stenting metod su izbora kod hemodinamski značajnih stenoza, subokluzija i segmentnih okluzija potključne arterije. KSBP je indikovan u slučaju difuznih okluzivnih lezija, ukoliko je PTA i stenting neuspešan ili ako se iskomplikuje.

Ključne reči:
a. subclavia; a. subclavia, sindrom promene toka krvi; angioplastika; stentovi; krvni sud, proteza; endovaskularne procedure; hirurgija, operativne procedure.

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Introduction

A phenomenon of subclavian steal is caused by occlusion or stenosis of the proximal subclavian artery with subsequent retrograde filling of the subclavian artery via the ipsilateral vertebral artery. Contorni was the first to recognize and describe this retrograde flow in 1960 using angiography in a patient who had an absent radial pulse [1]. In 1961, Reivich et al. [2] showed that there is a relationship between cerebral ischemic attack and retrograde vertebral flow. The term "subclavian steal syndrome", was coined by Fisher [3], after he reviewed Reivich et al. [2] article and observed that the anomaly caused the ipsilateral subclavian artery to receive retrograde flow from the contralateral circulation at the expense of the vertebro-basilar circulation.

The management of subclavian artery disease has evolved a great deal over the years, and a variety of therapeutic options are now available, including transthoracic bypass grafting or an endarterectomy, subclavian-carotid transposition, carotid-subclavian bypass and carotid-axillary bypass. The transthoracic approach is invasive and is rarely used in elderly population because of the high incidence of complications. Subclavian-carotid transposition and carotid-subclavian bypass grafts (CSBG) are the interventions used by most surgeons [4].

Percutaneous transluminal angioplasty (PTA) and stenting of the subclavian artery provides another tool for treating patients who have stenosis or occlusion. Endovascular treatment of subclavian artery is now commonly used for treating subclavian artery stenosis (total occlusion of the subclavian artery poses a special challenge and is technically demanding).

The aim of this study was to compare surgical CSBG and endovascular methods (PTA and stending) for subclavian artery disease from the aspect of immediate and long-term results.

Methods

Study design

This single center study included 70 patients treated at the Clinic for Vascular and Endovascular Surgery of Clinical Center of Serbia in Belgrade from January 1st, 2010 to March 1st, 2014. Thirty patients were treated by the surgical (CSBG) procedure, while 40 were treated endovasculary using the PTA and stent implantation. Data were collected prospectively with retrospective analysis. The indication for treatment was made on the basis of clinical and ultrasound examination of vertebral, carotid and upper limb arteries.

The clinical examination was used in order to determine the difference between radial arterial pulse. Arterial pressure gradients were measured on both arms and the following parameters were evaluated: the presence of symptoms of vertebrobasillar insufficiency (VBI) (vertigo and loss of consciousness while using the arm for physical activity, gait instability, amaurosis fugax) and disabling arm ischemia (functional weakness and paresthesia related to arm exercise, sensation of cold, rest pain, arm claudication discomfort), presence of anginal disturbances (in cases of mammary graft), need for repeated PTA and stenting or surgery.

PTA/Stent and bypass procedures

Endovascular procedures were performed under local anesthetic. Arterial access was obtained via common femoral and/or axillary arteries. A 6 F sheath was used during the procedures. The lesions were crossed using a 0.035-inch hydrophilic wire with a curved catheter providing directional control and support. The reference vessel diameter was determined with quantative angiography. Self-expandable and balloon-expandable stents were used. Neptun (Balton Medical) balloon-expandable stent was implanted in 7 (28%) patients. The following types of self-expandable stents were used: ev3 GPS (eV3) – 8 (32%) patients; SMART (Cordis) – 4 (16%) patients; Jaguar (Balton Medical) – 24% of patients; and Wallstent (Boston Scientific) – 24% of patients. Technical success for endovascular procedures was defined as residual diameter stenosis < 30% on the post-intervention arteriogram. After the procedure, all patients were prescribed lifelong aspirin (100 mg/d), whereas, clopidogrel (75 mg/d) or ticlopidine (100 mg twice a day) was continued for at least 1 month.

The carotid-subclavian bypass procedures were performed by 6 and 8 mm diameter polytetrafluoroethylene grafts (3 patients) and Dacron grafts (27 patients). In 20 (66.67%) patients the procedure was performed in general anesthesia, while regional anesthesia was used in 10 (33.33%) patients. A skin incision was made 1 cm above the clavicle, extending from the sternoclavicular joint to the lateral portion of the supraclavicular region for about 6 to 8 cm. An exposure of the carotid artery and subclavian artery was then carried out. Next, the carotid artery was isolated and surrounded with vessel loops. After systematic heparinization, the distal subclavian anastomosis was first performed in an end-to-side fashion to minimize the amount of graft material used. The carotid anastomosis was completed in end-to-side fashion. Lifelong aspirin (100 mg/d) therapy was prescribed after open surgery treatment. In 6 (20%) patients, ipsilateral eversion carotid endarterectomy was performed simultaniously with carotid-subclavian bypass in the same act.

Follow-up examinations

Graft and PTA-stent patency were determined by the presence or absence of peripheral pulses and confirmed by duplex ultrasound evaluation of vertebral arteries and upper limb arteries performed 1 month, 6 months, 12 months for the first year and annually thereafter. It included: determining segment systolic arm pressures, qualitative and quantitative analysis of doppler spectrums, duplex and color duplex scan. In symptomatic patients, angiographic examination in multiple projections was performed as well. Perioperative complications were any sequel that took place within 30 days following the procedure.

Statistical analysis

Perioperative complications, relief of symptoms, primary patency, and overall survival were compared

between the endovascular and surgical groups. Data were statistically analyzed using Exact Wilcoxon Rank Sum Test, Fisher exact test and Exact Wilcox Signed Rank Test for testing the difference between the treated groups and within the groups, as well as Log-rank test and Kaplan-Meyer product-limit method for testing patency difference and for presenting patencies within the two patient groups during the follow-up period. The results were considered statistically significant at level of $p < 0.05$. Calculations were performed using the statistical package STATA, version 8.2.

Results

The average age of both groups of patients was comparable. The proportion of women was higher in the group of endovasculary treated patients. In both groups, more than 50% of patients were smokers and had arterial hypertension, while diabetes mellitus was observed in 6 (20%) of surgically treated and 10 (25%) endovascular treated patients respectively. In 8 (26.67%) surgically treated patients previous carotid endarterectomy was performed, and within the group of endovasculary treated patients, there were 6 patients with previous carotid surgery. In both surgically and endovasculary treated groups, carotid lesions were symptomatic, and because of that these patients were treated with open surgery procedures. There was no significant difference between the two groups regarding cardiovascular comorbidities and previous surgeries (Table 1).

Symptomatic lesions were present in 67 (95.71%) of all patients. They included VBI during physical activity involving the arm, and disabling arm ischemia, as well as the combination of the two. In all three (4.29%) asymptomatic patients, hemodynamically significant subclavian stenosis was found accidentally during preparations for aorto-coronary bypass surgery using internal thoracic artery. There was no significant difference between the two treated groups regarding patient distribution by symptoms ($p = 0.254$).

The average length of a lesion was 17.33 ± 6.26 mm in the group of patients with CSBG, and 16 ± 7.77 mm in patients in which PTA and stenting had been performed. There was no significant difference between the two groups of patients ($p = 0.177$). The left subclavian artery was more commonly involved than the right in both groups (90% in both groups).

In the CSBG group, 29 patients had complete subclavian artery occlusion. Among patients treated endovasculary, 1 patient had subclavian artery occlusion, 25 patients had near total occlusion, while 14 patients had hemodynamically significant subclavian stenosis. Significant difference was observed between the two groups regarding patient distribution by grade of stenosis/occlusion ($p < 0.05$).

There were no fatal outcomes within the initial 30 days or during the follow-up period. Both methods significantly improved patient condition regarding pre-procedure complaints. The rate of remaining pre-procedure symptoms was insignificant. In the group of surgically treated patients, only one (3.33%) patient still had VBI, and the same was found in the group of endovasculary treated patients where only one (2.5%) patient still had arm-related complaints. During the early post-procedure period, within the CSBG group, 29 (96.67%) patients had palpable radial pulse. This ratio was 38 (95%) *vs* 2 (5%) within PTA/stenting group. No significant difference was found by comparing the two groups of patients regarding radial pulse palpability during early post-procedure period ($p = 1$). The difference between brachial pressure of the healthy and treated arm was significantly smaller when compared before and after the procedure, both in surgically and endovasculary treated groups.

Immediately after the procedure, 2 (6.67%) surgically

Table 1

Demographic characteristics, risk-factors and associated diseases in patients with subclavian steal syndrome

Parameter	Treatment		*p*
	surgical	endovascular	
Gender			
males, n (%)	16 (53.33)	18 (45)	0.489
females, n (%)	14 (46.67)	22 (55)	
Mean age (years), $\bar{x}$ ± SD	60.1 ± 8.25	57.75 ± 6.15	0.268
Risk factors, n (%)			
arterial hypertension	26 (86.67)	29 (72.5)	0.239
cigarette smoking	20 (66.67)	23 (57.5)	0.435
diabetes mellitus	6 (20)	10 (25)	0.622
hyperlipidemia	4 (13.33)	3 (7.5)	0.451
Associated diseases, n (%)			
PVD	10 (33.33)	17 (42.5)	0.435
ischemic heart disease	9 (30)	18 (45)	0.201
carotid endarterectomy	8 (26.67)	6 (15)	0.227
AAA	5 (16.67)	5 (12.5)	0.622
CVI	4 (13.33)	3 (7.5)	0.451
CABG	1 (3.33)	1 (2.5)	1

PVD – peripheral vascular disease; AAA – abdominal aortic aneurysm; CVI – cerebrovascular insult; CABG – coronary artery bypass grafting; n (%) – number (%) of patients; $\bar{x}$ – mean value; SD – standard deviation.

treated patients developed transient ischemic attack (TIA), and the symptoms disappeared after 24 hours. One endovascular treated patient developed dissection. The repeated angioplasty was unsuccessful. The same patient was also diagnosed angiographicaly with distal axillary artery embolization. Both complications were successfully managed using carotid-subclavian and subclavian-axillary bypass grafts. One endovascular treated patient developed groin hematoma requiring surgical treatment. No significant difference was observed between the two treatment groups regarding total number of early complications (Table 2).

The follow-up period after the procedure ranged from 6 months to 3 years, while the average follow-up time was 22.37 ± 11.95 months. During the follow-up period, no significant rate of symptom reappearance was observed (2 surgically and 3 endovascular treated patients). The comparison of the efficacy of the methods did not show any significant difference. When comparing two treatment groups, no significant difference was found regarding palpability of radial arterial pulse at the long-term follow-up. After the follow-up period, the difference between brachial pressure of healthy and treated arm was significantly smaller when compared to the time before the procedure in both groups.

Graft occlusion was observed in 2 (6.66%) surgically treated patients. One patient underwent new bypass surgery, while the other did not undergo surgery because of the symptoms, age, clinical presentation, and comorbidity. Within the group of endovascular treated patients, 3 (7.5%) restenosis were observed. In 1 case, stent was implanted primarily, while in 2 cases, only PTA without stent implantation. Balloon dilatation was performed in 1 out of these 3 patients, while the stent was implanted in 2 patients. No significant difference was found between the two groups regarding patient distribution by localization and frequency of long-term complications (Table 3).

Also, no significant difference in primary patency was found between the two groups of patients (Figure 1).

Table 2

The results during the initial 30 postoperative days in surgically and endovascular treated patients with subclavian steal syndrome

Parameter	Treatment		*p*
	surgical	endovascular	
Clinical characteristics, n (%)			
asymptomatic	29 (96.67)	39 (97.5)	0.677
Radial pulse palpability			
palpable	29 (96.67)	38 (95)	1
The difference in systolic brachial pressure on healthy and treated arm (mmHg), $\bar{x}$ ± SD			
pre-procedure	42.6 ± 14.5	41.2 ± 15.35	0.959
post-procedure	4.75 ± 12.94	3.58 ± 5.83	0.371
Early complications, n (%)			
TIA	2 (6.67)	0 (0.0)	
dissection	0 (0.0)	1 (2.5)	1
distal embolization	0 (0.0)	1 (2.5)	
groin hematoma	0 (0.0)	1 (2.5)	

TIA – transient ischemic attack; n (%) – number (%) of patients; $\bar{x}$ – mean value; SD – standard deviation.

Table 3

Follow-up results in surgically and endovascular treated patients with subclavian steal syndrome

Parameter	Treatment		*p*
	surgical	endovascular	
Clinical characteristics, n (%)			
asymptomatic	28 (93.3)	37 (92.5)	0.494
VBI	0 (0)	2 (5)	
disabling arm ischemia	2 (6.67)	1 (2.5)	
Radial pulse palpability, n (%)			
palpable	27 (90)	36 (90)	1
The difference in systolic brachial pressure on healthy and treated arm (mmHg), $\bar{x}$ ± SD			
pre-procedure	42.6 ± 14.5	41.2 ± 15.35	0.959
after (22.37 ± 11.95) months	6.45 ± 15.41	5.33 ± 8.48	0.835
Long-term complications, n (%)			0.715
graft occlusion	2 (6.66%)	0 (0.0%)	
restenosis	0 (0.0%)	3 (7.5%)	

VBI – vertebrobasillar insufficiency; n (%) – number (%) of patients; $\bar{x}$ – mean value; SD – standard deviation.

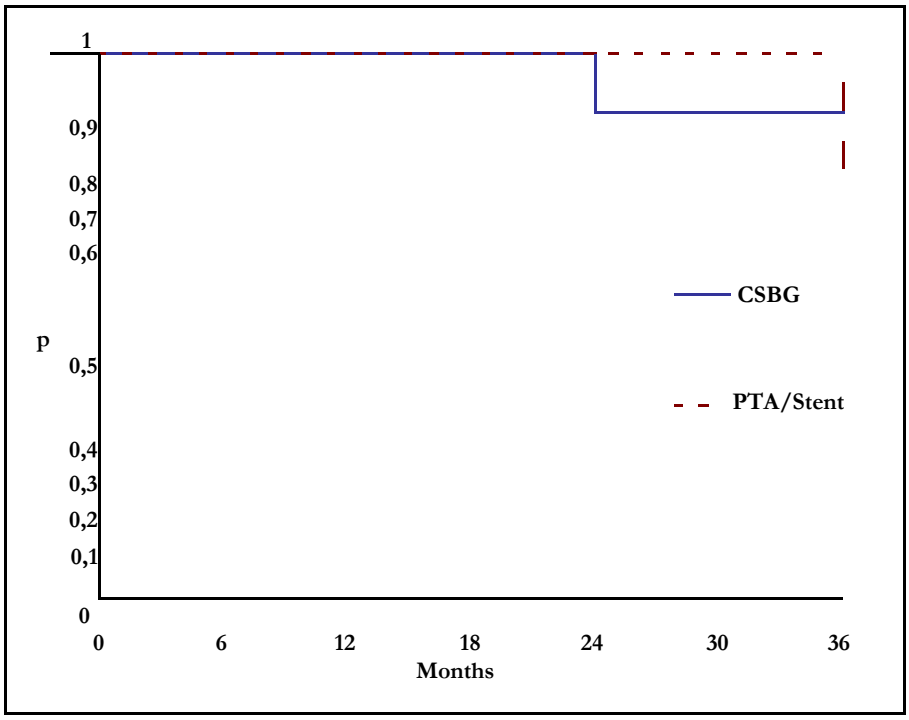

Fig. 1 – Long-term patency in treatment groups (Kaplan-Meyer curve).
CSBG – carotid-subclavian bypass grafts; PTA/Stent – percutaneous transluminal angioplasty and stenting of subclavian artery.

Discussion

The incidence of subclavian artery obstructive disease is difficult to determine as most of the lesions appear asymptomatic. Based on the available data, the incidence ranges from 0.5–2% and is mostly present in polyvascular patients [5–7].

Initially, subclavian artery lesions were treated surgically. Transthoracic approach was primarily used that is now abandoned method due to high complication and mortality rates [8]. In 1964, Parrott [9] was the first to describe subclavian artery transpositioning technique. In 1967, Diethrich and Koopot [10] presented extraanatomical bypass from carotid to subclavian artery. Today, the CSBGs and subclavian artery transposition are characterized by similar early and long-term results [7, 11, 12]. In 1980, Bachman and Kim [13] performed the first subclavian artery PTA. The numerous studies showed significant increase in success rates in early and long-term results in endovasculary treated patients, especially in those undergoing stent implantation [14]. Therefore, the opinions about the choice of treatment for this region changed, and PTA, with or without stent implantation, became method of choice besides extrathoracic surgical revascularization [5, 15]. Endovascular procedures are important especially in high risk patients. The majority of patients included in this study were polyvascular patients with numerous risk factors and comorbidities. Taking risk factors into account, AbuRahma et al. [16] treated patients with several comorbidities using PTA and stenting, considering their risk was too high to be treated surgically. Patients with high grade stenosis/near total occlusion and those with subclavian artery occlusion cannot be considered equal, nor can those with segmental and diffuse lesions. In our study, as many as 29 (96.67%) patients with subclavian artery occlusion were treated surgically. Over the past years, numerous studies showed significantly increased technical success rate of endovascular treatment of chronic occlusions [6, 17].

In the PTA/stenting group, we mostly used transfemoral approach (92%), while transaxillary approach (8%) was used in cases of changes in aorto-iliac region and in cases of total arterial occlusion of proximal segment of subclavian artery. Apart from these two approaches used to treat most of their patients, Sagić et al. [18, 19] and Babic et al. [20] described patients treated with "snare" technique using combined axillary and femoral approach. A bidirectional approach is mostly used for chronic total occlusions and much calcified lesions [21]. Within the proximal segment of subclavian artery, stents have to be implanted carefully in order to prevent blockage of vertebral artery ostium [6]. Balloon-expandable stents enable more accurate implantation compared to self-expandable stents. Therefore, in the study published by Brountzos et al. [22] balloon-expandable stents were used in the majority of patients (90%). Conversely, Wang et al. [23] implanted self-expandable stents in all 60 (100%) of their patients. Within our study, in 7 (28%) patients we used balloon-expandable stents, while 18 (72%) patients received self-expandable stents. In some recent studies, the use of balloon expandable covered stents to treat occlusive disease has been proposed as a method to reduce intimal hyperplasia and improve patency rate [24].

Although PTA and stenting is a minimally invasive technique, a comparable complication rate is seen in the PTA and stenting group (2.9–14.9%) [25], vs. the bypass group (5.9–14.2%) [12, 16, 26]. In a group of surgically treated patients, there was no case of graft infection, nor pseudoaneurysms were found in the PTA/stenting group. No significant difference regarding complications was found between the two groups.

Only five studies have been published so far comparing the results of CSBG and endovascular procedure at the same institution [16, 25–28]. Farina et al. [26] analyzed 15 patients with CSBG and 21 patients treated by balloon dilatation without stent implantation. Periprocedural complications were similar in both groups, while the long-term success, in contrast to our study, was significantly higher in the group of patients treated with bypass grafts (84% compared to 54%, respectively). Modarai et al. [25] compared early and long-term results of PTA and stenting of subclavian artery with extraanatomical bypass procedures on supraaortic branches and obtained more favorable long-term results in surgically treated patients. AbuRahma et al. [16] compared 121 patient treated with PTA and stenting, and 51 patient with CSBG, and confirmed better long-term results in CSBG patients. Linni et al. [27] compared 34 patients with subclavian-to-carotid artery transposition with 40 patients treated by stent supported PTA. According to their experience, they recommend endovascular treatment for subclavian stenosis and surgery for subclavian occlusions. The most recent comparative study was established in 2012 by Song et al. [28]. They analyzed the immediate and long-term outcomes in 148 patients treated with balloon-expandable stents, and 104 patients treated with extrathoracic surgical bypasses and concluded that both endovascular stenting and extrathoracic surgical bypass are safe and effective treatments for subclavian steal syndrome in the short and medium term, however, extrathoracic surgical bypasses are more durable in the long term. In our study, we showed similar long-term patency in both groups; stents were as durable as bypass grafts (Table 4).

Endovascular treatment, such as PTA and stenting, may have several advantages over surgical approach, including its minimally invasive nature, avoidance of general anesthesia and shorter length of hospital stay. Also, most in-stent restenosis can be treated with endovascular therapy. Today, we routinely select subclavian artery stenting first for subclavian steal syndrome. When, endovascular therapy is unsuccessful, surgical revascularization can provide durable treatment option.

Our study has some limitations. First, more occlusions were treated in the bypass surgery group, that can be partially based on selection bias by the endovascular specialist, and also it has to be noted that endovascular procedure was tried in some of them and abandoned due to difficulties in passing wire through the lesion after which they were treated surgically. Second, this was a retrospective, nonrandomized study. Prospective randomized studies are needed to compare the current treatment modalities for subclavian steel syndrome.

Conclusion

Both CSBG and PTA/stenting of subclavian artery are safe, efficacious and durable procedures. Using the PTA/stenting procedures is a method of choice in the treatment of hemodynamically significant stenosis, near total occlusions and segmental occlusions of subclavian artery. CSBG is indicated in case of diffuse occlusive lesions and when PTA and stenting do not succeed or cause complications.

Table 4

Studies comparing surgical and endovascular subclavian artery procedure

Author (year of publication)	Patients (number)		Follow-up period		Periprocedural complications		Long-term success/period	
	PTA/stent	Surgical	PTA/stent	Surgical	PTA/stent	Surgical	PTA/stent	Surgical
Farina et al. [26] (1989)	21	15	30 ± 24 months	40 ± 25 months	4.76	13.33	54% (5 years)	87% (5 years)
Modarai et al. [25] (2004)	41	35 (14 CSBG)	20 years		9.75	14.3	82% (4 years)	97% (5 years)
AbuRahma et al. [16] (2007)	121	51	41 (12–108) months	92 (12–80) months	14.9	5.9	70% (5 years)	96% (5 years)
Linni et al. [27] (2008)	40	34	50.1	52.6 months	5	11.7	95% (5 years)	100% (5 years)
Song et al. [28] (2012)	148	104	67 months	101 months	6.1	9.6	67% (5 years)	95% (5 years)
Our study Cvetic et al. (2017)	40	30	22.37 ± 11.95 months		6.67	7.5	92.5%	93%

PTA – percutaneous transluminal angioplasty; CSBG – carotid-subclavian bypass grafts.

Conflict of interest statement

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Association between Val158Met COMT, TNF-α -857 C>T, TNFR1 36 A>G, IL-1α 4845 G>T and IL-10 -1082 A>G Polymorphisms and Risk of Early-Onset Preeclampsia and Its Complications

Povezanost genskog polimorfizma Val158Met COMT, TNF-α -857 C>T, TNFR1 36 A>G, IL-1α 4845 G>T i IL-10 -1082 A>G sa rizikom od pojave rane preeklampsije i njenih komplikacija

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Abstract

Background/Aim. Preeclampsia (PE) belongs to the group of hypertensive disorders in pregnancy with the global average incidence of 2.16%. It is considered as one of the leading causes of maternal and neonatal morbidity and mortality worldwide. The goal of this study was to assess the potential association between Val158Met catechol-o-methyltransferase (COMT), tumor necrosis factor-alpha (TNF-α) -857 C>T, tumor necrosis factor receptor 1 (TNFR1) 36 A>G, interleukin-1alpha (IL-1α) 4845 G>T and interleukin-10 (IL-10) -1082 A>G polymorphisms and risk of early-onset preeclampsia (PE) and its complications. **Methods.** The study included 47 early-onset PE patients, which were grouped by disease severity and by size for gestational age and 47 control cases. The Val158Met polymorphism was genotyped by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) analysis and inflammatory cytokine polymorphisms by the Sanger sequencing method. **Results.** The COMT Met allele as well as IL-1α T showed a protective role, decreasing the risk of early-onset PE after age and body mass index (BMI) adjustments. The detected interactions between the COMT Met and IL-10 A alleles, as well as between the COMT Met and TNF-α T alleles were insignificant after age and BMI adjustments. **Conclusion.** COMT and IL-1α may be used as candidate genes for early-onset PE and its severe form and small for gestational age (SGA) complications.

Key words:
comt protein, human; cytokines; pre-eclampsia; polymorphism, genetic.

Apstrakt

Uvod/Cilj. Preeklampsija pripada grupi hipertenzivnih poremećaja u trudnoći sa prosečnom incidencom od 2,16% i predstavlja jedan od vodećih uzroka morbiditeta i mortaliteta majki i novorođenčadi širom sveta. Cilj ove studije bio je da se ispita potecijalna povezanost između polimorfizma Val158Met katehol-O-metiltransferaze (COMT), faktor nekroze tumora-alfa (TNF-α) -857 C>T, receptora 1 za faktor nekroze tumora (TNFR1) 36 A>G, interleukin-1 alfa (IL-1α) 4845 G>T i interleukin-10 (IL-10) –1082 A>G sa rizikom od pojave rane preeklampsije (PE) i njenih komplikacija. **Metode.** Ova studija obuhvatila je 47 bolesnica sa ranom PE, grupisanih prema težini oboljenja i prema veličini za odgovarajuću gestacionu starost, i 47 zdravih osoba. Polimorfizam Val158Met je genotipiziran analizom *polymerase chain reaction – restriction fragment lenght polymorphism* (PCR-RFLP), a polimorfizam inflamatornih citokina Sangerovom metodom sekvencioniranja. **Rezultati.** Aleli COMT Met i IL-1α T pokazali su protektivnu ulogu, smanjujući rizik rane PE nakon korekcije za starost i indeks telesne mase (BMI). Uočena interakcija između alela COMT Met i IL-10 A, kao i između alela COMT Met i TNF-α T nije bila statistički značajna nakon korekcije za starost i BMI. **Zaključak.** COMT i IL-1α se mogu koristiti kao geni kandidati za otkrivanje rane PE i njenih komplikacija, teškog oblika rane PE i PE sa zastojem u rastu.

Ključne reči:
comt protein, humani, citokini; preeklampsija; polimorfizam, genski.

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Introduction

Preeclampsia (PE) belongs to the group of hypertensive disorders in pregnancy with global average incidence of 2.16%. It is considered as one of the leading causes of maternal and neonatal morbidity and mortality worldwide [1–3]. Special attention should be paid to the early and severe form of PE, due to its correlation with a very high morbidity rate and a frequent occurrence of serious health complications [2, 4].

The mechanism that initiates PE development is still unknown. The hypothesis is that combined over-expressed inflammatory response and angiogenic imbalance potentially cause an endothelial dysfunction [5]. By analyzing epidemiological data, it has been emphasized that genetic factors are one of the main risk factors for PE development, and numerous candidate gene studies and linkage analyses have been carried out in this area [6].

Recently, one of the genes whose expression showed to have potential as a candidate gene for PE and could be connected with angiogenic imbalance is the COMT gene [7]. COMT is among the major enzymes responsible for the inactivation of catechol-estrogens, which play an important role in pregnancy management and fetal development [8]. Different studies showed a correlation between the Val158Met COMT polymorphism and increased risk of PE in different patient groups [9]. A more detailed investigation showed that fetal Val158Met COMT polymorphism was correlating with increased risk of PE, and maternal Val158Met COMT polymorphism showed a protective role [10]. Furthermore, in line with the theory of excessive inflammatory response, several polymorphisms of inflammatory cytokines showed to be associated with PE [11–13].

Recently we demonstrated that material COMT Met-Met genotype was associated with decreased risk of early-onset PE including its severe form as well as risk of small-for-gestational-age (SGA) complicating PE [14]. In contination of this study we investigated examine the potential correlation between the Val158Met COMT, TNF-α -857 C>T, TNFR1 36 A>G, IL-1α 4845 G>T and IL-10 -1082 A>G polymorphisms and the risk of early-onset PE, the risk of a severe form of early-onset PE and risk of SGA complicating early-onset PE. Furthermore, the investigation included the assessment of potential interaction between associating polymorphisms in order to determine their potential synergistic or antagonistic effect.

Methods

Subjects

The study was conducted at the Clinic of Gynecology and Obstetrics, Clinical Centre of Serbia, Belgrade, in the period between September 2012 and December 2013. The official approval for this study was obtained from the Ethics Committee of the Clinical Centre of Serbia. All patients and control subjects were informed beforehand about this study and they provided their written informed consent to participate.

The study included two groups of participants: the group of 47 early-onset PE patients and the control group of 47 healthy cases. The early-onset PE group was divided into two subgroups: severe early-onset PE of 33 patients and mild early-onset PE of 14 patients. Based on the second criterion, all 47 early-onset PE patients were divided into two subgroups: appropriate-for-gestational-age (AGA) subgroup with 12 patients and SGA subgroup with 35 patients.

PE, early-onset PE and severe PE were defined according to the American College of Obstetricians and Gynecologists Task Force on Hypertension in Pregnancy [15]. SGA and AGA were defined according to the national birth weight distribution of the Serbian population [16]. The excluding criteria were any of the following: pregnant women with known abnormal fetal karyotype or chromosomal abnormalities, multi-fetal gestation, gestational hypertension without proteinuria, chronic hypertension, diabetes mellitus, cardiovascular disease, autoimmune disease and renal disease.

The control subjects were defined as healthy singleton pregnancies, having come to the Clinical Centre of Serbia for delivery, and delivering a healthy neonate at term (37 weeks of gestation or more) without medical or obstetric complications.

Antenatal care was provided according to hospital guidelines and protocols. One aliquot of analyzed ethylenediaminetetraacetic acid (EDTA) whole blood was kept frozen on -70°C for deoxyribonucleic acid (DNA) extraction and genotyping. At delivery, the type of delivery was recorded; gestational age was calculated; birth weight was measured, and the Apgar score was assessed.

DNA preparation and genotyping

Isolation of genomic DNA from 200 μL of peripheral blood was done with the commercial kit for isolating genomic DNA (Roche Diagnostics), in accordance with the manufacturer's instructions. The detection of mutation presence in the gene for inflammatory cytokines and Val158Met COMT was performed by a polymerase (PCR) chain reaction amplification of DNA. The amplification was carried out in a PCR instrument (Termocycler-in) GeneAmp PCR System 9700 (Applied Biosystems). The product of PCR reactions for inflammatory cytokine polymorphism was than digested by EXOsap-IT enzyme. The Sanger sequencing method was used to analyze the expected polymorphism by the Genetic Analyzer Applied Biosystem 3130. Sequences were analyzed in the Software Sequencing Analysis 5.2 with a 36 cm capillary and polymer POP7. The product of PCR reactions for COMT polymorphism was digested by enzyme NlaIII (Hin1II Thermo SCENTIFIC). Electrophoretic separation was performed on 2.5% agarose gel, containing ethidium bromide. After digestion the enzyme fragments were visualized under ultraviolet light on transillumination (Wilber Lourmat).

Statistical analysis

General clinical characteristics between cases and controls were compared using Student's *t*-tests or the Wilcoxon rank sum test where appropriate. Genotype frequencies were

tested against theoretical Hardy-Weinberg equilibrium (HWE) by χ^2 contingency table analysis (degree of freedom = 2). Allele and genotype frequencies were compared between all cases of PE and their controls by contingency tables or by the Fisher's exact probability test, and odds ratios (OR) and 95% confidence intervals (CI) were computed. The frequency of homozygote for the common allele was considered as the reference for comparisons (OR = 1). Under a dominant model and a rare allele frequency of 0.34, our study sample had a power $1-\beta = 0.78$ to detect a genetic effect resulting in an OR = 0.2 at a type I error of 0.05. Power calculations were performed using the online tool Genetic Power Calculator [17].

The polymorphisms individually associated with PE (with $p < 0.05$ as entry criteria) were included in logistic regression models to test for joint multi-locus association with PE, both unadjusted and adjusted for clinical co-variables.

All the computation was done in R language and environment, version 3.1.0 [18].

Results

Clinical characterization

As shown in the previous study [14] significant differences were observed between early-onset PE patients and the control group in maternal age, body mass index (BMI), systolic and diastolic blood pressure (BP) and in gestational age at delivery. There was a significantly higher risk of SGA neonate delivery in patients with early-onset PE (Table 1). Deviataion from Hardy-Weinberg equlibrium (HWE) – D values for interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α) and tumor necrosis factor receptor type I (TNFRI) were significant in investigated loci. In contrast, genotypes for COMT and interleukin-1 alpha (IL-1α) in the investigated population respectively comply with the HWE proportions (Table 2).

COMT Val158Met genotyping

The Met allele decreased the risk for early-onset PE development and early-onset PE SGA development. There was no statistically significant difference between the mild and severe form of early-onset PE (Table 3). The strongest statistically significant influence was noticed in the allele recessive model. The MetMet genotype was decreasing the risk of early-onset PE and PE complications (Table 4).

IL-1α4845 G>T genotyping

T allele decreased the risk for early-onset PE development, severe early-onset PE and early-onset PE SGA development (Table 5). GT and TT genotypes were associated with decreased risk of early-onset PE and PE complications (Table 6).

IL-10-1082 A>G, TNF-α -857 C>T and TNFR1 36 A>G genotyping

There was no statistically significant association between IL -1082 A>G, TNF-α -857 C>T or TNFR1 36 A>G genotype and early-onset PE, severe early-onset PE or early-onset PE SGA observed ($p > 0.05$).

Multinomial logistic regression x̄

Older age and increased BMI significantly led to early-onset PE development. Even after age and BMI correction, a

Table 1

Clinical characteristics of examined patients and controls

Characteristics of patients	Controls n = 47, x̄ ± SD	Early-onset PE n = 47, x̄ ± SD (*p*)	Severe early-onset PE n = 33, x̄ ± SD (*p*)	Mild early-onset PE n =14, x̄ ± SD (*p*)	Early-onset PE SGA n = 35, x̄ ± SD (*p*)	Early-onset PEAGA n = 12, x̄ ± SD (*p*)
Age (years)	29.44 ± 4.49	32.14 ± 5.52 (0.009767)	32.45 ± 5.22 (0.007997)	31.42 ± 6.3 (0.2244)	32.06 ± 6.09 (0.03021)	32.41 ± 3.55 (0.03643)
Gestational age (days)	275 ± 9	225 ± 28 (1.346×10^{-14})	218 ± 23 (3.142×10^{-15})	241 ± 33 (2.398×10^{-5})	219 ± 24 (5.357×10^{-14})	240 ± 34 (2.119×10^{-6})
BMI (kg/m^2)	24.16 ± 4.11	27.77 ± 3.93 (0.0002266)	27.9 ± 4.25 (0.0007854)	27.46 ± 3.18 (0.01384)	27.0 ± 3.37 (0.003876)	30.03 ± 4.71 (0.0007481)
Systolic BP (mmHg)	108.62 ± 9.9	162.98 ± 18.61 (3.456×10^{-13})	171.36 ± 15.07 (1.037×10^{-11})	143.21 ± 8.23 (1.758×10^{-7})	163.57 ± 18.92 (8.828×10^{-12})	161.25 ± 18.35 (4.39×10^{-7})
Diastolic BP (mmHg)	70.17 ± 8.5	104.36 ± 11.68 (3.898×10^{-13})	109.24 ± 9.36 (9.147×10^{-12})	92.86 ± 8.02 (3.333×10^{-7})	104.57 ± 11.14 (7.54×10^{-12})	103.75 ± 13.67 (8.559×10^{-7})
Proteinuria (g/24 h)	/	3.74 ± 4.05	4.59 ± 4.41	1.73 ± 2.0	4.31 ± 4.12	2.07 ± 3.49
Birth weight (g)	3340.24 ± 445.28	1511.3 ± 784.2 (1.853×10^{-13})	1304.5 ± 536.2 (1.918×10^{-13})	1998.6 ± 1050.8 (8.639×10^{-5})	1206.3 ± 450.3 (7.715×10^{-14})	2400.8 ± 886.5 (0.0005678)

PE – preeclampsia; BMI – body mass index; SGA – small for gestational age; AGA – appropriate for gestational age; BP – blood pressure.

Table 2

Testing for Hardy-Weinberg Equilibrium (HWE)

HWE in the control group (n = 47)	Deviation from HWE (D)	χ^2	*p*
COMT	0.032	2.1942	0.3338
IL-1α	0.174	0.805	0.6686
IL-10	0.214	0.0474	1
TNF-α	0.215	0.0686	1
TNFRI	-0.219	0.0504	1

IL-1α – interleukin-1 alpha; IL-10 – interleukin-10; TNF-α – tumor necrosis factor-alpha; TNFRI – tumor necrosis factor receptor type I; COMT – catechol-o-methyltransferase.

Table 3

Distribution of COMT alleles in the investigated group of early-onset preeclampsia (PE) patients and the control group

COMT (allelic)	Controls (n)	Early-onset PE OR (n)	Severe early-onset PE OR (n)	Mild early-onset PE OR (n)	Early-onset PE SGA OR (n)	Early-onset PE AGA OR (n)
Val	(37)	(52)	(37)	(15)	(39)	(13)
Met	(57)	0.526(42)*	0.511(29)	0.565(13)	0.518(31)**	0.552(11)

***p* = 0.04057; ****p* = 0.0411;**
SGA – small for gestational age; AGA – appropriate for gestational age; BP – blood pressure; COMT – catechol-o-methyltransferase; n – number.

Table 4

The COMT allele recessive model in the investigated group of early-onset preeclampsia (PE) patients and the control group

COMT (under AR assumption)	Controls (n)	Early-onset PE OR (n)	Severe early-onset PE OR (n)	Mild early-onset PE OR (n)	Early-onset PE SGA OR (n)	Early-onset PE AGA OR (n)
Val-Val and Met-Val	(27)	(39)	(27)	(12)	(29)	(10)
Met-Met	(20)	0.281 (8)*	0.304 (6)**	0.229 (2)	0.284 (6)***	0.275 (2)

***p* = 0.01235, Fisher exact test; ****p* = 0.02928; *****p* = 0.01732;**
SGA – small for gestational age; AGA – appropriate for gestational age; BP – blood pressure; COMT – catechol-o-methyltransferase; n – number.

Table 5

Distribution of interleukin-1 alpha (IL-1α) alleles in the investigated group of early-onset preeclampsia (PE) patients and control group

IL-1α (allelic)	Controls (n)	Early-onset PE OR (n)	Severe early-onset PE OR (n)	Mild early-onset PE OR (n)	Early-onset PE SGA OR (n)	Early-onset PE AGA OR (n)
G	(62)	(83)	(58)	(25)	(61)	(22)
T	(32)	0.259 (11)*	0.269 (8)**	0.235 (3)***	0.288 (9)****	0.178 (2)*****

***p* = 0.0004281; ****p* = 0.0016; *****p* = 0.0173; ******p* = 0.0019; *******p* = 0.012**
SGA – small for gestational age; AGA – appropriate for gestational age; OR – odds ratio; n – number.

Table 6

The interleukin-1 alpha (IL-1α) allele dominant model in the investigated group of early-onset preeclampsia (PE) patients and the control group

IL-1α (under AD assumption)	Controls (n)	Early-onset PE OR (n)	Severe early-onset PE OR (n)	Mild early-onset PE OR (n)	Early-onset PE SGA OR (n)	Early-onset PE AGA OR (n)
GG	(22)	(38)	(26)	(12)	(27)	(11)
GT and TT	(25)	0.212 (9)*	0.241 (7)**	0.151 (2)***	0.265 (8)****	0.082 (1)*****

***p* = 0.001123; ****p* = 0.00532; *****p* = 0.0136; ******p* = 0.0067; *******p* = 0.0075;**
SGA – small for gestational age; AGA – appropriate for gestational age; OR – odds ratio.

statistically significant association between Val158COMT or IL-1α polymorphism and early-onset PE development still remained.

COMT-Met homozygous was showing a protective role by reducing the risk for early-onset PE development 3.2 times, as well as IL-1αT allele, of which one dose reduced the risk for early-onset PE development for almost six times (Table 7).

Interactions between Val158Met COMT, TNF-α -857 C>T, TNFR1 36 A>G, IL-1α 4845 G>T and IL-10 -1082 A>G polymorphisms

The presence of COMT Met allele and TNF-α T allele additionally increased the risk for early-onset PE development 2.765-fold in comparison to the simple multiplying of both OR. After age and BMI adjustment, this interaction be-

Table 7

Multinomial logistic regression including age, body mass index (BMI), COMT and interleukin-1 alpha (IL-1α) polymorphisms

Variable / polymorphism	Early-onset PE adjusted OR (95% CI)	Early-onset PE *p*-value
Age (years)	1.122(1.015–1.253)	0.030064
BMI (kg/m^2)	1.134(1.133–1.568)	0.000876
COMT (AR model)	0.308(0.091–0.960)	0.047627
IL-1α (AD model)	0.167(0.048–0.508)	0.002620

PE – preeclampsia; OR – odds ratio; CI – confidence intervals; BMI – body mass index; IL-1α – interleukin-1 alpha; COMT – catechol-o-methyltransferase.

came statistically insignificant. The interaction between COMT Met allele and IL-10 A allele was close to the statistical level of significance, uncorrected as well as corrected for age and BMI. Combined OR was significantly different (almost three times higher) in comparison to the multiple of those two OR, leading to the conclusion that IL-10 A allele presence reduced the protective effect of COMT Met allele 2.71-fold (Table 8).

Table 8

Interactions of COMT and inflammatory cytokines polymorphisms

Interactions	OR for interaction term – unadjusted for BMI and age	*p* – unadjusted for BMI and age	OR for interaction term, adjusted for BMI and age	*p* – adjusted for BMI and age	OR combined, adjusted for BMI and age
COMT (Met) : IL-1α (T)	0.618	NS	0.509	NS	0.190
COMT (Met):IL-10 (A)	0.369	0.0647	0.357	0.0827	0.937
COMT(Met):TNF-α(T)	2.765	0.0491	1.703	NS	0.490
IL-1α(T):IL-10(A)	0.799	NS	1.046	NS	0.159
IL-1α(T):TNF-α(T)	1.948	NS	2.486	NS	0.371
IL-10(A):TNF-α(T)	1.724	NS	1.470	NS	1.239

BMI – body mass index; IL-1α – interleukin-1 alpha; IL-10 – interleukin-10; TNF-α – tumor necrosis factor-alpha; COMT – catechol-o-methyltransferase; OR – odds ratio.

Another group of polymorphisms that were included in the scope of our investigation are inflammatory cytokine gene polymorphisms: TNF-α -857 C>T, TNFR1 36 A>G, IL-1α 4845 G>T and IL-10 -1082 A>G. Haggerty et al. [11] showed that IL-1α 4845 GG genotype significantly increased the PE risk (black OR 11,6; 95% CI 1,5-89,3; white OR 1,7; 95% CI 0,7–3,9). The combination of TNF-α -857C>T and TNFR1 36 A>G polymorphisms leads to 2.26-fold times increased risk of PE. The effect was even stronger in their joint presence with IL-1α 4845 G>T polymorphism, which together were associated with higher than 4 times higher risk of PE development (OR = 4.13; CI95: 2.16 – 7.89; p = 0.00002) [12]. The most frequently investigated polymorphism was IL-10 -1082 A>G. However, a meta-analysis performed by Lee et al. [13] showed a statistically insignificant association, after excluding the studies where the distribution of control subjects was deviating from HWE. In our study, we could only find a statistically significant association between IL-1α 4845 G>T polymorphism and the risk of early-onset PE, severe early-onset PE and early-onset PE with SGA. The potential reason could be the limited number of patients included in this study, as well as a potentially inappropriate control population selection for these polymorphisms, described by significant deviation from HWE for TNF-α, TNFR1and IL-10 in the control group.

Regarding the potential of the IL-1α 4845 G>T polymorphism to do the risk stratification of PE, to our knowledge this is the first study investigating this topic. Significant differences in the IL-1α 4845 G>T allele distribution between the mild and severe form of PE could be identified. We also showed the association between the IL-1α 4845 G>T polymorphism and early-onset PE associated with SGA.

For the statistically significant loci, adjustments for age and BMI was made in a multivariate logistic regression analysis. It is important to underline that we confirmed previously published data about positive association between

Discussion

Many genes and their polymorphisms have been examined in order to detect the potential markers for high-risk pregnancies [19, 20]. Due to the most recent highlights in the theories of 'excessive inflammation' and 'angiogenic imbalance' as the potential causes of PE, there is a dilemma whether the polymorphisms of genes associated with these could be a potential cause of PE development [5].

Recently, the animal COMT mice knockout model has shown to be useful in clarifying the significance of decreased COMT expression in PE [7]. Even though this study was revolutionary in the field of PE genetic models investigation, it was clearly limited with the absence of differentiation between maternal and fetal low COMT activity. Our study confirmed the protective role of low maternal COMT activity. The explanation could be found in a hypothesis that Hill et al. [10] proposed, which considered decreased maternal COMT activity as a protective role by stimulating the placenta to produce 2-ME. Placental low COMT activity is the key contributor for PE development. We are further supporting our finding with the fact that our control group is on HWE for COMT genotype.

Regarding the potential association of COMT allele distribution and early-onset PE complicated with SGA, our study showed that MetMet decreased the risk of early-onset PE SGA for more than 3.5 times. To the contrary, Sata et al. [21] showed that patients with homozygous COMT-L alleles had a 2.98 times higher risk of low birth weight (< 2,500 g) [20]. It is concluded that lower COMT activity may lead to the accumulation of catechol estrogens, and thus, cause oxidative DNA damage which may be associated with SGA [22]. These explanations could be applied to fetal low COMT activity and our suggestion is that low maternal COMT activity could show a protective role allowing higher fetal COMT activity. Further studies are needed to investigate these contradictory data.

age or BMI with PE development [23]. Even after age, BMI and other statistically significant polymorphism adjustments, each of the two polymorphisms still showed a statistically significant association with early-onset PE development.

Finally, we investigated the interactions between the previously mentioned polymorphisms and their association with early-onset PE, which is to our knowledge the first study investigating this topic. The allele model showed a statistically significant interaction between the COMT Met allele and TNF-α T allele which significance after age and BMI adjustments disappeared. Secondly, the interaction between COMT Met and IL-10 A alleles, unadjusted as well as age and BMI adjusted, were on the level of statistical significance. Further studies are needed in order to investigate this suggestion in the larger population size.

The main shortcoming of our study was the limited number of investigated patients, as we were primarily focused on difficult cases of early-onset PE (33 severe early-onset PE patients in comparison to 47 early-onset PE patients in total) with a mean systolic BP 162.98 ± 18.61 mmHg and diastolic BP 104.36 ± 11.68 mmHg. Further studies with a bigger sample size of early-onset PE patients should be conducted in order to investigate the significance of a potential interaction between COMT and inflammatory cytokines polymorphisms.

Conclusion

We showed that the distribution of COMT as well as IL-1α alleles between early-onset PE patients and control subjects was different, particularly between SGA early-onset PE patients and respected control subjects. The COMT Met allele as well as IL-1α T showed a protective role, decreasing the risk of early-onset PE after age and BMI adjustments. Interaction between COMT Met and IL-1α T did not show any statistically significance. Statistical significance was observed in interaction between the COMT Met and IL-10 A alleles, but it was not any more statistically significant after age and BMI adjustments. Interaction between the COMT Met and TNF-α T alleles were close to level of significance, both before and after age and BMI adjustments.

COMT and IL-1α may be used as candidate genes for detection of high-risk patients for development of early-onset PE and its severe form and SGA complications.

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Surgical stress response following hip arthroplasty regarding choice of anesthesia and postoperative analgesia

Izbor anestezije i postoperativne analgezije i sistemski odgovor na hirurški stres nakon aloartroplastike kuka

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Abstract

Background/Aim. Significant surgical stress response consisting of hormonal, metabolic and inflammatory changes can be initiated by the hip replacement surgery. Appropriate choice of anesthesia and postoperative analgesia should provide diminution of surgical stress response and may reduce number of perioperative complications. Surgical stress response after peripheral nerve blocks has not been studied extensively in patients who underwent hip replacement. The aim of the study was to investigate whether continuous lumbar plexus block can significantly reduce surgical stress response in comparison to other types of postoperative analgesia – continuous epidural analgesia and intravenous patient controlled analgesia (PCA) with morphine. **Methods**. Prospective study included 60 patients, scheduled for total hip arthroplasty. The patients were randomized into 4 groups: group CNB (central nerve block - epidural), group PNB (Peripheral nerve block - lumbar plexus block), SAM (Spinal anesthesia- PCA (anesthesia) morphine) and GAM (General anesthesia + PCA with Morphine). Serum levels of cortisol, thyroid hormones (T3, T4) and thyroid stimulating hormone (TSH), insulin, glucose and C-reactive protein (CRP) were measured in all groups – preoperatively, as well as 4 h, 12 h and 24 h after surgery. **Results**. The study showed that average serum cortisol levels were significantly lower 4 h after the operation in the groups where methods of regional anesthesia were performed intraoperatively (SAM, CNB, PNB); ($F = 19.867$; $p < 0.01$). Groups with postoperative continuous catheter analgesia (CNB, PNB) had significantly lower serum cortisol levels 12 h after the operation ($F = 8.050$; $p < 0.01$). The highest serum insulin levels were detected 4 h postoperatively in the CNB and PNB group, while the lowest were in the GAM group ($F = 5.811$; $p < 0.05$). Twelve hours after the operation, the lowest values of insulin were measured in the SAM group ($F = 5.052$; $p < 0.05$), while 24 h postoperatively, the lowest values were found in the SAM and GAM group ($F = 6.394$; $p < 0.05$). T3, T4 and TSH levels showed slight reduction in comparison to preoperative values without statistical significance. Blood glucose levels were significantly different among the groups 4 h after surgery with the highest values recorded in the GAM group and the lowest ones in the SAM group ($F = 10.084$; $p < 0.01$). On the other hand, 12 h after the operation significant rise in blood glucose levels was detected in the SAM group ($F = 7.186$; $p < 0.01$) Levels of CRP increased remarkably 12 h and 24 h after the surgery, but without significant difference among the groups. **Conclusion**. Administration of postoperative analgesia using continuous lumbar plexus block following hip arthroplasty reduces significantly stress response in comparison to postoperative PCA with morphine and has comparable effects on hormone release to epidural analgesia. Spinal anesthesia provides the best diminution of surgical stress response in the early postoperative period in comparison with other types of intraoperative analgesia.

Key words:
anesthesia, general; analgesia; anesthesia, spinal; anesthesia, conduction; intraoperative period; hydrocortisone; insulin; biological markers.

Apstrakt

Uvod/ Cilj. Zamena totalne proteze kuka može izazvati značajan sistemski odgovor na hirurški stres, koji uključuje hormonske, metaboličke i zapaljenske promene. Odgovarajući izbor anestezije i postoperativne analgezije bi trebalo da obezbedi slabljenje ovog odgovora i da utiče na smanjenje broja postoperativnih komplikacija. Istraživanja u oblasti sistemskog odgovora na hirurški stres, kod bolesnika kod kojih je primenjen periferni nervni blok nakon ugradnje totalne proteze kuka, nisu brojna. Cilj studije bio je da se ispita da li kontinuirani blok lumbalnog pleksusa može značajno da umanji sistemski odgo-

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vor na hirurški stres u poređenju sa drugim vrstama postoperativne analgezije – kontinuiranom epiduralnom analgezijom i intravenskom PKA (pacijent kontrolisanom analgezijom) morfinom. **Metode**. U prospektivnu studiju bilo je uključeno 60 bolesnika, predviđenih za aloartroplastiku kuka. Bolesnici su bili raspoređeni u četiri grupe u zavisnosti od primenjene vrste anestezije i postoperativne analgezije: grupa CNB (centralni neuroblok - epidural), grupa PNB (periferni neuroblok - blok lumbalnog pleksusa), SAM grupa (spinalna anestezija + PKA morfinom) i OAM (opšta anestezija + PKA morfin). Vrednosti kortizola, tiroidnih hormona (T3, T4) i tiroidni stimulišući hormon (TSH), kao i vrednosti insulina, glukoze i C-reaktivnog proteina (CRP) u serumu merene su kod svih grupa, 4 h, 12 h i 24 h u serumu nakon operacije. **Rezultati**. Istraživanje je pokazalo da su prosečne vrednosti kortizola u serumu bile značajno niže 4 h postoperativno u grupama gde je intraoperativno primenjena regionalna anestezija (SAM, CNB, PNB), ($F = 19.867$; $p < 0.01$). U grupama u kojima je primenjena kontinuirana analgezija preko katetera (CNB, PNB), nivo kortizola u serumu bio je značajno niži 12 h posle operacije; ($F = 8.050$; $p < 0.01$). Najveći porast nivoa insulina u serumu detektovan je 4 h postoperativno u grupama CNB i PNB, a najmanji u grupi OAM; ($F = 5.811$; $p < 0.05$). Dvanaest sati nakon operacije, najniže vrednosti insulina izmerene su u grupi SAM; ($F = 5.052$; $p < 0.05$), dok su nakon 24 h njegove najniže vrednosti bile u grupama SAM i GAM, ($F = 6.394$; $p < 0.05$). Nivoi T3, T4 i TSH bili su blago sniženi u odnosu na preoperativne vrednosti, 4 h, 12 h i 24 h nakon operacije. Vrednosti glikemije su se statistički značajno razlikovale među grupama, 4 h posle operacije, a najviše vrednosti su zabeležene u grupi OAM, dok su najniže bile u grupi SAM; ($F = 10.084$; $p < 0.01$). Nasuprot tome, 12 h nakon operacije, značajan porast u nivou glukoze u serumu detektovan je u grupi SAM, ($F = 7.186$; $p < 0.01$). Vrednosti CRP-a su primetno rasle 12 h i 24 h postoperativno, ali bez statistički značajne razlike između grupa. **Zaključak**. Primena kontinuiranog bloka lumbalnog pleksusa nakon aloartroplastike kuka značajno smanjuje sistemski odgovor na stres u poređenju sa postoperativnom PKA morfinom i po hormonskom odgovoru može se porediti sa epiduralnom analgezijom. Spinalna anestezija obezbeđuje najveće smanjenje sistemskog odgovora na hirurški stres u ranom postoperativnom periodu u poređenju sa drugim vrstama anestezije.

Ključne reči:
anestezija, opšta; analgezija; anestezija, spinalna; anestezija, provodna; intraoperativni period; hidrokortizon; insulin; biološki pokazatelji.

Introduction

Significant surgical stress response consisting of hormonal, metabolic and inflammatory changes can be initiated by the hip replacement surgery [1]. Triggering of this reaction is caused by surgical incision and includes release of local tissue activators from damaged tissue, such as interleukins, tumor necrosis factor (TNF), serotonin, kinins and some intracellular proteins. These local mediators may influence activation of complex autonomic, endocrine and biochemical response. Local tissue activators increase the sensitivity of nociceptors. Pain sensation is carried from nociceptors to spinal cord via fine unmyelinated C and myelinated Aδ nerve fibers. Afferent impulses are transmitted throughout spinal cord and reach reticular formation and limbic system. Impulses also travel to hypothalamus and cerebral cortex [2].

Activation of the neuroendocrine and immune system includes stimulation of hypothalamo-pituitary-adrenal axis, resulting in secretion of "stress hormones", predominantly adrenocorticotropic hormone (ACTH), cortisol and catecholamines. Increase in cortisol level represents the key result of the excitation of hypothalamus and secretion of ACTH. It is known that serum concentration of cortisol is doubled two hours after the surgical incision, while maximum of cortisol level (around 35 μg/dL) is reached eight hours after surgery. Thereafter, serum concentration of cortisol starts to decline, reaching 22μg/dL, 24 h postoperatively, and remains stable within the seven following days [3]. Production of thyroid hormones, thyroxine (T4) and tri-iodothyronine (T3) is affected by cortisol and catecholamines. Concentrations of total and free T3 decrease few days after surgery, while TSH levels fall within 2 h postoperatively [1, 4].

Secretion of insulin is significantly impaired during the operation and immediately after surgery, caused by α-adrenergic inhibition of β cell secretion. The plasma concentration of insulin, the key anabolic hormone, tends to decrease due to predomination of catabolic hormones (cortisol, glucagon and catecholamines). Period of reduced insulin secretion is followed by the period of the impairment of cellular sensitivity to insulin. This phenomenon, called "insulin resistance", starts on the day of surgery and may last up to three weeks after the operation [3, 5, 6].

Increase in blood glucose levels is the main metabolic consequence of gluconeogenesis and hepatic glycogenolysis, stimulated by cortisol and cateholamines. Furthermore, the rate of glucose utilization is impaired by reduced peripheral sensitivity to insulin [7–10].

Level of C-reactive protein (CRP), an acute phase protein, is expected to rise following hip arthroplasty, reaching its maximum 24 h postoperatively. It can be used as nonspecific marker of inflammatory response in these patients [11].

Influence of anesthesia on stress response to surgery has been well established in patients scheduled for major abdominal and cardiac surgery [12–14]. General anesthesia cannot diminish completely activation of hypothalamo-adrenal axis. Some of the medications, used for general anesthesia, e.g. propofol, sevoflurane and beznodiazepines are found to attenuate release of catabolic hormones [1].

On the contrary, regional techniques, such as spinal and epidural anesthesia cease the nocicepive signals from surgical wound, preventing their transfer to central nervous system. As the result, release of pituitary hormones is significantly attenuated. Blocking of efferent signals, caused by regional anesthesia is also an important factor of preventing hormonal release from the target organs [2]. Extensive use of anticoagulant therapy concomitantly with hip surgery during the past decades, raised the risk of spinal and epidural hematoma associated with neuraxial blockade in orthopedic

surgery [15]. Therefore, peripheral nerve blocks have gained the popularity, providing adequate analgesia with less side effects and complications [16–18]. Surgical stress response after peripheral nerve blocks, such as 3-in-1 block and lumbar plexus block, was not studied extensively in patients who underwent hip replacement. Intraoperatively, spinal anesthesia can be used as single method of anesthesia. Epidural anesthesia and peripheral blocks are more often used in combination with general anesthesia intraoperatively, whilst as single method for continuous postoperative analgesia via the epidural or peripheral catheter. In the early postoperative period, up to 24 h following general or spinal anesthesia, intravenous administration of narcotics, especially morphine, is the method of choice in the absence of peripheral or epidural catheters. Morphine should be used via patient controlled analgesia (PCA) pump, which enables precise delivery on patient's demand with the least side effects [19, 20].

Patients scheduled for hip replacement surgery are often elderly people, with significant comorbidity and chronic use of anticoagulant therapy. Therefore, appropriate choice of anesthesia and postoperative analgesia should provide decrease of surgical stress response and may reduce perioperative complications, such as myocardial infarction, stroke and organ dysfunction [21–23].

The aim of the study was to investigate whether continuous lumbar plexus block can significantly reduce surgical stress response in comparison with other types of postoperative analgesia – continuous epidural analgesia and intravenous PCA with morphine.

Methods

This prospective, randomized study was conducted in 60 patients [American Society of Anesthesiologists [(ASA) physical status II-III)], with hip osteoarthritis, scheduled for unilateral total hip arthroplasty, after obtaining Ethical Committee approval.

No significant differences were found among the groups, regarding age, gender, body mass index, type of implant prosthesis, duration of surgery and postoperative blood loss in 24 h.

Before inclusion, written informed consent was obtained from each patient. Exclusion criteria were: known allergy to local anesthetics and opioids, diabetes, chronic use of corticosteroids and opioids, neurological disorders and contraindications to central or peripheral nerve block (local skin infections, coagulation disorders). The patients were randomized into 4 groups of 15 patients each: the CNB group (central nerve block - epidural), the PNB group (peripheral nerve block - lumbar plexus block), the SAM group (spinal anesthesia + IV morphine) and the GAMa group (general anesthesia + IV morphine).

All patients received midazolam 0.03 mg/kg i.v. 20 min before planned surgery. Preoperatively, in the CNB group, epidural space was identified with normal saline, using an 18G epidural needle. Thereafter, the epidural catheter 20G (Braun, Meslungen, Germany) was inserted. Bolus of 3 mL chirocaine 0.5%, and fentanyl 50 μg was administered via epidural catheter before anesthesia induction. Intraoperatively, boluses of 5 mL levobupivacaine 0.5% were added on a regular basis, every 30 min. Anesthetic induction was performed using propofol 2 mg/kg, fentanyl 100 μg and rocuronium 0.6 mg/kg. Following endotracheal intubation, anesthesia was maintained using sevoflurane 1–2% in a 50%/50% mixture of oxygen and nitrous oxide (N_2O). Postoperative analgesia was maintained via epidural catheter during the first 24 h, by continuous infusion of a mixture - 0.1% levobupivacaine and fentanyl 2 μg/mL, 8–15 mL/h.

In the PNB group, lumbar plexus was identified by the nerve stimulator according to Capdevila's approach [24], using a 15 cm long needle for peripheral block. Contractions of quadriceps muscle ("dancing patella sign") were obtained using an initial current of 1–2 mA. After twitches were observed, the current was reduced to 0.5 mA. The peripheral catheter (Braun, Meslungen, Germany) was inserted into psoas compartment where lumbar plexus is situated. A total of 20 mL levobupivacaine 0.25% was administered. Following the catheter insertion, general anesthesia was performed in the same way as in the group CNB. Postoperative analgesia was maintained via the peripheral catheter during the first 24 h, by continuous infusion of 0.25% levobupivacaine, 5–10 mL/h. Initial titration was performed postoperatively, using a 10 cm visual analog scale (VAS) and providing pain score lower than 3 cm.

In the GAM group, all patients received general anesthesia, using the same protocol as it was used for the patients from the CNB and PNB group. Average duration of general anesthesia was 135 ± 52 min and did not differ significantly among the groups (CNB, PNB and GAM). Preoperatively, the patients included into the SAM and GAM group were informed about postoperative pain management using the PCA devices. In a recovery room, the patients from the SAM and GAM groups received initial intravenous boluses of morphine hydrochloride (5 mg doses at 5 min intervals), titrated manually until their pain score was lower than 3 on a 10 cm VAS. Thereafter, PCA was initiated. The PCA pump (μSP 6000, Arcomed ga, Switzerland) was connected and delivered 1 mg doses of morphine i.v. with a 7 min lockout period and a maximum dose of 20 mg over 4 h.

After surgery, the patients from all groups were transferred to the post-anesthesia care unit (PACU), and after a two-hour observation period to the orthopedic ward.

In the SAM group, all the patients received spinal anesthesia in sitting position, using 25G, 88 mm Quincke tip needles (Braun, Meslungen, Germany). A total of 12.5 – 17.5 mg of hyperbaric bupivacaine 0.5% was administered into subarachnodal space at L3-4 spinous level. Postoperative analgesia was administered intravenously using the PCA pump.

All operations were performed in the morning, taking into consideration circadian rhythm of hormone release. Serum levels of cortisol, thyroid hormones (T3, T4) and TSH, insulin, glucose and CRP were measured in all groups preoperatively, as well as 4 h, 12 h, and 24 h after surgery. Electrochemiluminescence immunoassay was used to determine serum concentrations of cortisol, thyroid hormones

(T3, T4) TSH and insulin. To estimate blood glucose levels, glucose oxidase enzymatic method was used. The CRP levels were determined by immunoturbidimetric assay. Non-invasive intermittent blood pressure monitoring was performed intraoperatively using measurements at 5 minute intervals. Postoperatively, arterial blood pressure was measured hourly. Episodes of hypotension were recorded if arterial pressure was below 100/70 mmHg.

Statistics

The methods of descriptive statistics were applied. The numerical variables were presented as: mean value, minimum, maximum, standard deviation, while the categorical ones as proportions (percentages). Dependence of the parameters in order to check the differences was analyzed using Pearson's $\chi 2$ test and Fisher's exact test. The differences were considered to be significant if $p < 0.05$.

Results

Data analysis showed that preoperative serum levels of cortisol did not differ significantly among the studied groups. Average value was within the normal range (around 18.0 μg/dL; F = 2.011; $p > 0.05$). However, 4 h after the operation, average values of serum cortisol level rose significantly, especially in the GAM group (F = 19.867; $p < 0.01$). The lowest serum levels of cortisol, still in normal range, were observed in the SAM group. The levels of serum cortisol in the PNB group were nearby the levels in the GAM group, whilst the values of cortisol in the CNB group were similar to the values from the SAM group.

Furthermore, 12 h after the operation, the average serum cortisol levels were significantly different among the groups. The highest values were observed in the GAM group, while the lowest were found in the CNB group (F = 8.050; $p < 0.01$). The serum levels of cortisol tended to decrease 12 h postoperatively when compared to values 4 h after the operation, except in the SAM group, where they were doubled.

Finally, 24h after the surgery, the serum cortisol levels returned to the preoperative values, except in the GAM group and this difference was significant (F = 3.894; $p < 0.05$) In the CNB group, the serum cortisol level was even lower compared with preoperative values, but still within the normal range (7–28 μg/dL) (Table 1).

The preoperative values of insulin in the serum did not show any significant difference among studied groups and the average value in each group was around 10 mLU/L; (F = 1.511; $p > 0.05$). After the operation, the serum levels of insulin were significantly changed. Four hours postoperatively, the highest serum insulin levels were detected in the CNB and PNB group, while the lowest ones were in the GAM group; (F = 5.811; $p < 0.05$). Twelve hours after the operation, the average serum levels of insulin showed significant differences among the groups, (F = 5.052; $p < 0.05$). The highest values were also observed in the CNB group, while the lowest one was found in the SAM group. The serum insulin levels in the PNB and GAM group were close to the levels in the CNB group. Twenty four hours after the surgery, there was a remarkable drop in the serum insulin levels in all groups. The CNB group had still the highest values, while the lowest ones were found in the SAM and GAM groups and this difference was statistically significant, (F = 6.394; $p < 0.05$) (Table 2). T3, T4 and TSH levels in the serum showed slight reduction 4 h, 12 h and 24 h postoperatively without statistical significance.

The preoperative values of blood glucose showed

Table 1

Serum cortisol levels in the CNB, PNB, SAM and GAM group preoperatively, and 4 h, 12 h and 24 h after the operation

Group	Mean serum cortisol level ± SD (μg/dL)				
	Preoperatively	4 h	12 h	24 h	Average †
CNB	18.70 ± 1.14	22.34 ± 2.21	19.17 ± 1.90	14.89 ± 1.26	18.77 ± 1.23
PNB	19.97 ± 1.51	29.94 ± 1.95	21.51 ± 1.43	19.10 ± 1.51	22.63 ± 1.60
SAM	15.83 ± 1.02	15.59 ± 0.99	33.39 ± 2.25	18.94 ± 1.41	20.93 ± 1.67
GAM	19.09 ± 1.26	33.66 ± 1.82	27.14 ± 2.77	22.03 ± 1.65	25.48 ± 1.92

†Average values of serum cortisol within the group; SD – standard deviation; CNB – central nerve block (epidural); PNB – peripheral nerve block (lumbar plexus block); SAM – spinal anesthesia + intravenous (i.v.) morphine; GAM – general anesthesia + i.v. morphine.

Table 2

Serum insulin levels in the CNB, PNB, SAM and GAM group preoperatively, and 4 h, 12 h and 24 h after the operation

Group	Mean serum insulin ± SD (mU/L)				
	Preoperatively	4 h	12 h	24 h	Average †
CNB	9.11 ± 4.84	17.08 ± 13.49	22.00 ± 18.38	19.04 ± 23.26	16.80 ± 13.22
PNB	12.36 ± 6.64	17.73 ± 7.17	20.35 ± 9.34	18.49 ± 10.42	17.23 ± 8.17
SAM	9.16 ± 4.51	11.21 ± 6.20	13.86 ± 4.30	12.04 ± 3.79	11.56 ± 5.11
GAM	9.27 ± 3.51	15.63 ± 9.14	20.06 ± 8.63	14.36 ± 5.59	14.83 ± 5.74

Note: For abbreviations see under Table 1.

normoglycemia, with similar results in all studied groups (around 5.5 mmol/L) ($F = 0.967$; $p > 0.05$) However, 4 h after the surgery, highly significant differences were found among the groups. The highest values were recorded in the GAM group, the lowest in the SAM group, while values in the CNB and PNB group were a bit higher than those from the SAM group; ($F = 10.084$; $p < 0.01$). On the other hand, 12 h after the operation significant rise in the blood glucose levels was detected in the SAM group, while these levels remained stable in the CNB and PNB group. The GAM group showed drop in the blood glucose levels; ($F = 7.186$; $p < 0.01$) Lowering of the blood glucose levels was noticed 24 h after the operation in all groups, with average values varied from 6.4–7.4 mmol/L, ($F = 2.246$; $p > 0.05$) (Table 3).

The CRP levels were found to be within normal range preoperatively in all groups, without significant differences. Postoperatively, 4 h after the operation, CRP showed slight increase in all groups. The level of CRP continued to increase remarkably in all groups 12 h after surgery. Twenty four hours postoperatively, the level of CRP became 10-20-fold higher, when compared with preoperative values, but without significant difference among the groups (Table 4).

The number of episodes of hypotension did not differ signifficantly among groups; 4 h ($\chi 2 = 3.057$; $p > 0.05$); 12 h ($\chi 2 = 2.243$; $p > 0.05$) and 24 h after the operation (0% in all groups) (Table 5).

and the mode of postoperative analgesia. The present study revealed that the markers for assessing surgical stress response were significantly lower if techniques of regional anesthesia and analgesia were performed.

Cortisol, also called "the stress hormone", has been extensively investigated in order to find the best choice of anesthesia and postoperative analgesia, capable of reducing the stress response. Some of intravenous anesthetics, such as propofol (1.5–2.5 mg/kg) may diminish release of cortisol after induction in anesthesia. Opioids (fentanyl and morphine) are also found to have significant influence on reduction of cortisol levels, but less than regional techniques [25]. Postoperative epidural analgesia is found to be extremely efficacious in prevention of increase in cortisol and catecholamine levels [26]. Our study confirmed that immediately after the operation, patients from the CNB, PNB and SAM group, where regional anesthesia techniques were performed, had significantly lower cortisol levels when compared to the GAM group. Especially in the SAM group, as long as spinal anesthesia lasted (around 4–5 h), cortisol levels were even lower than preoperatively. After recovering from spinal anesthesia, there was a sharp increase of the serum corisol levels. Values were even higher than the cortisol values in the GAM group, 12 h after the operation. Only if postoperative analgesia was continued using regional anesthesia techniques, significant increase in the cortisol levels was

Table 3

Serum glucose levels in the CNB, PNB, SAM and GAM group preoperatively, and 4 h, 12 h and 24 h after the operation

Group	Mean serum glucose levels ± SD (mmol/L)				
	Preoperatively	4 h	12 h	24 h	Average †
CNB	5.61 ± 0.63	7.50 ± 1.78	6.74 ± 1.24	6.60 ± 1.03	6.61 ± 1.14
PNB	5.33 ± 0.48	8.08 ± 1.24	7.08 ± 0.85	6.32 ± 0.62	6.70 ± 0.72
SAM	5.24 ± 0.60	6.49 ± 0.86	9.65 ± 2.61	6.85 ± 0.62	7.05 ± 1.14
GAM	5.38 ± 0.75	10.44 ± 3.36	8.45 ± 2.42	7.38 ± 1.90	7.91 ± 2.08

Note: For abbreviations see under Table 1.

Table 4

CRP levels in the CNB, PNB, SAM and GAM group preoperatively, and 4 h, 12 h and 24 h after the operation

Group	Mean CRP values ± SD (mg/L)				
	Preoperatively	4 h	12 h	24 h	Average †
CNB	2.65 ± 2.30	3.08 ± 3.22	14.85 ± 8.67	81.45 ± 38.34	25.50 ± 12.84
PNB	2.88 ± 1.38	2.82 ± 2.53	15.46 ± 8.57	78.72 ± 26.63	24.97 ± 10.09
SAM	4.57 ± 1.60	5.66 ± 1.80	21.28 ± 11.21	94.19 ± 34.88	31.42 ± 11.42
GAM	3.65 ± 2.20	4.39 ± 3.80	19.10 ± 8.80	84.92 ± 21.39	28.01 ± 9.17

CRP – C reactive protein.
Note: For other abbreviations see under Table 1.

Table 5

Incidence of hypotension 4 h and 12 h after the hip replacement

Group	Number (%) of patients with postoperative hypotension	
	4 h	12 h
CNB	5 (33.33)	3 (20)
PNB	3 (20)	1 (6.67)
SAM	1 (6.67)	1 (6.67)
GAM	1 (6.67)	0 (0)

Discussion

Surgical stress response depends on type of surgery and can be modulated by anesthetic technique intraoperatively

prevented (the CNB and PNB group). Rodgers at al. [27] in an extensive meta-analysis showed reduction in overall postoperative mortality in surgical patients undergone regional anesthesia in comparison to general anesthesia. They found out

reduction in mortality rate of 30% in the regional anesthesia group, due to pulmonary embolism, pneumonia, stroke, wound infections and cardiac events. The great majority of mortality causes were supposed to be connected to inability to diminish surgical stress response. When spinal and epidural anesthesia were compared, the differences in mortality were inconclusive. Mortality rate was low, whether spinal or epidural anesthesia was continued postoperatively or not. Kehlet et al. [21] recently confirmed that a lack of properly designed prospective studies, which compared modern general and regional anesthesia for hip and knee arthroplasty, enabled setting of recommendations and the anesthesia protocols for this type of surgery.

Donatelli et al. [28] investigated insulin resistance in 60 patients underwent hip and knee arthroplasty under epidural anesthesia/postoperative analgesia or general anesthesia followed by intravenous PCA. The insulin resistance was estimated using the homeostatic model assessment (HOMA) score. HOMA score was calculated in the following way - fasting insulin (microU/mL) x fasting glucose (mmol/L)/22.5. The authors did not find significant difference in insulin resistance in any group of patients who were not previously insulin resistant. We found significant differences in the serum insulin levels among our studied groups 4 h and 12 h postoperatively, with the highest insulin concentration in the CNB and PNB group. Despite the elevation of serum insulin concentrations, the blood glucose levels were above normal range in all groups, confirming the theory that peripheral utilization of insulin is impaired after normalization of insulin secretion. According to our results, neuraxial block (epidural and spinal anesthesia) can positively influence insulin secretion, but does not affect the insulin resistance, which was present in all studied groups.

Hyperglycemia induced by the insulin resistance may influence a surgical outcome after hip replacement significantly, leading to a higher risk of wound infections, sepsis and organ failure [29–31]. In spite of the large body of evidence that has confirmed negative effects of the insulin resistance following surgery, Hahn and Ljunggren [32] found out reduction in number of early side effects after hip replacement under spinal anesthesia in non-diabetic patients, estimated as the insulin resistant preoperatively, using the oral glucose tolerance test (OGTT). Therefore, episodes of nausea, vomiting and hypotensive events (systolic arterial pressure < 80 mmHg) were recorded. The patients with the preoperative insulin resistance were found to have significantly lower number of early side effects – nausea and vomiting ($p < 0.04$) and hypotension ($p < 0.05$). Our study showed higher number of hypotensive episodes (systolic arterial pressure < 100 mmHg) in the patients with spinal and epidural anesthesia. The highest percentage of episodes of hypotension was recorded in the CNB group, 4 h postoperatively (33.3%) and 12h postoperatively (20%). Hypotension episodes, recorded 12 h after the operation in the CNB group, were more likely associated to the sympathetic blockade than to lack of insulin resistance.

Our study showed similar results with other authors, confirming the slight reduction in thyroid hormones and TSH in the early postoperative period (up to 24 h) without significance among the groups [33].

Impaired glucose metabolism after hip replacement is strongly associated with wound infection, cardiac events and thromboembolic complications [34]. Even mild changes in serum glucose concentrations perioperatively have been found to influence postoperative complications significantly, especially in elderly population [35–37]. Therefore, the appropriate choice of anesthetic technique, could have positive effects on glucoregulation and, consequently, number of complications, by reduction of stress response. According to recommendations of American Diabetes Association from 2015, values 7.8–10 mmol/L are considered as hyperglycemia. If values rise above 10 mmol/L, antihyperglycemic treatment is needed, because higher blood glucose values are connected with the higher number of postoperative complications [38]. Lattermann et al. [9] investigated the differences in intraoperative and postoperative plasma glucose concentrations in patients scheduled for hip replacement after combined spinal-epidural (CSE) or general anesthesia. Postoperative analgesia was maintained in the CSE group via the epidural catheter. General anesthesia was followed by intravenous PCA with opioids. Plasma glucose concentrations were significantly lower in the CSE group intraoperatively and immediately postoperatively ($p < 0.05$). On the first postoperative day, values were similar in both groups. Recent study of Gottschalk et al. [39] confirmed that the blood glucose levels were significantly lower immediately after surgery and 1 hour after surgery in non-diabetic patients following hip replacement, when spinal anesthesia was performed in comparison to general anesthesia ($p < 0.05$).

The present study showed that groups where regional anesthesia was performed and postoperative analgesia was maintained via peripheral or epidural catheter, had superior glycemic control. Ceasing regional anesthesia postoperatively, as it was in the SAM group, resulted in sharp rise in glucose levels, 12 h after the operation. These values were even higher in comparison to the GAM group. Furthermore, our study also confirmed results of Lattermann et al. [39] on the first postoperative day (24 h postoperatively). According to our findings, there were not significant differences in the blood glucose levels, 24 h after the operation, among the studied groups.

Study of Carli [40] showed that the treatment of postoperative pain was closely connected to stress induced hormonal release and represented an important factor in postoperative glucose metabolism and the insulin resistance. The present study confirmed that both the blood glucose levels and insulin levels were significantly lower in groups where anesthesia was maintained continuously in the postoperative period via epidural or peripheral catheter (the CNB and PNB group).

The scientific evidence, which confirmed influence of an anesthesia technique on inflammatory response after hip arthroplasty, has been inconclusive. Larsson et al. [41] found out significant rise in the CRP levels after elective orthopedic surgery (hip and knee arthroplasty and microdiscectomy) without influence of an anesthetic technique among other

possible causes such as - bleeding, transfusion and operation time. On the other hand, Bagry et al. [42] showed that the CRP and leukocyte count were lower if continuous peripheral nerve block was performed after knee arthroplasty in comparison to postoperative PCA with morphine ($p < 0.05$).

Chloropoulou et al. [43] showed that epidural anesthesia followed by epidural analgesia produced less inflammatory response in comparison to spinal anesthesia followed by intravenous morphine analgesia in the patients with total knee arthroplasty. The blood samples were collected preoperatively, immediately after the operation and 24 h postoperatively. They concluded that some novel markers, especially leukocyte activation molecules CD11b and CD62l showed more sensitivity in comparison with CRP and interleukins. Present study is in line with the findings of Larsson et al. [41], showing continuous rise in the CRP levels in all groups, independent of a type of intraoperative anesthesia or postoperative analgesia.

Conclusion

Administration of postoperative analgesia using continuous lumbar plexus block following hip arthroplasty reduces significantly stress response in comparison to postoperative PCA analgesia with morphine and has comparable effects on hormone release to epidural analgesia. Spinal anesthesia provides the best decrease of surgical stress response in comparison with the other types of intraoperative analgesia. In the postoperative period, after recovery from spinal anesthesia, the stress response cannot be attenuated successfully using patient controlled analgesia with morphine.

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CASE REPORTS

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Emphysematous pyelonephritis – case report and review of literature

Emfizematozni pijelonefritis

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Abstract

Introduction. Emphysematous pyelonephritis (EPN) is a severe, potentially fatal necrotizing infection of the kidney with the clinical picture ranging from the mild abdominal pain and discomfort to the septic shock and multiorgan failure. We presented here a case of EPN in a poorly controlled diabetic patient that was the first registered case of EPN in our clinic for more than ten years. **Case report.** A 78-year-old diabetic male patient was referred to the Clinic for Nephrology and Clinical Immunology of the Clinical Center of Vojvodina, Novi Sad, Serbia, with weakness, malaise, abdominal discomfort and reduced daily urine volume. After complete physical exam, laboratory work up, echosonographic and computed tomography scanning we diagnosed the patient with EPN class IV, according to the Huang and Tseng classification, with the presence of 5 risk factors for mortality (systolic blood pressure below 90 mmHg, altered consciousness, thrombocytopenia, elevated serum creatinine level, bilateral disease). Treatment with conservative therapy and percutaneous drainage was not successful, further deteorioration of the patient status ensued so the patient passed away on the 8th day of hospitalization due to the development of septic shock with multiorgan failure that was refractory to all measures that were instituted. **Conclusion.** EPN is a severe, potentially fatal necrotizing inflammation of the kidney and surrounding tissue. Management and prognosis of this disease depends on the clinical status, risk factors and radiological classification of the disease.

Key words:
pyelonephritis; emphysema; diabetes mellitus, type 1; drainage; treatment outcome.

Apstrakt

Uvod. Emfizematozni pijelonefritis je teška, potencijalno fatalna, nekrotizirajuća infekcija bubrega sa kliničkom slikom koja može varirati od blagih abdominalnih bolova do septičkog šoka sa multiorganskom insuficijencijom. Prikazan je slučaj emfizematoznog pijelonefritisa kod višegodišnjeg dijabetičara, što je prvi registrovan slučaj ovog oboljenja na našoj klinici u periodu dužem od deset godina. **Prikaz bolesnika.** Muškarac star 78 godina, sa višegodišnjim, loše regulisanim dijabetesom, hospitalizovan je na Klinici za nefrologiju i kliničku imunologiju Kliničkog centra Vojvodine u Novom Sadu, sa tegobama u vidu slabosti, malaksalosti, bolova u stomaku i smanjene količine mokraće. Nakon fizikalnog pregleda, laboratorijskih analiza, ehosonografskog pregleda i kompjuterizovane tomografije abdomena postavljena je dijagnoza emfizematoznog pijelonefritisa klase IV po klasifikaciji Huanga i Tsenga. Kod prikazanog bolesnika verifikovano je postojanje pet faktora loše prognoze (sistolni krvni pritisak ispod 90 mmHg, poremećaj svesti, trombocitopenija, povišen nivo serumskog kreatinina, bilateralna bolest). Primenjena konzervativna terapija i perkutana drenaža nisu rezultirale povoljnim ishodom i došlo je do daljeg pogoršanja stanja bolesnika. Bolesnik je preminuo osmog dana hospitalizacije usled razvoja septičkog šoka i multiorganske insuficijencije koja je bila refrakterna na sve primenjene mere. **Zaključak.** Emfizematozni pijelonefritis predstavlja tešku, potencijalno fatalnu nekrotizujuću inflamaciju bubrega i okolnog tkiva. Terapija i prognoza ove bolesti zavise od kliničkog statusa, prisutnih faktora rizika i radiološke klasifikacije bolesti.

Ključne reči:
pijelonefritis; emfizem; dijabetes melitus, insulin zavisni; drenaža; lečenje, ishod.

Introduction

The first case of kidney infection with gas accumulation in kidneys and the surrounding tissues was described by Kelly and MacCallum [1] in 1898. The term emphysematous pyelonephritis (EPN) was introduced in clinical practice in 1962, when Schultz and Klorfein [2] used it to point to the connection between kidney tissue infection and formation of gas accumulation.

EPN is a severe, potentially fatal necrotizing kidney infection with the clinical picture ranging from the mild abdo-

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minal pain and discomfort to septic shock and multiorgan failure. Most cases of EPN occur in poorly controlled diabetic patients, while fewer cases of these potentially fatal infections occur in patients with urinary tract obstruction or some other malformation of the kidneys, like polycystic kidneys [3, 4]. Treatment of EPN consists of a combination of conservative measures among which the most important is an antibiotic therapy, and invasive, surgical procedures ranging from percutaneous drainage to nephrectomy. We presented a case of EPN in a patient with poorly controlled diabetes. It was the first registered case of EPN in our clinic for more than ten years.

Case report

A male patient, aged 78 years, was admitted to the Clinic for Nephrology and Clinical Immunology of Clinical Center of Vojvodina, Novi Sad, Serbia, with complaints of fatigue, malaise, abdominal discomfort and reduced daily urine volume. The symptoms began two days prior to admission, and worsened on the day of admission. The patient had diabetes for decades and had been receiving insulin therapy for the past 6 years. He had a poorly controlled primary illness with the hemoglobin A1c (HbA1c) values of 10.2%. The patient`s vital signs showed normal body temperature (36.9 Cº), sinus tachycardia of 110 beats per minute (bpm), arterial hypotension with blood pressure of 90/60 mmHg and number of respirations of 21/min. Physical examination found extensive pain on succusion of left lumbar region as well as on palpation of left paracolic region. The patient`s general condition was poor, he was somnolent, with Karnofsky performance status of 30, Glasgow Coma Scale of 12, and he was oligoanuric in the previous 12 hours.

Laboratory analyses done upon admission showed the existence of leukocytosis with leukocytes measuring 21.8 × 10^9/L [normal range (nr) 4.5–10.0 × 10^9/L] and 83% of granulocytes (nr 50–70%), thrombocytopenia measuring 42 × 10^9/L (nr 150–450 × 10^9/L), C-reactive protein level was 224 mg/L (nr < 10 mg/L), serum procalcitonin level was 84.52 pg/mL (nr less than 50 pg/mL), as well as elevated serum creatinine level measuring 418 μmol/L (nr 70–120 nmol/L) and elevated blood urea level measuring 28.3 mmol/L (nr 2.5–7.1 nmol/L). Urine analysis showed the presence of a pyuria and bacteriuria, and the urine sample was sent for a microbiological analyses. Echosonography of the abdomen found suspected presence of gas accumulation on both kidneys, but predominantly on the left side with the presence of free fluid in the perisplenic and hepatorenal recessus. The patient was hospitalized and started on a dual antibiotic therapy of ciprofloxacin and ceftazidime, with other supportive measures. Considering the high serum levels of urea and creatinine as well as oligoanuria, a dual lumen dialysis catheter was inserted into the inner jugular vein on the patient`s right side. This was followed by a computed tomography (CT) scan of the abdomen which confirmed the presence of extensive gas accumulation in the left kidney parenchyma, calyceal system on the same side as well as in the left pararenal space. Gas collection was found in a right kidney as well, but the collection was limited to the parenchyma (Figure 1).

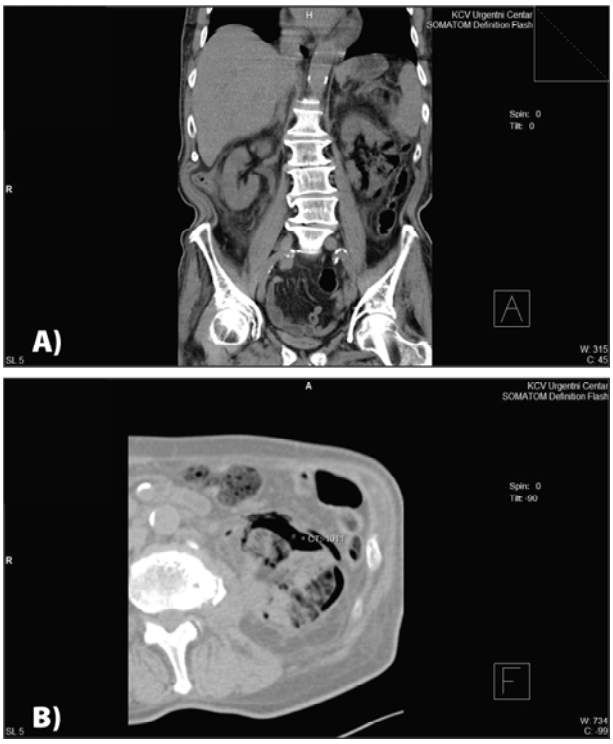

Fig. 1 – Computed tomography (CT) scan of abdomen: A) longituidinal view, showing minimal gas accumulation in the right kidney and extensive gas accumulation in the left kidney, collecting system on the left side and in the left pararenal tissue; B) transversal view, showing extensive gas accumulations in the left kidney, collecting system on the left side and in the left pararenal tissue.

Based to the CT findings and clinical status of the patient, left side lumbotomy with the intention of performing nephrectomy, was performed on the second day of hospitalization. Due to the patient's extremely poor condition accompanied by hypotension and profuse bleeding caused by thrombocytopenia, nephrectomy was abandoned, and only percutaneous drainage of left pararenal space was performed. As the patient's condition continued to deteriorate, he was transferred to an Intensive Care Unit. Measures of advanced life support, assisted ventilation and supportive measures among which infusion of platelets, crystalloid solutions and plasma expanders as well as positive inotropic drugs were instituted. On the third day of hospitalization and upon receipt of the results of the urine cultures which showed the presence of *Escherichia coli* resistant to then available antibiotics, piperacilin-tazobactam was introduced in the therapy. On a sixth day of hospitalization the patient started having diuresis in a range of 500–1,000 mL, but we continually performed renal replacement therapy (RRT) with continuous veno-venous hemodiafiltration (CVVHDF), due to the inadequate kidney function and volume status of the patient. In the course of further treatment, the patient`s status deteriorated and he developed septic shock with multiorgan failure, refractory to therapeutic measures, which caused a fatal outcome on the 8th day of hospitalization.

Discussion

EPN is a necrotizing infection of the kidney and surounding tissue where gas is formed in kidney parenchyma, collecting system and perinephric tissue. More than 90% of cases of EPN occur in patients with poorly controlled diabetes [5]. Other predisposing factors for EPN are urinary tract obstruction, polycystic kidney disease, immunosuppresive therapy and end stage renal disease [3, 4]. This disease has a higher predilection for a female patients [4], which is in accordance with the higher incidence of urinary tract infections in females, in general.

The pathogenesis of EPN depends on several factors which include the existence of bacteria capable of producing gas, high level of tissue glucose favorable for the fast growth of bacteria, disruption in tissue perfusion and inadequate immune response of the patient due to disrupted vascularization. The most frequent causes of EPN are *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* and *Pseudomonas aeruginosa* [6, 7], the same bacteria that most frequently causes urinary infections in general. Anaerobic bacteria very rarely cause EPN. The gas is formed because of the fermentation of glucose and lactate which produces large amounts of carbon dioxide and hydrogen, which are then accumulated in places where inflammation occurred. Clinical manifestations of EPN are similar to cases of "ordinary" pyelonephritis, most frequently accompanied by elevated body temperature and pain in the loins and abdomen. Depending on the severity of the clinical picture, nausea and vomiting may occur, as well as different levels of altered state of consciousness. Laboratory analyses are dominated by leukocytosis with neutrophilia, pyuria and parameters pointing to an infection such as elevated levels of C-reactive protein, erythrocyte sedimentation rate and procalcitonin level. Impaired kidney function may occur, or an existing poor kidney function may deteriorate.

A number of different methods are available for the visualization of the changes in kidney parenchyma. Although echosonography is not a preferred method of proving the presence of gas accumulation in kidneys and surrounding tissue, it is used to initially examine the upper urinary tract if an infection is suspected. It can, to a large extent, confirm or rule out the existence of a urinary tract obstruction. CT is the method of choice for diagnosing EPN [3, 6]. There are several staging systems based on the radiological findings that can have a great significance in the prognosis and treatment of EPN [3, 8, 9]. According to the classification by Huang and Tseng [3] (Table 1), which is the one most widely in use, the patient treated at our clinic had class IV EPN. Aside from the radiological classification of EPN, other important prognostic factors, i.e. risk factors, that are associated with outcome of EPN are systolic blood pressure below 90 mmHg, altered state of consciousness and initially elevated sera creatinine level (> 221 μmol/L or 2.5 mg/dL). In addition, the presence of thrombocytopenia (< 100×10^9/L) and the existence of bilateral EPN also lead to a less favorable outcome [5].

The treatment of EPN depends on the evaluation of the patient's general condition and the morphological report based on the CT scan. A decision on the treatment of each individual patient is made on the basis of evaluation of the condition including risk factors for a less favorable prognosis and the outcome of the illness, as well as on morphological diagnostics by Huang and Tseng [3]. Conservative treatment with antibiotics and measures aimed at stabilizing the patient is justified in all cases [10]. It is combined with surgical procedures ranging from percutaneous drainage [3, 7, 11, 12] to nephron-sparing surgery [13] and nephrectomy [14], depending on the patient's condition and the extent of changes visualized by CT scan. One of the first study on EPN, found an average mortality rate of 31% in those patients. Mortality rate was as high as 80% for patients who received conservative therapy only, 60% for patients treated with percutaneous drainage, and 20% in nephrectomized patients [15]. Shokeir et al. [4] registered a mortality rate of 20% in their series of nephrectomized patients. Procedures that are less invasive and have a goal to preserve kidney mass are justifiable, but only for a less extensive cases of EPN. In a meta-analysis of the different treatment strategies, the most successful one was combination of medical therapy and percutaneous drainage, with the mortality rate of around 13% [12]. In cases where advanced changes are evidenced by a CT scan (class III and IV by Huang and Tseng [3]), and especially if there are two or more risk factors, nephrectomy is recommended as the combination of conservative therapy and percutaneous drainage does not lead to very good outcomes, resulting in mortality in over 92% of patients [7].

Table 1

Classification system for emphysematous pyelonephritis (EPN) by Huang and Tseng [3]

Radiological test	Class of the disease
Computed tomography	I: Gas in collecting system only
	II: Parenchymal gas only, without extension to extrarenal space
	III: Extension of gas or abscess to pararenal space
	IV: EPN in solitary kidney or bilateral disease

Conclusion

EPN is a very serious necrotizing infection of the kidneys and the surrounding tissue which, in a number of cases, can result in a fatal outcome. This is especially true in cases of extensive gas accumulation in the kidney and surrounding tissue, as well as when two or more factors of unfavorable prognosis are present. Therapy for EPN consists of combination of conservative measures and surgery, and the decision for the every patient is individual, based upon radiological findings and clinical status.

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CASE REPORT

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Ultrasound in diagnosis of nontraumatic lower extremity pain syndromes: A case report

Uloga ultrazvuka u dijagnostici bolnog sindroma donjeg ekstremiteta

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Abstract

Introduction. Musculoskeletal impairment is the most prevalent impairment in people over 65. The spine involvement is the most frequently reported. However, common regional pain syndromes usually arise from undefined mechanical, musculoskeletal or soft tissue disturbances. Use of imaging methods is becoming a mandatory tool in the clinical practice in order to achieve the prompt and accurate disease definition. **Case report.** The Caucasian 79-year-old-male patient attended with the history of an acute, piercing pain along the postero-medial side of the right Achilles tendon and the medial side of the right sole. According to the spine magnetic resonance imaging, X-rays and electrodiagnostic studies, diagnoses of the radicular, neurogenic pain and plantar fasciitis was suspected. However, the popliteal groove ultrasound assessment revealed the presence of hypertrophied semimembranosus-gastrocnemius bursa, compressing the popliteal neurovascular bundle. **Conclusion.** In patients with a symptomatology of peripheral neuropathy, use of imaging techniques such as ultrasound may be essential for accomplishing patients' diagnostic approach.

Key words:
musculosceletal pain; knee; osteoarthritis; diagnosis; ultrasonography; synovial cyst.

Apstrakt

Uvod. Degenerativna oboljenja muskuloskeletnog sistema su najčešća oboljenja kod ljudi starijih od 65 godina, posebno oboljenja međupršljenskog diska u lumbalnom regionu. Međutim, uobičajeni regionalni bolni sindromi obično nastaju kao posledica nedefinisanih mehaničkih, muskuloskeletnih ili mekotkivnih poremećaja. U cilju brzog i preciznog definisanja bolesti primena vizuelizacionih metoda postala je neophodna u svakodnevnoj kliničkoj praksi. **Prikaz bolesnika.** Muškarac star 79 godina, belac, prijavio se na ultrazvučni pregled donjeg ekstremiteta usled nesnosnog, oštrog bola koji se širio duž dorzo-medijalne strane desne Ahilove tetive i medijalne ivice desnog stopala. U skladu sa magnetnom rezonanacom lumbalne kičme, rendgenom kolena i stopala kao i rezultatima elektrodijagnostičkih ispitivanja, postavljena je dijagnoza plantarnog fasciitisa kao i dijagnoza degenerativnog oboljenja međuprškjenskog diska sa posledičnim radikularnim, neurogenim bolom i radijacijom u desni donji ekstremitet. Međutim, ultrazvučni pregled poplitealne jame ukazao je na prisustvo uvećane i dilatirane poplitealne ciste, koja je uzrokovala pritisak na poplitealni neurovaskularni splet uzrokujući navedene simptome. **Zaključak.** Upotreba ultrazvuka kod bolesnika sa simptomima periferne neuropatije može biti esencijalna za utvrđivanje uzroka neuropatije i postavljanje konačne dijagnoze.

Ključne reči:
bol, mišićno-skeletni; koleno; osteoartritis; dijagnoza; ultrasonografija; cista, sinovijalna.

Introduction

Degenerative spondylarthropathy includes a vast spectrum of symtomatology due to joints and their surrounding soft tissues involvement. Clinical course can last for many decades of profound disability and may be compatible with a variety of presentations.

Use of ultrasound (US) in the musculoskeletal system assessment has proved to be an accurate mean in the joint, ligament and synovial evaluation, so that calcifications, bone, tendon and ligament lesions may be assessed even when the X-ray exams are negative [1, 2]. Improvement of the US equipment has established the US technique as an indispensable tool in the clinical management of degenerative and inflammatory musculoskeletal system diseases [3–7]. Lately, there has been a huge progress in the assessment and management of peripheral nerves [8–15].

In this case presentation, the US evaluation will be decisive in the differential diagnosis and therefore in the following therapeutic choice.

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Case report

The Caucasian 78-year-old male patient attended with a history of an acute, piercing, devastating pain along the posteromedial side of the right Achilles tendon, particularly of its distal-third, the ankle, heel and the medial half of the same side sole. The most painful point was localized around the tip of the medial malleolus. Pain aggravation was observed during the midnight hours. Patient also reported the prolonged whole-body morning stiffness (an hour) without body-weight loss, general weakness or pyrexia.

No swelling, bruising, skin breaks, palpable prominences or defects in the heel and mid-foot region, were detected. No tenderness at the points of peroneal and tibial tendons' insertions was noticed either. The Lasègue's sign, crossed Lasègue's sign, Bell, hyperextension and the Cough tests were negative [16–18]. Heel pain was increased when the plantar fascia was stretched by ankle and toes' passive dorsiflexion. The pronation (active or passive) was extremely limited and painful. The heel pain was accompanied by neuropathic symptoms such as a tingling, burning and numbness, so the tarsal tunnel assessment was performed (nerve percussion) and the Tinel's sign proved positive.

During the last two decades the patient has been suffering from the bilateral knee osteoarthritis. Varus alignment, tenderness, swelling, crepitus and limited range of motion were detected bilaterally. Enlarged Baker cysts were palpated in popliteal grooves.

Blood tests indicative of systemic inflammation (blood sedimentation rate and C-reactive protein) as well as the rest of biochemical markers were in the normal range.

Nerve conduction velocities and electromyography (EMG) indicated a radiculopathy due to pathological involvement of lower lumbar roots. Lumbosacral X-rays depicted severe, degenerative lumbar scoliosis. The scoliotic curve was associated with intervertebral osteochondrosis (vacuum phenomena, disc space narrowing) and large bone spurs at the vertebral epiphyseal edges and facet joints. Magnetic resonance imaging (MRI) scan showed multiple herniated discs.

Ankles and knee X-rays were performed and the both side calcaneal spur as well as a severe knee osteoarthritis (OA), grade 4 – according to Kellgren and Lawrence [19] were detected.

Musculoskeletal US examination of involved joints and periarticular soft tissues was conducted, by using a standardized scanning technique and international definitions of pathology [20, 21] [Ultrasound Subcommittee of the European Society of Musculoskeletal Radiology [22] and the European League Against Rheumatism (EULAR) guidelines [20]]. A General Electric Logiq P6 Pro machine, equipped with a 10–13 MHz linear transducer was used, depth was adjusted depending on the joint size just at the point of interest, gain was set at the 55–65%; high power Doppler (PD) frequency was used (6.7 MHz), pulse-repetition frequency (PRF) was set to 500 Hz, gain was set just below the level at which colour noise appeared below the bone. Colour priority was maximised and the wall filter was decreased to minimum 60–80 Hz.

Medial and anterolatrel knee scans revealed typical OA findings (Figure 1).

Posterior left knee scans revealed enlarge hypertrophied both-side Baker cyst (Figure 2).

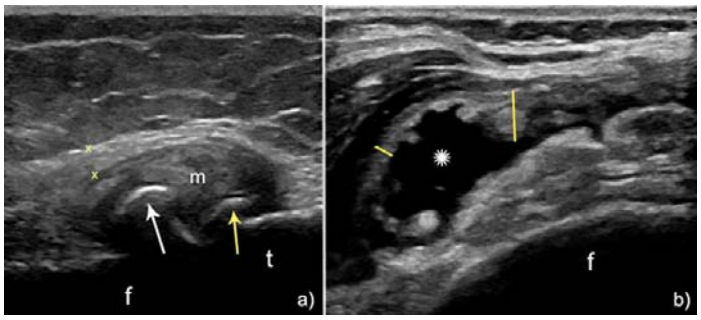

Fig. 1 – Right knee scans – a) Medial longitudinal scan; b) Transverse scan of lateral recess: m – medial meniscus – hypo/hyperechoic image of bulged medial meniscus base; f – femur; t – tibia – border points of medial collateral ligament; white arrow – enlarged femoral osteophyte; yellow arrow – enlarged tibial osteophyte; asterisk – presence of fluid inside the recess; yellow line – cross section of the hypertrophied synovial layer.

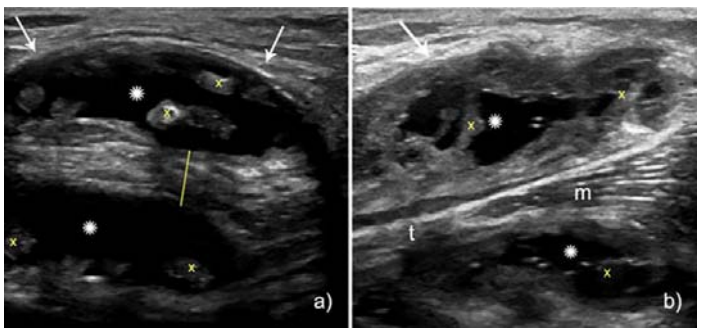

Fig. 2 – Left Baker-cyst scans: a) Posterior transverse scan; b) Posterior longitudinal scan: asterisk – cyst cavity – hypertrophied synovial tissue; yellow line – cross section of the large cyst septum; white arrow – Backer cyst wall; t – tendon of the medial head of the gastrocnemius muscle; m – muscular part of the medial head of the gastrocnemius muscle.

The right Baker cyst was compressing the popliteal neurovascular bundle (Figure 3). The compressed and dislocated tibial nerve appeared swollen and surrounded by an incomplete-anechoic hallo due to the presence of fluid between the nerve and its epineurium (Figures 4 and 5).

Medial longitudinal plantar scans revealed the large, both side calcaneal spur, accompanied by a respective plantar fasciitis (Figure 6).

US guided aspiration of the right Baker cyst was performed and 40 mL of serous fluid was removed. The fluid

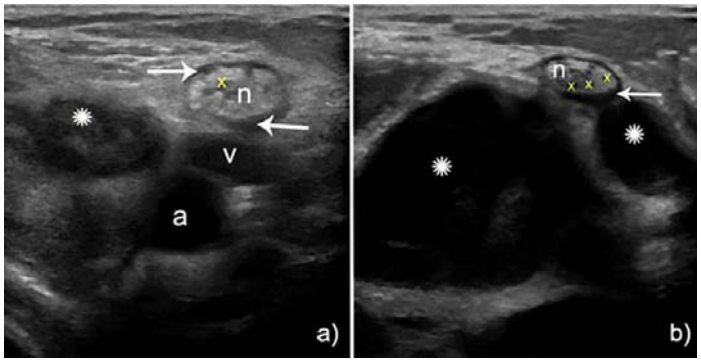

Fig. 3 – Right Baker-cyst transverse scans: a) Proximal part, b) Distal part (asterisk – cyst cavity with a cyst's hypertrophied synovial tissue: a – popliteal artery; v – popliteal vein; n – tibial nerve – hypoechoic, swollen nerve fascicles; white arrow – anechoic space in-between the epineurium and the nerve fascicles).

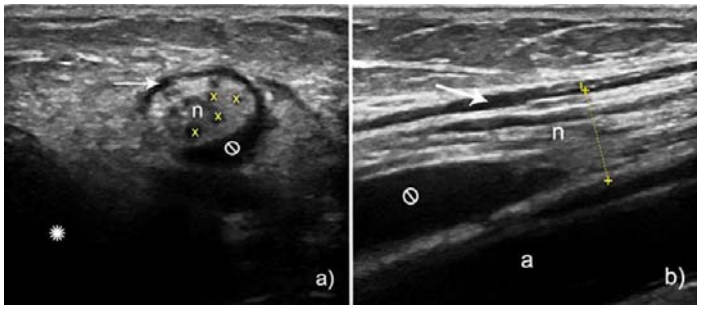

Fig. 4 – Right tibial nerve scans – proximal part: a) Transverse scan, b) Longitudinal scan: asterisk – Backer cyst cavity; n – tibial nerve – hypoechoic swollen nerve fascicles; white arrow – anechoic space in-between the epineurium and the nerve fascicles; circle – huge anechoic space underneath the epineurium (longitudinally spindle shaped).

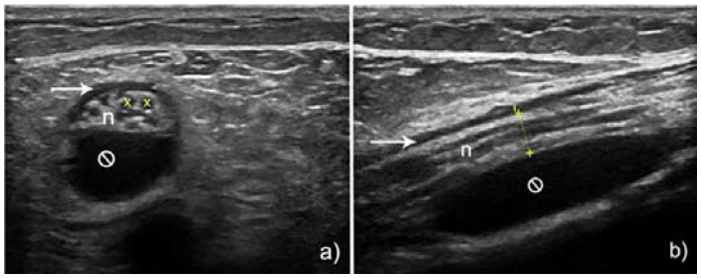

Fig. 5 – Right tibial nerve scans – distal part: a) Transverse scan, b) Longitudinal scan: asterisk – Backer cyst cavity; n – tibial nerve – hypoechoic swollen nerve fascicles; white arrow – anechoic space in-between the epineurium and the nerve fascicles; circle – anechoic space underneath the epineurium.

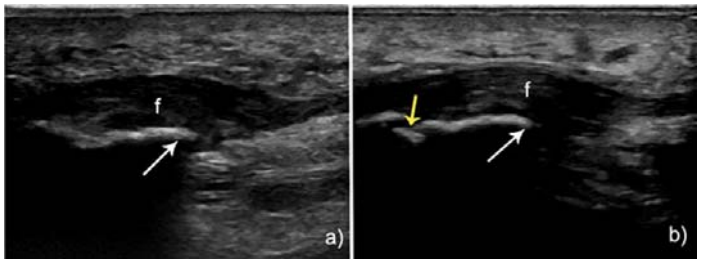

Fig. 6 – Calcaneal spurs and respective plantar fasciitis – plantar longitudinal scans: a) left; b) right: f – enlarged, swollen, hypoechoic plantar fascia (mainly the left side one); white arrow – calcaneal spur; yellow arrow – large depression (erosion) over the plantar aspect of the calcaneal tubercle.

laboratory tests were compatible with common inflammatory fluids (low viscosity, low white cell count/mm^3 and low percent of polymorphonuclear cells). No glucose, rheumatoid-factor or antinuclear-antibody was detected either. Betamethasone (10 mg) was injected within the bursal cavity and the symptoms resolved. While the similar signs and symptomatology reappeared 12 days later, the cyst aspiration was performed again. Four aspirations were necessary for the patient's full recovery. At patient's last visit, the right knee US evaluation revealed no fluid within the hypertrophied Baker cyst and the tibial nerve appeared normal. Ultimately, the patient was reported for the total right knee arthroplasty.

Discussion

Many different conditions can cause low-back and lower-extremity pain. While patients may have more than one disorder, it is necessary to undergo further testing to confirm or rule out these diagnoses. There is no gold standard for the diagnosis of degenerative spondylarthropathy and radiculopathy, so the combination of history, physical examination, imaging and EMG is used to obtain the diagnosis. The lack of the EMG sensitivity is its biggest limitation: though positive, the EMG is unable to ascertain the exact level of the involved root.

The peripheral nerve compression can occur acutely or chronically. In acute compression, the distal nerve portion retains normal function which tends to resolve after a decompression. Prolonged ischemia may cause significant damage of the myelin-sheath and/or axonal degeneration due to the intraneural microvascular supply and venous congestion that may produce endoneural edema. Increased fluid pressure in between the fascicles induces the microcompartment syndrome, producing the clinical image of the nerve impairment [23].

Thus, taking into account the patient's symptoms, blood tests, MRI, EMG and X-rays findings, the patient had been assumed as a patient with a lumbal radiculopathy and heel spur symptomatology correlated with plantar fasciitis. However, the cause of his symptomatology was clarified by means of the US-imaging.

US seems to be powerful weapon for clinicians, more sensitive and accurate method comparatively to the clinical examination. It is also relatively cheap, bed-side, not time-consuming and with no any radiation technique. The accuracy of the US-guided aspirations and local injections is also its great advantage [24–29].

The US is an efficient, appropriate and quick technique for diagnostic and treatment purposes (aspirations, injections) [30, 31] as well as for the patients' follow-up. Specifically, in patients with a symptomatology of peripheral neuropathy, use of imaging techniques such as ultrasound may be essential for patients' diagnostic accomplishment [8–15].

Conclusion

In patients with a symptomatology of peripheral neuropathy, use of imaging techniques such as ultrasound may be essential for accomplishing patients' diagnostic approach.

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Conflict of interest

The authors declare that they have no conflict of interest.

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CASE REPORT

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Preoperative misdiagnosed gastrointestinal stromal tumor surgical "transferred" into gastric duplication cyst

Preoperativno pogrešno dijagnostikovan gastrointestinalni stromalni tumor hirurški „preveden" u duplikacionu cistu želuca

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Abstract

Introduction. Gastrointestinal duplications are rare congenital anomalies, especially in the adult population which can occur along the entire gastrointestinal tract. The rarest among them are gastric duplications, making up 2–8% of all gastrointestinal duplications. Unusual embryonic malformations can be found even in the adult population and should always be considered in the differential diagnosis of thoraco-abdominal tumors. Unclear findings during preoperative diagnostic procedures are the reason for presenting the case. **Case report.** We present a 33 years old female, with tumorous lesion in the stomach wall, which had the endoscopic ultrasound features of gastrointestinal stromal tumor. During surgery, it was determined that the lesion was actually a cystic formation with gelatin content and histological examination confirmed the diagnosis of gastric duplication cyst. **Conclusion.** Preoperative diagnosis of gastric duplication cyst has always been a challenge for clinicians, since its morphological appearance may vary. That is the reason why these anomalies are often misdiagnosed as solid tumorous lesions by imaging methods and even by the most superior ones, and set up the surgery as a part of diagnostic algorithm.

Key words:
gastrointestinal stromal tumors; stomach; cysts; diagnosis, differential; digestive system surgical procedures; treatment outcome.

Apstrakt

Uvod. Gastrointestinalne duplikature su retke kongenitalne anomalije naročito u odrasloj populaciji koje mogu nastati duž čitavog gastrointestinalnog trakta. Najređe su želudačne duplikature koje čine 2–8% svih gastrointestinalnih duplikatura. Neobične embrionalne malformacije mogu se naći i u odrasloj populaciji i zbog toga bi uvek trebalo da budu razmotrene u diferencijalnoj dijagnozi torako-abdominalnih tumoroznih promena. Nejasni nalazi dobijeni tokom preoperativne dijagnostike su razlog prezentacije ovog slučaja. **Prikaz bolesnika.** Prikazali smo pacijentkinju, staru 33 godine sa tumoroznom promenom u zidu želuca koja je prema endoskopsko-ultrazvučnim karakteristikama izgledala kao gastrointestinalni stromalni tumor. Intraoperativno je ustanovljeno da je promena u stvari cistična formacija sa želatinoznim sadržajem, a histološkim pregledom je potvrđena dijagnoza želudačne duplikacione ciste. **Zaključak.** Preoperativna dijagnoza želudačnih duplikacionih cista je uvek izazov za kliničare. Morfološki izgled ovih duplikatura je različit. To je razlog da se ove anomalije *imaging* metodama, čak i onim najsuperiornijim, pogrešno dijagnostikuju tako da se time hirurgija premešta u sastavni deo dijagnostičkog algoritma.

Ključne reči:
gastrointestinalni stromalni tumori; želudac; ciste; dijagnoza, diferencijalna; hirurgija digestivnog sistema, procedure; lečenje, ishod.

Introduction

Gastrointestinal duplications are rare congenital anomalies, with an incidence of 1 in 4,500 births [1], and are very rare in the adult population. They can be formed along the entire gastrointestinal tract, but the least common are gastric duplications, making up 2–8% of all gastrointestinal duplications [2]. The most common symptoms are epigastric pain or discomfort, vomiting, loss of weight, rarely bleeding. Due to the absence of symptoms, they are usually discovered incidentally during upper endoscopy and endoscopic ultrasound or other abdominal imaging methods, performed from

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other reasons [3, 4]. Diagnosis of these cystic duplicatures before surgery is complicated due to mimicry with other diseases. Differential diagnosis includes gastrointestinal stromal tumor (GIST), neuroendocrine tumor, pancreatic heterotopy, pancreatic pseudocyst and neurogenic tumor [4, 5]. There may be unusual complications such as recurrent pancreatitis or biliary fistula, chronic inflammation, formation of local peptic ulceration inside the cyst, obstruction, and rarely rupture/perforation or compression of the surrounding structures [2, 6]. Although extremely rare, malignant transformation of these lesions is also possible [4–6]. Accurate diagnosis is confirmed by surgery, followed by histopathological examination [7]. There are several methods of treating gastric duplication cysts, however enucleation with minimal disruption of normal anatomy, can be considered as the first choice procedure.

In the case under review here, the tumorous lesion of the gastric wall is presented, presurgically appeared as GIST, while during the surgery it appeared as cysitic formation. The diagnostic algorithm is also discussed, regarding preoperative misleading and unclear findings.

Case report

A thirty-three years old female patient turned to gastroenterohepathologist to determine the cause of pain below the right costal arch, nausea and vomiting. Previous medical history was unremarkable. Physical examination was normal, as well as laboratory analyzes. Abdominal ultrasound was normal as well. Serology, i.e. IgG antibodies for *Helicobacter pylori* with ELISA method were positive (13.1 U/L; negative for < 5.0). The eradication therapy lasted ten days, however there was no improvement at all. A year later, due to persistent complaints, esophagogastroduodenoscopy was performed and showed a protruding mass, 2 cm in size, localized in cardia, near gastroesophageal junction, with intact mucosa.

In diagnostic algorithm, a multislice computed tomography (MSCT) (16 slice) was performed, and clearly formed tumorous mass in gastric wall was found, circa 2 cm in size, with intact surrounding organs, but without possibility to differentiate if the mass was solid or cystic (Figure 1).

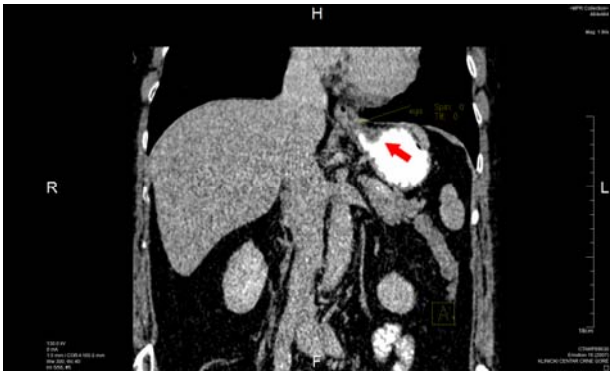

Fig. 1 – Multislice computed tomography (MSCT) with contrast showed unclear picture of tumorous lesion (red arrow) in gastric wall, near esophagogastric junction.

Endoscopic ultrasonography (EUS) with 7.5 and 12 MHz probe, showed hypoechogenic, homogeneous lesion 2.5 × 1.5 cm in diameter, which belonged to the fourth gastric wall layer, near the gastroesophageal junction (Figure 2).

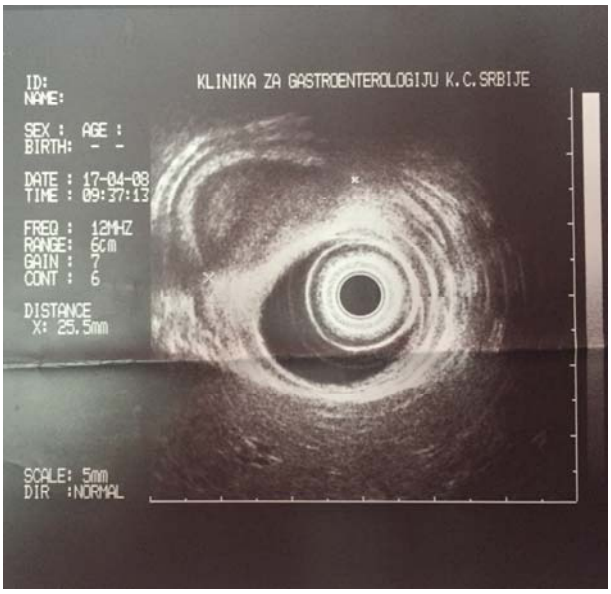

Fig. 2 – Endoscopic ultrasonography shows hypoechogenic, homogenous lesion belonging to the fourth gastric wall layer near gastroesophageal junction.

Based on the EUS characteristics, GIST was suspected. During the open surgery, the protruding mass was removed entirely, and noted that it has a cystic formation, with a gelatinous whitish content inside. Histopathological examination of the resected gastric wall was covered by smooth mucosa with preserved folds. On the section, the specimen had a recognizable stratification, with cystic formation in the muscle layer, without communication with the surface of the stomach wall, nor with peritoneum. The cyst had a smooth internal surface and it was filled with dense, light brown liquid content. Histology: surface was covered by corpus type mucosa; within the muscle layer there was a cystic formation, covered by squamous epithelium with stratification, pseudostratified ciliary epithelium, and single row cylindrical epithelium (parts which were covered with single-row cylindrical epithelium had the morphology of gastric type of mucosa). The cyst contained mucus, erythrocytes and macrophages. The wall of the cystic formation was made of mucosa, submucosa and muscle layer. In some parts *lamina muscularis mucosae* was incorporated into the muscle layer of the stomach wall (Figure 3).

The postoperative course of the patient was without complications.

Discussion

Preoperative diagnosis of gastric duplication cysts is always a challenge. Gastric duplication cysts are the rarest among gastrointestinal duplications, and among eldery usually asymptomatic.

In most of the cases, as it was in our report, the lesion is localized in the upper part of the stomach: in the level of the cardia, close to the cystic formation 20 mm in diameter junction, on the front or back wall of the fundus [7, 8].

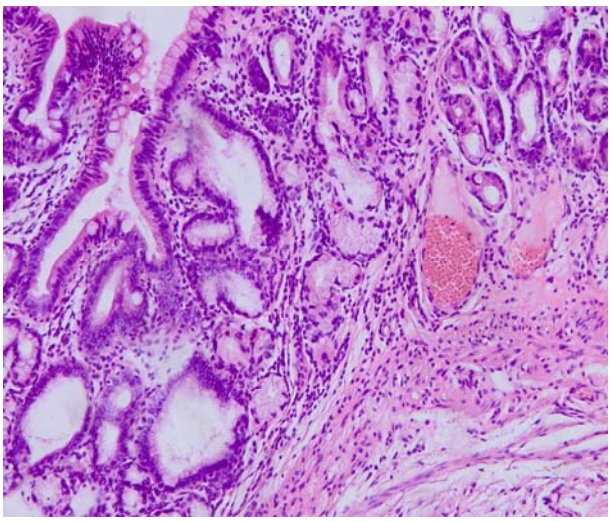

Fig. 3 – Mucus, single row cylindrical epithelium (gastric type of mucosa covering one part of the cyst wall). [Hematoxylin-eosin (HE) staining, ×100].

Abdominal ultrasound, computed tomography and magnetic resonance imaging are useful diagnostic methods [4, 6, 7]. However, in presented case, the abdominal ultrasound did not show the existence of the cyst, since MSCT image was not useful for determination if the mass was solid or cystic. Literature data shows that MSCT may indicate the presence of solid instead of cystic lesions in the 43% to 70% of cases [8]. The explanation may be related to the different amount of highly concentrated proteins in the cystic content which may be appeared as solid tumor [9]. It is often difficult to distinguish gastric duplication cysts from other subepithelial changes in the gastric wall because the morphological appearance of the cysts varies. The finding depends on the density of its content. The cysts that contain solid, thick secretions are often misdiagnosed as a solid mass by imaging techniques. EUS is also superior to computed tomography in the differentiation of cystic and solid masses [8]. Both methods may be useful for determining the localisation, size and affecting the surrounding structures [10]. EUS is useful in determination intramural versus extramural gastric lesions [11]. However, the accuracy of EUS findings is greatly affected by intracystic content – cystic lesions with thick mucoid content most often are diagnosed as GIST, which is the most common subepithelial lesion [3, 8, 12]. This occasion happened in presented report. Based on the findings of EUS, the patient was surgically treated under the suspicion that tumorous lesion was GIST. Certainly, we must not forget that the GIST can be presented as cystic degeneration as well. Also, fine needle aspiration biopsy is not recommended in any suspicious case on GIST, because of the risk of peritoneal dissemination.

Communicating gastric duplications usually do not require intervention if both lumens (the cystone, and the gastric one) are connected, unlike non-communicating duplications that require surgical treatment [2].

Our report states that the correct diagnosis was confirmed by surgical intervention, which has been emphasized in the literature as well [7].

Gastric duplication cysts are often diagnosed during surgery in elderly patients, but histology still remains the most important method for determination of the tissue of origin of the duplication cyst. The essential characteristic of gastric duplication cysts is that the wall is surrounded by at least one layer of smooth muscle, covered by mucous membrane, which contains gastric epithelium in most cases [1].

Laparoscopic surgery was not an option for us, since the cyst was situated near vagal nerve and its branches whose injury would cause serious complication and urge to extend surgical procedure.

Every subjective patient's complains disappeared after surgical recovery. Control endoscopies performed once per year in next two years, as well as clinical appearance showed normal finding.

Conclusion

Despite the progress in diagnostic imaging methods, including endoscopic ultrasound, preoperative diagnosis of gastric duplication cysts in the adult population is still difficult and challenging due to their rarity in one hand, and the absence of specific findings on the other hand. Cystic lesion with dense mucoid content may be misdiagnosed as GIST. It is important to emphasize that unusual embryonic malformations can occur in the adult population and therefore must be considered in the differential diagnosis of thoraco-abdominal tumorous lesions.

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CASE REPORT

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To wait for a spontaneous recovery of the third cranial nerve palsy occurring after the coiling of a PComA aneurysm or to implement surgical treatment? – A case report.

Da li treba čekati spontani oporavak slabosti trećeg kranijalnog nerva nastale nakon koilinga PComA aneurizme ili sprovesti operativno lečenje?

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Abstract

Introduction. In the last two decades a method of endovascular embolization has been imposed as a method of choice in the treatment of unruptured intracranial aneurysms. Therefore, the problem of treating posterior communicating artery (PComA) aneurysms presenting with the third cranial nerve (TCN) palsy has become even more complex. The case of a patient reported in the paper itself has presented a dilemma of whether to wait for spontaneous resolution of ophthalmoplegia developed after the coiling of a PComA aneurysm or whether to implement an early surgical treatment. **Case report**. An unruptured saccular aneurysm, directed inferolaterally in the right internal carotid artery (ICA) segment in the position of the PcomA origin, was diagnosed in a 58-year-old male patient. The aneurysm was measuring 9 mm in diameter while the neck was measuring 5 mm. The day before the planned embolization, the patient developed ipsilateral ophthalmoparesis, whereas the first day after the endovascular procedure was completed, the patient developed right-sided complete ophthalmoplegia. Ten weeks after the endovascular embolization our team decided to perform a microsurgical treatment including aneurysm clipping and coil extraction. Eighteen months after the surgery, the patient made a full recovery of the functions of *musculus* (*m) levator palpabrae*, *m. rectus medialis* and pupillary function, with a partial recovery of the functions of *m. obliqus inferior*, *m. rectus inferior* and *m. rectus superior*. **Conclusion**. According to medical research and literature, the partial recovery of the TCN palsy is expected to happen in the first few weeks after embolization. Despite the completion of endovascular treatment progression of ophthalmoparesis to ophthalmoplegia without any simptoms of clinical improvement after 10 weeks is considered to be an indicator of long-standing TCN compression, which can lead to irreversible nerve damage. Despite the increase in the use of an endovascular embolization method in the treatment of PComA aneurysms preceeded by the TCN palsy, neurosurgical treatment is believed to have been necessary. Still, there is one question left to be answered - did we react too late in this particular case?

Key words:
intracranial aneursym; oculomotor nerve; embolization, therapeutic; neurosurgical procedures; ophthalmoplegia; recovery of function.

Apstrakt

Uvod. U poslednje dve decenije metoda endovaskularne embolizacije nametnula se kao metoda izbora u lečenju nerupturiranih intrakranijalnih aneurizmi. Stoga je problem lečenja aneurizmi u regiji zadnje komunikativne arterije (PComA), udružene sa slabošću trećeg kranijalnog nerva (TKN), postao još kompleksniji. Slučaj bolesnika prikazanog u ovom radu stvorio je dilemu da li treba čekati spontani oporavak oftalmoplegije koja je nastala nakon embolizacije aneurizme na PComA ili je potrebno sprovesti rano operativno lečenje? **Prikaz bolesnika**. Kod bolesnika muškog pola životne dobi od 58 godina dijagnostikovana je nerupturirana inferolateralno orijentisana bilobarna aneurizma na desnoj unutrašnjoj karotidnoj arteriji – *arteria carotis interna* (ACI) u regiji ishodišta PComA, dijametra 9 mm i širine vrata 6 mm. Dan pre planirane embolizacije kod bolesnika se javila ipsilateralna oftalmopareza, a prvog dana nakon endovaskularne procedure došlo je do razvoja kompletne desnostrane oftalmoplegije. Nakon 10 nedelja perzistentne oftalmoplegije doneli smo odluku da se sprovede operativno lečenje u vidu

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klipsovanja aneurizme i ekstrakcije koilova. Nakon 18 meseci od operacije došlo je do potpunog oporavka funkcije *musculus* (m.) *levator palpabrae*, *m. rectus medialis* i pupilarne funkcije, sa parcijalnim oporavkom funkcije *m.obliqus inferior*, *m. rectus inferior* i *m.rectus superior*. **Zaključak**. Prema literaturi, očekivano vreme, barem delimičnog oporavka TKN nakon embolizacije je tokom prvih nekoliko nedelja. Progresija oftalmopareze u ofalmoplegiju uprkos sprovedenom endovaskularnom lečenju, a bez kliničkog poboljšanja nakon 10 nedelja od sprovođenja istog, shvaćena je kao indikator izražene kompresije TKN koja bi mogla dovesti do ireverzibilnog oštećenja nerva. Uprkos sve većoj učestalosti metode endovaskularne embolizacije u lečenju aneurizmi u regiji PComA koje su praćene preoperativnom slabošću TKN, smatramo da je operativno lečenje bilo neophodno. Ostaje pitanje da li je naša reakcija u ovom slučaju zakasnila?

Ključne reči:
aneurizma, intrakranijalna; n. oculomotorius, embolizacija, terapijska; neurohirurške procedure; oftalmoplegija; funkcija, povratak.

Introduction

The question of an effective treatment alternative of unrupted intracranial aneurysms poses a particular problem encountered by neurosurgeons, especially nowadays when the detection rate of aneurysms during routine neurodiagnostic examinations has been increased [1]. Particular neurosurgical approaches have always been required for the treatment of posterior communicating artery (PComA) aneurysms. These aneurysms reported to occur in 13–30% of all cases cause the third cranial nerve (TCN) palsy [2]. Therefore, even before the era of 'high definition' neuroimaging, aneurysms was detected prior to the occurence of spontaneous subarachnoid hemorrhage due to aneurysmal rupture. The clinical presentation of unruptured PComA aneurysms includes symptoms associated not only with the TCN palsy, but also with retrobulbar pain [3]. Considering the wide use of endovascular coil embolization to treat unruptured intracranial aneurysms [4], particularly in the last decade, the problem regarding optimum management of unruptured PComA aneurysm became even more complex.

Possible mehanisms for the occurrence of the TCN palsy caused by a PComA aneurysm are the following: aneurysm induced mass effect and consequent compression of neighboring nerve, pulsatile effects of aneurysm and the combination of the latter two mechanisms. Coiling of PComA aneurysms was considered to affect the compression effect on the TCN, and aneurysms in this region were believed to require surgical treatment. However, in the past few years there was an increasing number of reports speaking in favour of complete symptomatic recovery or partial relief of the TCN after coil embolization [5–7]. Also, they refered to the fact that no significant differences were recognized in the clinical outcome observed while following treatment compared with surgical clipping aneurysms [8–10].

The patient's case presented in our paper has initiated a dilemma of whether to wait for a spontaneous recovery of the TCN palsy which progression is observed immediately after endovascular coiling of an unruptured PComA aneurysm. Once more, the reported case put an emphasis upon the complexity of pathology manifested within the case itself along with the emergence of taking a patient-centred approach which should be tailored to each individual. Other possible complications relating to the management of a PComA aneurysm have also been discussed within the paper itself.

Case report

A 58-year-old male patient was admitted to the outpatient clinic of the Department of Neurology because of repeated episodes of right-sided headaches followed by ipsilateral retrobulbar pain. The computed tomography (CT) scan of endocranium revealed no evidence of intracranial hemorrhage or any other pathological lesions. During the further course of examination, magnetic resonance (MR) angiography was performed, complete with the digital subtraction angiography (DSA) study. With 3D DSA of the cerebral vessels, an unruptured aneurysm was recognized (Figure 1A). It revealed a bilobed saccular aneurysm of the right internal cartoid artery (ICA) proximal to the PComA origin, directed inferolaterally. A diameter of the aneurysm sac was 9 mm, with a proximal neck diameter of 6 mm and

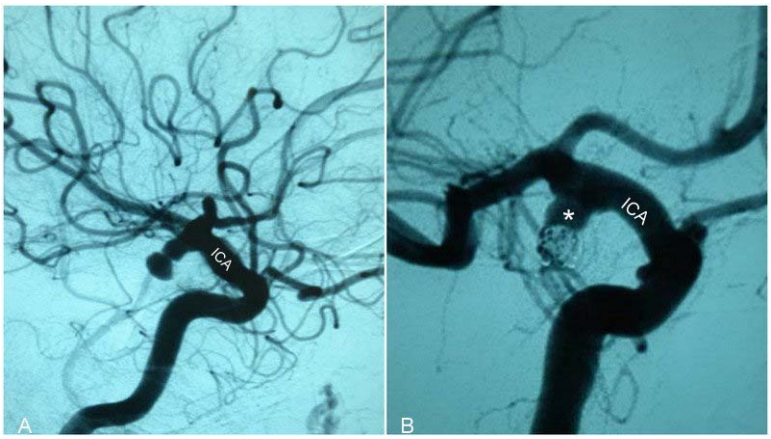

Fig. 1 – Preoperative cerebral digital subtraction angiography (DSA) shows: A) bilobed saccular aneurysm of posterolateral wall of the internal carotid artery (ICA), communicating segment, aneurysmal sac was measuring 9 mm in diameter while the neck was measuring 6 mm in diameter, with a fundus which was directed inferolaterally; B) DSA immediately after coil endovascular embolization shows that approximately 75% of the total aneurysm sac volume was filled (arrow), with a neck residue (asterisk).

a dome-to-neck ratio of 1.5. Endovascular coil embolization was recommended as an initial treatment. It was also planned along additional placement of a stent for the finally recovery of aneurysm.

The day before the scheduled endovascular embolization the patient developed a TCN palsy causing ptosis which was accompanied by lateral deviation in the right eyeball. Although it was followed by ophthalmoparesis, the procedure of endovascular embolization (EE) was administered in order to relieve compression of TCN by relieving pulsating effect of the aneurysm.

The EE was performed and after treating the intracranial wide-necked aneurysm, approximately 75% of the total aneurysm sac volume was filled, which was revealed by the immediate postprocedural DSA images (Figure 1B). However, in the immediate postprocedural course the patient developed right-sided ophthalmoplegia, including drooping of the upper eyelid (ptosis), pupilary dilation (mydriasis) and paralysis of the bulbomotor muscles innervated by TCN.

Eight weeks after procedure there was no regression of neurological deficit. Taking into consideration that it was not possible to completely fill the aneurysm and that in the postprocedural course the patient exhibited the progression to complete ophthalmoplegia, our clinical team decided to perform neurosurgical operative treatment 10 weeks after the endovascular embolization.

After the right-sided pterional craniotomy, due to the level of magnification increased by an operating microscope it was enabled to present the right *nervus (n) opticus* and right ICA in the first place. In a microdissection procedure a wide-necked aneurysm was first identified on the posterolateral wall of the ICA proximal to the PComA origin, with a fundus which was directed inferolaterally, causing compression of the TCN (Figure 2A). *N. oculomotorius* presented a flatter surface, while the fundus caused its slightly elevated prominence and convex outer surface. The two clips were placed across the neck of the aneurysm – a slightly curved clip, measuring 9 mm and the straight one, measuring 8.3 mm (Figure 2B). Then, microdissection of the aneurysm fundus was carried out. Using the microscissors, the fundus was cut sharply and the placed coils were gradually removed (Figure 2C and 2D). The TCN compression was relieved completely, but nerve was bent and arch-shaped and it was made thinner.

In the early postoperative period after surgery the patient awoke with right ophthalmoplegia. Immediate postoperative angiography showed the absence of aneurysm rest (Figure 3).

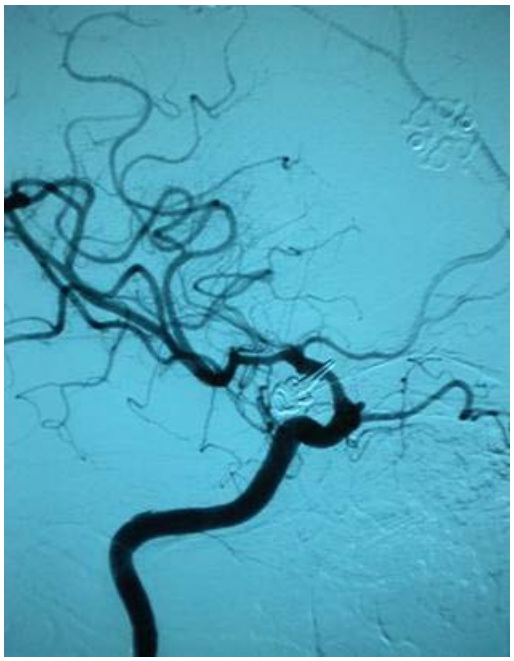

Fig. 3 – Immediate postoperative cerebral digital subtraction angiography shows complete obliteration of the aneurysm.

The postoperative course was uneventful. Examination performed two months after surgery revealed regression rate of neurological deficit. The patient had a full recovery, with resolution of the eyelid ptosis first, which was followed by the recovery of external ocular movement and then improvement in pupillary function. Follow-up data concerning the patient's neurological state were collected during a six-month period. Eighteen months after the surgery the patient had a full

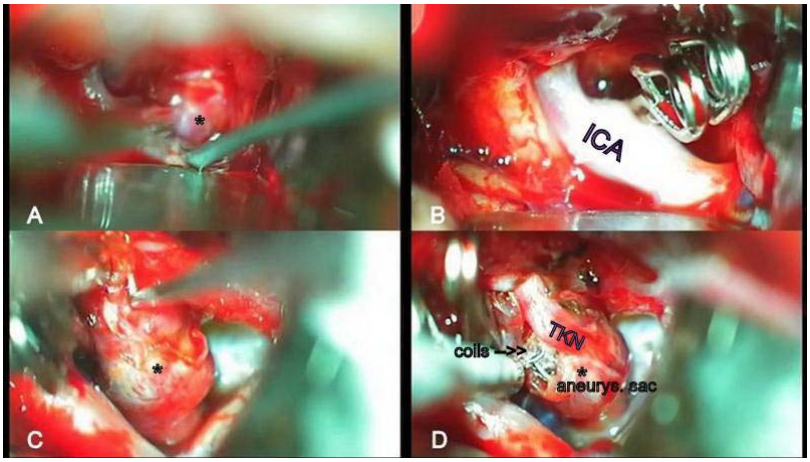

Fig. 2 – Intraoperative images under magnification (× 8) of an operating microscope show: A) microdissection of an aneurysmal sac (asterisk); B) by placing two clips at aneurysmal neck which is at posterolateral wall of internal carotid artery (ICA) followed by the (C) aneurysmal sac opening and (D) removing of coils from aneurysmal sac with third cranial nerve (TKN) decompression.

recovery of the functions of *m. levator palpebrae*, *m. rectus medialis* and pupil function. It also demonstrated that the patient had a partial recovery of the functions of *m. obliquus inferior*, *m. rectus inferior* and *m. rectus superior*.

Discussion

Although it can be stated that the adequate management of unruptured intracranial aneurysms is still one of the most controversial topics [11], unruptured PComA aneurysms, presenting with TCN palsy, require urgent treatment to maximize the potential of functional recovery and prevent subarachnoid hemorrhage [12]. A surgical procedure of aneurysm clipping used to be a method applied in a standard treatment of TCN palsy caused by an expanding PComA aneurysm [13]. Also, the mechanism of recovery by surgical clipping was known to be effective by relieving the mass effect. As the method of endovascular embolization has been more widely used over time, more cases have been reported along with a minor case series of patients who have made a functional recovery of TCN after coiling of an PComA aneurysm [5–7]. Recovery mechanism of TCN following coil embolization is related to loss or decrease of aneurysmal pulsation, despite the fact that the mass effect was not completely relieved. There is also evidence indicating that after embolization the aneurysm volume decreases by 30% within the period lasting from 2 to 12 months [14].

Although some studies suggested that surgical clipping was associated with a higher incidence of recovery of TCN and higher recovery level in comparison with endovascular treatment [15], meta-analysis of all similar available studies showed that there were no statistically significant differences in clinical outcome and rate of complete recovery between the two groups of patients following coil embolization and surgical clipping [9, 10]. The findings suggested that oculomotor nerve palsy may result not only from mechanical compression by coils but also from inflammation induced by perpendicular thrombosis occurring immediately after endosaccular embolization [11].

The prognosis of the TCN palsy mainly depends on the degree of preoperative deficit. Gender, age and size of the aneurysm had no influence on the functional recovery of the nerve [6, 7, 10, 16, 17]. The importance of interval between the onset of palsy and the time of operation/embolisation seems to be contradictory when compared to the recovery of the nerve according to the results obtained by various authors [7, 12]. Patients usually experienced complete functional recovery of TCN within 3 months of surgery/embolization. However, full recovery may also take two years. [6, 7, 17].

Ptosis is generally the first symptom, and it frequently shows the earliest recovery of all other disturbed oculomotor functions after surgery. The restitution of the single ocular muscle functions shows a fairly constant course: the *musculus* (*m) levator palpebrae* and the *m. rectus medialis* show rapid recovery. The parasympathetic fibres follow next, but normal function of elevation and depression of the ocular bulb (*m. rectus sup*., *m. obliquus inf*. and *m. rectus inferior*) is often delayed. The above mentioned clinical course of the TCN recovery in patients after clipping completely correlates with the clinical data of the patient reported here, regarding his/her functional recovery [13].

Endovascular embolization of PComA aneurysms imposes a problem of anatomical specificity of the location of an aneurysm, based on which the following types of aneurysms can be identified: „true PComA aneurysms", aneurysms which arise on the posterolateral wall of the ICA located proximal to the origin of the PComA and ICA communicating segment aneurysms distal to the origin of the PComA [18]. In the first aneurysmal type neck of the aneurysm originate of the PCom artery itself and is often associated with large or fetal PComA arteries. In the most common second type the neck of the aneurysm can partially incorporates the PComA artery [9], which was not present in our reported patient, although the aneurysm neck was closely related to the PComA origin. Although it is a seemingly irrelevant classification of a small segment of blood vessels at the base of the brain, its significance is highlighted in the era of endovascular embolization. As for recommendations given for the first two aforementioned subgroups of aneurysms, microsurgical treatment performed by placing adequately modified clips is being recommended. Endovascular embolization of the mentioned aneurysms is often incomplete due to the efforts invested in order to save the PComA origin. This is followed by aneurysm recurrence, while stent placement may compromise perforant branches. The location of the branches cannot be determined without performing neurosurgical microdissection. ICA communicating segment aneurysms distal to the origin of the PComA can be adequately treated by microsurgical treatment and endovascular embolization [18].

An increasing number of aneurysms demanding operative treatment immediately after the performed procedure of endovascular embolization, has been reported in the past few years. In our viewpoint, it is a result of inadequate diagnostic indications for aneurysm coiling procedures. Based on the literature, the most common indications for an operative treatment of previously coiled aneurysms were the following: incomplete aneurysm occlusion, aneurysmal regrowth and coil herniation [19]. A few case series of patients demonstrated that microsurgical treatment of the given aneurysms was associated with a low incidence of serious complications and favourable clinical outcome in most cases. When reviewing the mass effect of an aneurysm, numerous authors consider utilizing a aneurismal sac evacuation technique to be their first choice regardless of the aneurysm location and nerve deficits it causes [20]. Taking into consideration all the available literature, we have not been able to identify a case of microsurgical treatment of a PComA aneurysm where ophthalmoparesis progressed to acute TCN palsy immediately following the coiling procedure.

Due to all mentioned above, we are facing the following clinical dilemma: to wait for a spontaneous recovery of developed ophtalmoplegia after the coiling of a PcomA aneurysm, or not, and how long we should wait. In addition, is it necessary for patients to undergo operative treatment as soon as possible? Or, to rephrase our question: can our patient's state be led to the point of the irreversible TCN

damage by taking an expectation approach? The literature published so far has not given clear guidelines related to a reasonable time frame to expect the recovery of the nerve after endovascular embolization. Although it has been noticed that the recovery of the TCN palsy can take even a whole year after the embolization was performed, it is all about cases where initial regression of weakness symptoms has appeared in the first few weeks after the intervention [6, 7]. Taking into consideration all the findings revealed up to this point, we believed that concerning our patient's case we had run out of time and that microsurgical intervention was necessary. The progression of ophthalmoparesis to ophthalmoplegia that did not improve within ten weeks, was considered in our opinion to be an indicator of long-standing compression of the TCN. In the following postembolization course, it could lead to the irreversible TCN damage. Having completed further analyses of the clinical course and intraoperative findings, we concluded that the right decision, related to the further patient's treatment was made at the given moment. The decision was made for the purpose of prevention of permanent nerve deficit. Still, one question remains to be answered - did we react in a timely manner? Additionally, is the progression of ophthalmoparesis to ophthalmoplegia after coiling considered to be a early predictor of unfavourable outcome of the recovery of TCN which demands operative treatment in the first few days? All the additional questions that may arise concerning the given topic remain unanswered for the state being due to the lack of reported cases of patients with the clinical course similar to the one presented in our case report.

Conclusion

Despite the increasing frequency of use of endovascular embolization in the treatment of unruptured PComA aneurysms presenting with the TCN palsy, it is our viewpoint that concerning the cases with weakness showing marked progression towards complete ophtalmoplegia after EE is performed – the patients are required to undergo surgery. The surgery is to be performed by placing a clip over the neck of an aneurysm and by the use of the technique of coil extraction in order to decompress the oculomotor nerve. Simultaneously, it is neccessary to take into account the time factor, because the acute complete ophtalmoplegia occuring after the coiling of a PComA aneurysm should be taken as an indicator of a predominantly expressed compression of the TCN. The compression may lead to irreversible nerve damage, resulting in permanent nerve deficit. In the case of the aforementioned clinical course microsurgical procedure is advised, by means of which the aneurysm mass effect could be relieved. Unfortunately, based on our own experience and the given literature, we have to state that nothing can be said about the period of time recommended for the patient to undergo surgery which is considered to be more invasive treatment modality. However, in this case, according to the authors of the paper itself – the utilized modus of treatment was highly necessary.

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Military Hospital of Valjevo, from its beginnings until the Great War

Valjevska vojna bolnica, od prvih dana do Velikog rata

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Key words:
history of medicine; military medicine; history, 19th century; world war I; hospitals, military; military personnel; serbia.

Ključne reči:
istorija medicine; medicina, ratna; istorija, 19. vek; prvi svetski rat; bolnice, vojne; kadar, vojni; srbija.

Introduction

The general, well-known name of the Hospital of Valjevo does not apply to any particular hospital facility or a hospital as institution, as far as the first years of the WWI are concerned, but it rather denotes the time process. As part of a series of specific events, due to which the whole city at the end of 1914 and beginning of 1915 became one large hospital, there were two permanent and six auxiliary hospitals, distributed in dozens of central or associated facilities in Valjevo together with the changing number of field hospitals in the city and its surroundings. Previous historiographic research has been focused on the situation as a whole emphasizing the general development and care of the wounded and diseased [1–3].

Thus, the details related to the two earlier hospitals, which had existed before the wars, were largely neglected, and they are: the Civilian District Hospital of Valjevo and the Military Hospital of Drina Division. While there is more information about the Civilian Hospital due to the written records, which partially systematize its beginning and its development [4–5], setting up of the Military Hospital has eluded the attention of the researchers; some details about it were mentioned casually and could be found in some works related to other military topics [6–7]. This is the reason why the aim of this paper is to open up the topic with the first records and then follow the history of the hospital over the first three decades of its continuing existence, that is, from its founding in 1883 until the start of the WWI. This may be a way to lay the foundations for further systematic research, if some unknown archive documents have evaded our research efforts.

The history of the Military Hospital of Valjevo is inevitably connected with the strategic position of Valjevo city situated in the background of the Drina River and historical environs along its banks which could or have formed continuous, hostile challenges starting from the first days of the uprising in the early 19th century until the escalation of WWI in 1914. Being located in the immediate background of possible and real warfare, Valjevo was recognized by the military command not only as a place of military headquarters, but also as a convenient gathering place for the reception of wounded and sick soldiers.

There are three different periods in the history of Military Hospital of Valjevo. The first period is related to the crisis caused by the education of the Serbian National Army in 1860 and the Serbian- Turkish incident in Belgrade in 1862. The second period covers the Serbian-Turkish wars 1876–78, and, finally, the third one was the time of great reforms in the army which were being carried out by King Milan Obrenovic starting from 1883. The hospitals from the first and the second period, and the second and the third period, had no observable continuity, while a hospital established in the third period had continued to exist up to WWI, and even after it, although throughout the first 15 years, it was officially designated to be a temporary, and then, from the beginning of 20th century, as a permanent one.

Volunteer Hospital at the time of the crisis in 1862

One of the basic tasks Prince Mihailo Obrenović challenged himself after his second coming to power in 1860 was to liberate Serbia from the Turkey's dominance. That is why, in August 1961, the Law on Standing People's Army was adopted, which, in addition to the existing few army units, projected the formation of a national, mobilizing military

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force capable of embarking on the liberation war. This led to intensively tense relations with Port which culminated in an incident on Čukur Česma on June 3rd to 15th 1962, Turkish bombing of Belgrade from the city fortress on June 5th to 17th. The tensions were expected to be solved by arms and it seemed likely that they would spread over the other Balkans nations. The escalation of the conflict, however, ended by a diplomatic intervention of the great powers, which in the following year led to the emigration of Turks from Serbia (except the Danube cities of Belgrade, Šabac, Smederevo and Kladovo).

Before reaching the agreement, during the period of uncertainty, Prince Mihailo was preparing for the possible outcome of the war, and among other things, he initiated the formation of so-called "Volunteer Corps" consisting of 3,000 people, mostly Serbs, volunteers coming from Serbia, Habsburg Monarchy and the Ottoman Empire, as well as representatives of other Slavic nations under command of the former Austrian officer Antonije Orešković. One of the two legions of this corps, called "Borderline Corps", had been stationed in the improvised military cottages in Valjevo since the end of June, so that they could carry out the armed activities in Bosnia and agitate people to an uprising. Another one, called "Bulgarian Corps" was in Aleksinac. However, since there was no escalation of the conflict because of the diplomatic decisions brought at Kanliš Conference on September 30 – October 11, at the request of the great powers, the corps was dismissed at the end of October and early November 1862, but a certain number of educated officers and soldiers remained in the Serbian army [8–10].

In addition to volunteers, in 1862, a field battery of the People's Army was stationed in Valjevo, and they were accommodated in the cannon sheds which were built according to the order given by the Minister of Internal Affairs N. Kristić on November 15–27 1862 [11]. Previously, besides the old gunpowder storage located in Nenadović Kula, a new one was made. A quite common road run between these two, as can be seen from a report of the Minister of Construction representative, a French officer H. Mondin on October 13–25 [12].

The above mentioned military preparations for the eventual conflict were accompanied by the organization of the medical service. In addition to organizing a temporary hospital in Belgrade with 800 beds, the present Director of the military medical service, Dr Beloni, ordered to appoint the doctors and define the locations for hospitals all around Serbia so that patients and the wounded could be admitted, especially in border areas [13]. One of the hospitals was also located in Valjevo and its main purpose was the care of volunteers. This "Volunteer Hospital" was formed according to the order of the Minister of the Military, Mondin on July 27 - August 8, 1862. That was why an empiricist Dimitrije Kaparis, the Captain 2nd class, was sent to Valjevo with "all necessary medicines and instruments". In the order of the Ministry of the Interior from August 2–14, you can read: "A hospital home for hundreds of patients will have to be determined by the district authorities, and exactly 400 blankets, 100 straw-bags, 100 pillows, and 400 shirts should be procured" [14]. Immediately after that, the Minister of the Military sent further instructions to military authorities on August, 7–19: Everything that people of that district have acquired will be abandoned in present and handed over to their district authorities in due time, and this will be used to put up a district hospital" [15]. In the report of the commander of the Volunteer Corps from August 28th / September 9th, 1862, as Vladan Djordjević wrote: "This year (1862) volunteers have started to gather in Valjevo and the Military Minister sent Dimitrije Kaparis to Valjevo to be a doctor, and beside treating volunteers, he was a corps doctor. Immediately upon his arrival to Valjevo, he examined the entire volunteer corps and, after that, 154 volunteers were discharged as incapable of military service" [16]. Among the volunteers, there was a certain Danišević, a medical student from Russian Poland, "who had escaped from the Kronstadt fortress" [17], but it is not known if he joined the hospital.

In all likelihood, Valjevo Volunteer Hospital was closed down when the Volunteer Corps was dissolved in autumn 1862, and Dimitrije Kaparis, who arrived to Valjevo from a small Military Hospital of Požarevac (with only 20 beds), continued his service and at the beginning of 1864 we could find him working as a doctor in a temporary military hospital in Ćuprija [18].

Regarding the further existence of any military medical service in Valjevo, in the period starting from the closure of the Volunteer Hospital in autumn 1862 until the formation of a war auxiliary hospital from the period of the Serbian-Turkish wars from 1876 to 1978, the available information are meagre and does not allow to get a complete historical picture. According to the data from that period regarding the state model of engaging officers and planning military activities [19–21], it can be seen that, as before, no units of Standing Army were stationed in Valjevo, at least not in the long run, but there was still an artillery battery of the People's Army established in 1862. It was a part of the Drina and Sava Command with the Headquarters in Šabac. The plans of the military hospitals show that there were hospitals in Belgrade, Kragujevac and Ćuprija, however, in the tabular presentation of the number of physically treated soldiers, Vladan Djordjević pointed out to other hospitals, apparently auxiliary or temporary medical objects, which number changed yearly, as well as their locations. Thus, as we saw, in 1862, when there was a Volunteer Hospital in Valjevo, there were also five hospitals in Serbia: Belgrade, Topčider, Kragujevac, Zaječar and Valjevo, in which 58 soldiers were treated. In 1863, Valjevo was not mentioned, but in the review of 1864, Valjevo appeared to be one out of ten places where patients were hospitalized. That year, as one of the places for treatment, Šabac, the Headquarters of the Drina and Sava Command was said to have only 2 patients. In the next 2 years, in 1865 and 1866, Valjevo was not mentioned, but Šabac, where 12, i.e. 8 patients were treated. In 1866, Užice was found to be listed as a treatment place, and it was also included in the Drina and Sava Command. In 1867, (when a Civilian District Hospital was established in Valjevo), Valjevo was mentioned as a place where 48 soldiers were treated, and there was no word about Šabac, while in the following year, in 1868, there

was information about Šabac, Loznica, Užice and Valjevo where 44 patients were treated out of which 1 patient passed away. The same places were mentioned in 1869 when 22 soldiers were treated in Valjevo. Over the next two years, Šabac, Loznica and Užice were also mentioned, but there was no information about Valjevo. Then, again, in 1872, along with already mentioned places, Valjevo appeared as a place with 29 patients. The same situation occurred in 1873 when 30 patients were treated and 2 of them died in Valjevo; in 1874 there were 8 patients, while in 1875, the data referred only to Belgrade and Kragujevac, "because, in the time when this report was being written, Dr Laza Dokic took the rest of other records with him" [22].

Reserve Military Hospital of Valjevo from the time of the Serbian-Turkish wars from 1876 to 1878

The warfare between Serbia and Turkey, which Prince Mihailo began to prepare with the aim of a total liberation, occurred during the reign of Prince Milan Obrenović. In the period from 1876 to 1878, there were two wars. The first one started in July and lasted until November 1876. After the initial success on the south-eastern front, the Serbian troops were overwhelmed and due to the diplomatic intervention of great powers Serbia was saved from a total defeat. The Serbian army, with its police-like (or, gendarmerie model of organization), weakly armed and trained, despite the 2,500 Russian volunteers, officers, and almost 200 different medical profiles, starting from doctors and pharmacists to nurses, was not able to resist superior Turkish army which was larger, better trained and equipped with modern German weapons. Yet, as the great Serbian statesman Jovan Ristić said, "blood of Serbs and Russians spilt together and in that way fortified the fraternity of our two peoples." During that time, the Western Serbian Army, the Drina Division (the Director of medical care was Major Dr Josef Holec) had somewhat more success in fighting around Zvornik and Semberija, temporarily conquering Janja, but unsuccessfully attacking Bijeljina.

There was a need for medical care of the wounded at the beginning of the hostilities and a temporary military hospital was opened in Valjevo. Dr Aleksa Djukić, the Captain 2nd class, a volunteer from the western Srem was appointed to be a Director. He arrived to Valjevo with his young Russian wife on April 21 – May 2. County and municipal authorities were ordered to designate buildings in which 400–800 wounded and sick people could be accommodated if needed, and to establish a local committee to assist the authorities. In the hospital, Dr Djukić's wife worked as a nurse and helped her husband who had been sick with tuberculosis when he came. A doctor Vojislav J. Subotić was sent to join the staff in July, a doctor Djordje Šainović in August, and a Russian doctor Dr Perestrajtkovic with two male nurses in September [23–24].

The second Serbian-Turkish war started on 7/19 December 1877 to the insistence of Russia which in April started the war with the Ottoman Empire, liberated Bulgaria, conquered the fortress Plevna after the siege and reached the gate of Constantinople. Niš, Pirot, Leskovac, Vranje, were freed, and Serbs stepped onto Kosovo soil where they were stopped because a treaty was signed. The provisional peace treaty between Russia and Turkey was signed in March 1878 in San Stefano, with very unfavourable conditions for Serbia, because Russia forced the constitution of the Great Bulgaria. The treaty was revised at the Berlin Congress in the middle of the same year when Serbia gained full independence, and besides, keeping the support of Austria, it also maintained the inclination of the Chancellor Count Bismarck with regard to conquered territory.

Although the focus of military activities during the war was on the southern and south-eastern border with Turkey, a high level of readiness, there were occasional skirmishes on the western border of the river Drina, which was protected by Drina Corps counting about 20,000 soldiers. The Head of the corps' medical care was medical Major Dr Stefan Nedok. The military command formed two temporary military hospitals, one in Šabac, where a doctor was a young Berlin student of Medicine Laza Lazarević, and the other one in Valjevo, in which Dr Herman Kraus was appointed a Director. A little more information about the work of the medical service in Valjevo during the Second Serbian-Turkish War was recorded in the third book written by Dr. V. Djordjević under the title "Reserve Hospitals in Valjevo" based on a report of its Director, Dr Kraus: "in a storage, the whole medical supply was left after the last war in such quantity that not only the 1st and 2nd classes of the People's Army could be provided to satisfy their needs for some time, but also the hospital with 200 patients. As far as the hospital building is concerned, it was difficult at the beginning, and for that reason, the patients had to move several times: from the barracks to a school building, and when the school started on March 1st, then from the school again to Kasino and the District Court … Now there is a school again in the former cannon barracks, where 30 beds could be installed, and in the District Court there are places for 26 patients. In the second building, there is a place for both, a pharmacy and a hospital office. The hospital personnel consisted of a Director of the hospital, 1 medical assistant, 1 pharmacist, commissioner and subcommittee as well as other necessary staff. The first patients were admitted on December 1st, and by the end of May, they were dismissed; 72 in total, 383 total number of patients and wounded, 279 sent to command, 72 sent home, 8 died" [25].

This war was devastating for weak Serbian medical resources: till the cease-fire agreement, 7 doctors out of 60 died: Majors Dr Stefan Nedok and Aleksandar Verminski and Captains 2nd class Dr Aleksa Djukić, Dr Adam Derman / German /, Dr Rade Petrović, Dr Periša Šljivić and Dr Andrej Bikl, and later on, the eighth – Captain Dr Ilija Milijić [26].

At the end of the war, since 1879, the Standing army was stationed in Valjevo: the Headquarters of the Drina Artillery Regiment and its two field batteries. At that time, according to the state model, military hospitals, either permanent or temporary, existed in Belgrade, Kragujevac and Niš, and temporary in Ćuprija, Kuršumlija, Kladovo, Prokuplje, Vranje, Leskovac and Karanovac (Kraljevo) [19]. Reserve Military Hospital of Valjevo was, in all likelihood, dissolved at the end of the war conflicts, and was re-established

in 1883, when the prince, then the King since 1882, Milan Obrenović, started to implement new military reforms.

Valjevo Military Hospital of the Drina Division

After the peace was made, there was a break in development activities until 1883, when 3 "Laws" were published in the "Official Military Letters", which enabled gradual progress in the organization of the Serbian army, including the field of medical care, and with attained level of development, it entered Balkan Wars (1912) and WW1 (1914). By the first law, the "Law on the Organization of the Ministry of Defence", the Medical Section gained autonomy; by the second, "Law on the Arms of the Army", professional medical personnel was defined, and by the third one, "Law on the Formation of the Army", the internal organization of medical care was determined. This third law defined the territorial division of the state in 5 divisions and certain centres (Moravska - Niš, Drinska - Valjevo, Dunavska - Belgrade, Šumadijska - Kragujevac and Timočka - Zaječar). Thanks to its geostrategic position, Valjevo became the headquarters of the Drina Division, the location of its commanding staff and a part of the operational units [6–7].

In the first year of implementation of the reforms, besides the command of the Drina Division, Valjevo had 2 units out of 12, deployed in 3 infantry battalions. Since 1885, there were already 4 infantry troops in Valjevo, and after the new military reforms, at the beginning of the 20th century, 24 infantry troops and artillery were located in Valjevo [6, 19].

As you can see from this review, not rich in details, during the two decades, Valjevo grew into a strong garrison centre with developing military infrastructure. In 1885, at the edge of Valjevo city, on Ilidža, a construction of pavilion complex of the first barracks was already completed (later on, the 17th Regiment barracks, namely, Kadinjača barracks after the WWII). Afterwards, in 1899, on the Krušik hill, a construction of a monumental barrack of the 5th Infantry Regiment of the Drina Division was completed, and during the first decade of the 20th century, at the exit from Valjevo, beside the Belgrade road, a pavilion complex of artillery barracks with storages was built. Then, they built a building for the Drina Division Command in the center of the city, and on the west from the Krušik hill, a number of storages and other auxiliary and ancillary facilities, including a building for the accommodation of the Drina Division hospital unit [27–28].

The transformation of Valjevo into a garrison centre implied the formation and development of military medicine and an opening of the Drina Military Hospital. In 1883, Dr Jovan Djokić, the Captain 2nd class, became its first Director being at the same time a medical desk-officer of the Drina Division [29]. In addition to the hospital in Valjevo, the Drina Hospital Company with a driving squadron was established, and since then a Commander of the Hospital Company had also been a hospital commissar.

By examining the established state and military models (taking into account that the situation shown in the scheme for a particular year actually corresponds to the situation from the previous year, during which the published data were collected), it was noted that in 1883 and 1884, in the first years of existence, the Military Hospital of Valjevo was named as permanent. Then, for a long period of 15 years, from 1885 to 1899, it was run as a temporary, but since 1900, after the implementation of the reforms, completion of the construction of a large barrack for the 5th Regiment and considerable growth in a number of soldiers, the Military Hospital of Valjevo began to function again as permanent. By the WWI, during the three decades of its existence, there were 12 Directors of the Military Hospital, with some of them performing the duty alongside the duties of medical desk-officers of the Drina Military District.

As we pointed out, the first Director of Military Hospital of Valjevo was a medical Captain 2nd class, Dr Jovan Djokić. In 1884, he was acting as a medical desk-officer of the Drina Division Command. The Assistant Director of the District Hospital was Dr Svetozar Atanasijević, a physiologist, and there was also Waclaw Sypniewski, a contractual medical assistant 1st class. In the following years, from 1885 until June, the duty of the Director of the Military Hospital was performed by Major Dr Vasa Brentović. When the Serbian-Bulgarian War started, the regiments of the Drina Division were also engaged. Its military desk-officer Major Dr Ljubomir Vesović was killed during the Battle of Slivnica which the unprepared Serbian army lost. During the war, Major Dr Alois Kumer stayed in Valjevo, in the Military Hospital and Regional Command, and after signing the peace and according to a peacetime schedule of 1 – 13 March 1886, remained in the office performing the same duties [30].

During the three-year period from 1886 to 1888, a sanitary major Dr Oswald Hajnc was the Director of the Military Hospital, as well as the desk-officer for the Department of the Drina Regional Divisional Command, while Sub-Lieutenant Ljubomir Subotić was an apothecary. Soon after that, during 1888, the function of the Director was temporarily performed by a medical Captain 2nd class, Dr Mihailo Lorenc, replacing the sick Hajnc, who died at the beginning of 1889, from "incarcerated hernia" [31]. In 1889, both positions were held by Dr Jevta Ristić, a medical Captain 1st class, while Dr Lorenc was still working with him as a doctor. A similar situation remained during the following 5 years, from 1890 to 1894, according to the state scheme, Dr Ristić performed only the duties of a sanitary desk-officer in 1890, whereas, in 1890 Dr Lorenc was the acting Director of the hospital while in 1891 that position was vacated (from 1892 to 1894), and Dr Ristić (since 1893) resumed both functions, and Dr Lorenc (attained the rank of Captain 1st class in 1892) still remained a doctor. During this period, a doctor assistant was Pavle Oško, a contracted Sub-Lieutenant, and Ljubomir Subotić, a physiotherapist, was an assistant. Since 1890, there was a notion about a position of a doctor for the Garrison Headquarters, which was occupied by young doctors, but it often used to be vacant. During the next two years, from 1895 to 1896, Major Dr Ristić was again at the position of the medical Director of the Drina Regional Divisional Command, and Dr Jevgenije Branovački, Lieutenant Colonel, replaced him in 1897, while Dr Svetozar Arsenijević (attained the rank of Major in 1896), who previously, in 1891, as a Lieutenant, worked as a troop doctor for the Garrison Headquarters. Besides him, there was a pharmacist

employed in the hospital, but not another doctor, while young doctors were replacing each other out of the hospital, as a Garrison Headquarters troop doctors, and one of them, medical Lieutenant Andreja Liška, a medicine undergraduate, became a doctor in the Military Hospital in1897.

Over the next three years, from 1898 until 1900, the position of the Director of the Military Hospital is taken over by the medical Lieutenant Colonel Dr Roman Dalmajer, who in 1900 took over from Dr Branovački the position of the District medical desk-officer. At that period, in the hospital, beside an administrator and pharmacist (former physiotherapist) there was always one doctor (occasionally two), and, out of the hospital, one troop doctor either at the Garrison Headquarters, or at the King Milan 5th Infantry Garrison, yet not every year. Since 1906, these positions are taken over by hospital doctors as additional duties.

From 1901 to 1903, the positions of a medical desk-officer for the Drina Regional Divisional District and a Director of Drina's permanent military hospital were joined again into one and performed by a sanitary Lieutenant Colonel, Dr Josif Hrnjiček who worked together with a hospital physician and pharmacist. However, there were no special troop doctors outside the hospital. From 1904 to 1909, Dr Milan Pecić, a medical Major (attained the rank of Lieutenant Colonel in 1908), was in charge of the Headquarters of the Drina permanent Military Hospital. He was also appointed a medical desk-officer in 1905 replacing Dr Hrnjiček, who in 1904 ceased to perform this duty. Since 1906 until the beginning of the war in 1914, the medical desk-officer was Lieutenant Colonel Dr Vladimir A. Popović (attained the rank of Colonel in 1912) and beside him, there were one pharmacist and one doctor engaged in the hospital, too.

In the second year of Dr Pecić's managing the hospital, in summer 1905, in the Units of Valjevo, primarily in the 17th Infantry Regiment Garrison located in the barracks on Ilidža, a large epidemic of abdominal typhus spread. There were 136 soldiers from the 17th Garrison (16% out of a total), 5 out of 5th Garrison, 1 driver and 9 male nurses (out of 40); 9 males died. Three doctors fought the epidemic, one of them also performed a duty of a sanitary desk-officer, and otherwise small capacities of the hospital were increased from 35 to 50 beds. That is why many soldiers were in one of the pavilions of the 17th Garrison barracks, while officers were treated at their homes [32–33]. The second epidemic, but with fewer consequences, was the epidemic of dysentery and was recorded in summer 1909 [34–35].

Since 1910, Dr Jordan T. Stajić, a medical Major (attained the rank of Lieutenant Colonel in 1912) became the Director of the Department of Surgery, while Dr Pavle Vojteh, a Captain 1st Class, was at the Internal Department and Melhior Artiko a pharmacist. Dr Stajić was absent for a few months, from April to the beginning of the wars in the fall, and he was replaced by Lieutenant Colonel Dr Negosav Velizarić.

Military Hospital of Valjevo in Balkan Wars

Before the horrors Valjevo went through during the WWI, Valjevo hospital had been in the rear lines, and accepted not only Serbian, but also wounded enemies during the Balkan wars in 1912–13, when besides the permanent hospital, the two reserve military hospitals were opened to operate in several city facilities [36]. After the war started, all military doctors went to military units, while in Valjevo, a district doctor Dr Selimir Djordjević and a young Valjevo citizen, a Berlin student Dr Draginja Babić, were working in the military hospital. But many foreign doctors, who already came to Valjevo, were also helping them. Some of them wrote their memories at this time; such was a young Swiss adventurer, Dr Hans Vogel, who three decades later described his too romanticized and rich in imagination his observations from the First Balkan War [37]. Varaždin City physicist Dr Stjepan Stanković was much more objective and more precise when sharing his observations with readers of the local newspaper "Our Right". From his articles, we learned that 'besides 2 Serbian doctors in the District Hospital in Srebrenica', a man and woman (apparently Dr Selimir Djordjević and Dr Draginja Babić), a total of 11 doctors worked in Valjevo. Six of them were foreign volunteers (3 Danes, 2 Czechs and he, a Croat) along with 5 old Serbian doctors and 8 medical practitioners (4 Czechs). We also found out that the wounded, who came from all war actions led against the Turkish Vardar Army (Kumanovo, Merdare, Prilep, Bitola), were put up in the buildings of 5th Garrison (400 beds, the Director Dr S. Stanković, 1 Czech doctor and 3 Czech medical practitioners), in gymnasium and elementary school which together with a small military and district hospital, counted 1,100 beds. Dr Stanković wrote that wounded soldiers from the first battles were in better condition because they had been wounded by bullets, while from the battles that took place later, due to a long transport and poor conditions, especially after the Battle of Bitola, they came exhausted and with more additional complications. We also found out that there were 2,000 Turkish prisoners in Valjevo. There were also medical technicians among them and they were treated well [38–40].

Similarly to Dr Stanković whose records referred to the First Balkan War, a young Dutchman Dr Arius van Tienhoven also wrote for the newspaper readers from his homeland about events in Valjevo during the Second Balkan War. He had already been in Serbia during the previous war, and when the war with Bulgaria began, he responded to the invitation of the Serbian Red Cross. He returned to Belgrade again with an operating sister de Groote, Dr Van Hammel, 2 "half-doctors" and 9 nurses, with the material help organized by "Balcan Committee" founded by his father and his friends. He was sent from Belgrade to Valjevo with an X-ray apparatus and arranged a hospital for one day (he did not describe in which building), and that on the first evening he admitted 230 wounded. He worked in Valjevo until September when the last patient was dismissed, and after being proclaimed a honorary citizen of Valjevo, he returned to the Netherlands [41–42].

After the end of the Balkan Wars, the Military Hospital of Valjevo returned to its former state and Dr Jordan Stajić, as its Director, remained at this position until the beginning of the WWI. During this short interlude from 1913 to 1914, Major Pavle Vojteh, a Russian contracted Lieutenant, Dr

Ivan Vladisavljević, and a pharmacist Melhior Artiko worked at the Internal Department. The doctor of the garrison was the contractual Captain 2nd class, Dr Simeon Swiff [43].

The location of the Military Hospital of Valjevo

The question of the location of the Military Hospital of Valjevo has not been the subject of historical research so far, nor was question about the oldest purposefully built buildings for the Civilian Hospital of Valjevo which has appeared recently [44]. According to the memories of the old Valjevo citizens, the Military Hospital in the period between the two World Wars was located in the city centre, at the initial part of the Pop Lukina Street, which after the WWI was renamed into Dr Stajić Street, after its long-time Director. The remaining question is whether the Military Hospital was before and during the War at the same place or perhaps some other one, including the barracks in Ilidža and Krušik.

Previously, there was a scarce information which could not help with locating the place of the Hospital and this was presented in a template report sent by Dr Mijailo Cvijetić, a Valjevo district doctor, in February 1904 to the Ministry of Internal Affairs, where we can read that there were two hospitals in Valjevo and that one "district, suits pretty much while the other permanent Military one, does not at all. " [45]. But there was no information about the position of this inadequate hospital. Additional information, very valuable, but again with the ambiguous description of its exact position was given by one of the military hospital directors, Dr Milan Pecić, in his article describing the above mentioned epidemic of the abdominal typhus in 1905. Dr Pecić pointed out that a small, but unsuitable hospital was located 500 meters from the barracks, "going through the main street - a centre", located in a rented tavern (mehana) nearby a market and that there was a hotel just across the tavern/hospital, and that "a patient in the hospital is dying while across the street loud music plays" [32].

This determinant, bearing in mind the content of the urban milieu of Valjevo, could indicate that the tavern/hospital was not far away, neither from the hotel Grand, nor from the hotel Sekulić, since both the town hotels were then located on smaller town squares how the word "market" should be properly understood. Therefore, it is more likely that it goes on about the Sekulić Hotel, since it was located about 500 meters from the barracks, while there were about 800 meters from the Grand Hotel to the barracks; it was also because Sekulić was at the beginning of the Pop Lukina Street, where later the Military Hospital of Valjevo was located, as it was already pointed out. The definitive confirmation of this assumption was a text published on 8/20 November 1909 in the Belgrade newspaper Večernje Novosti, which read as follows: "Harmful to health: The military hospital in Valjevo is in the middle of the town, opposite the biggest hotel of Drago Sekulić, where parties are frequent and where the theatre plays. They shoot in the theatre, Gypsies sing and play in the tavern, sometimes the military music, which does a harm to the peace of the patient. Hygiene does not allow this, and it's time for the hospital to be where it should be once and for all" [46].

As we can see, the Military Hospital was probably in 1904, and certainly, in 1905 and 1909 located at the same place where it was after the WWI - at the beginning of the Pop Lukina Street, i.e. the Stajiceva Street, across the road from the Sekulic Hotel. And even during the WWI, there are some records from the memoirs of an Austro-Hungarian prisoner, Fr. Gabro Cvitanovic who described where he was working during 1914-1915 and said the following: "I was in the Military Hospital of Drina near the Sekulic Hotel" [47] (Figure 1).

After all, from the moment the Military Hospital became permanent if not earlier, it was located in the mentioned facilities which used to be a tavern (mehana), and, according to the practice of that time, had lodging rooms as well as a yard with facilities for cattle and goods. It probably should have been an initial, provisional solution, but it turned into long-term one, which could also be seen at the example of organizing the temporary Civilian Hospital of Valjevo in 1867 [5, 44].

By further research, we came across a photograph took in Valjevo in 1914/15 by a Dutch surgeon Dr Arius van Tienhoven, where the yard of Military Hospital of Valjevo can be seen. It was published in the 1st Dutch edition of his book of memory of war events 1914/15 [48], but it was not published in the Serbian edition [49]. There could be seen a part of the courtyard of the Military Hospital with a longitudinal ground-based facility. It was just a part of the hospital complex, and the whole could be recognized on the geodetic plan of Valjevo from 1926, where this yard was also recognized [50] (Figure 2).

Fig. 1 – a) The building where the Military Hospital was situated (Photography archive of the National Museum in Valjevo), and b) today's view of the same building after it has been completely reconstructed; on the right side, you can see the building where the Hotel Sekulić used to be (photography: V. Krivošejev).

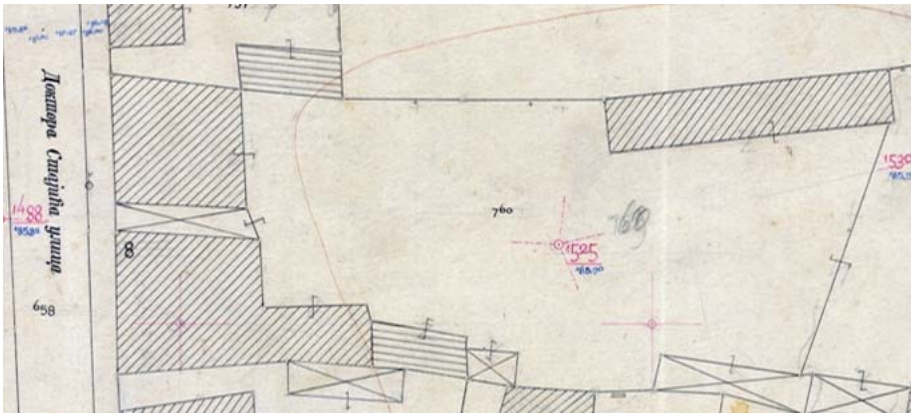

Fig. 2 – A plot of land of the Military Hospital from the Cadastre plan of Valjevo in 1926 (Digitalized cadastre diameter of Valjevo from 1926. Valjevo: Immovable Property Cadastre, Valjevo).

Fig. 3 – a) A view on the Military Hospital from the inner yard on the photography taken by Arius van Tienhoven in 1914 (A. van Tienhoven De gruwelen van der oorlog in Servie – het dagboek van den oorlogs-chirurg. Den Haag; 1915), and b) today (photography: V. Krivošejev).

Finally, it is necessary to emphasize that the mentioned complex in which the Military Hospital of Valjevo used to be, still exists. The street-side building belongs to the company Erozija, and it has the same dimensions; it was fundamentally reconstructed during the 1980s, and in the depth of the yard there was a municipal plot with a car park and offices in the building that can be seen on the Van Tienhoven's photograph (Figure 3).

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E R R A T U M

In the article:

Arsić S, Perić P, Stojković M, Ilić D, Stojanović M, Ajduković Z, Vučić S. Komparativna analiza linearnih morfometrijskih parametara humane mandibule dobijenih direktnim i indirektnim merenjem [Comparative analysis of linear morphometric prameters of the **humane mandibula** obtained by direct and indirect measurement]. Vojnosanit Pregl 2010; 67(10):839-46.

there was an error in the title in English.

This title should have read: Comparative analysis of linear morphometric prameters of the **human mandible** obtained by direct and indirect measurement.

The correction has been made to the online version of that issue of the Journal which is available at: http://www.vma.mod.gov.rs/vsp_10_2010-1.pdf .

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

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Primeri referenci:

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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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