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LETTER TO THE EDITOR / PISMO UREDNIKU

Dr. Charles Richard Drew (June 3, 1904 – April 1, 1950), an American surgeon was particularly involved in blood transfusion research. He developed improved blood storage techniques which were used to develop large blood banks during World War II. He also improved the process of collecting blood with the aim of avoiding possible contamination, as well as the way of transporting donated blood.

Otherwise, every year, on June 14 the World Blood Donor Day is celebrated.

Dr Charles Richard Drew (3. jun, 1904–1. april, 1950), američki hirurug, posebno se bavio istraživanjima u oblasti transfuzije krvi. Razvio je poboljšane tehnike čuvanja krvi koje su korišćene za razvoj velikih banaka krvi tokom Drugog svetskog rata. Takođe, poboljšao je i proces prikupljanja krvi sa ciljem izbegavanja eventualne kontaminacije i unapredio način transporta donirane krvi.

Inače, svake godine, 14. juna obeležava se Svetski dan dobrovoljnih davalaca krvi.

ORIGINAL ARTICLES

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https://doi.org/10.2298/VSP180417101D

Interatrial conduction time is early marker of disturbed impulse propagation in adults with slightly elevated blood pressure

Kašnjenje električnog impulsa između dve pretkomore je rani marker usporene propagacije impulsa kod odraslih osoba sa blago povišenim krvnim pritiskom

Dijana Djikić*, Nebojša Mujović*†, Vojislav Giga*†, Milan Marinković*, Goran Trajković†‡, Snežana Lazić§, Vedrana Pavlović†‡, Vladan Perić§, Dragan Simić*†

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Abstract

Background/Aim. Interatrial conduction time is early marker of disturbed impulse propagation in adult with elevated blood pressure. The aim of our study was to evaluate significance of noninvasive echocardiographic marker of slow sinus impulse propagation (atrial conduction time) for the identification of persons with slightly elevated blood pressure and hypertension in adults. **Methods.** One hundred and forty nine adults with normal and elevated blood pressure were studied: 46 normotensive adults (group 1), 28 adults with elevated blood pressure and hypertension stage 1 (group 2) and 75 adults with hypertension stage 2 (group 3), based on the Joint National Committee 8 (JNC-8) hypertension guidelines. We studied P wave dispersion, reservoir function of the left atrium (LA), total emptying volume of the LA and total emptying fraction of the LA (LATEF). The atrial conduction time (ACT) was evaluated by the pulsed tissue Doppler, and expressed as interatrial and intraatrial conduction time. **Results.** The LATEF decreased progressively from the group 3 (64.8 ± 4.4%) to the group 2 (59.8 ± 5.2%) and the group 1 (55.6 ± 7.3%) ($p < 0.001$). The P wave dispersion (55.1 ± 9.8 ms vs. 46.8 ± 3.1 ms vs. 43.1 ± 2.6 ms; $p < 0.01$) and intra ACT were significantly prolonged only in the group 3 compared to the other groups (22.7 ± 11.0 ms vs. 8.4 ± 4.7ms vs. 5.6 ± 2.4 ms, respectively; $p < 0.001$). Inter ACT significantly increased from the group 1 to the group 2 and the group 3 (15.6 ± 3.9 ms vs. 24.6 ± 5.7 ms vs. 50.4 ± 20 ms, respectively; $p < 0.05$). Using a cut-off level of 19.5 ms, inter ACT could separate adults in the group 2 from the group 1 with a sensitivity of 85%, and specificity of 89% [area under receiver operating characteristic (ROC) curve 0.911]. **Conclusion.** Prolonged ACT estimated with the tissue Doppler may be useful for identification persons with slighty elevated blood pressure, and hypertension stage 1.

Key words:
blood pressure; hypertension; echocardiography, doppler; electrocardiography; diagnosis.

Apstrakt

Uvod/Cilj. Kašnjenje električnog impulsa između dve pretkomore je rani marker usporene propagacije impulsa kod odraslih osoba sa povišenim krvnim pritiskom. Cilj ovog rada bio je da se, koristeći novu ehokardiografsku metodu za procenu vremena provođenja impulse kroz pretkomoru, identifikuju odrasle osobe sa blago povišenim krvnim pritiskom i arterijskom hipertenzijom. **Metode.** Ispitivano je 149 odraslih osoba sa normalnim i povišenim krvnim pritiskom: 46 normotenzivnih zdravih osoba (grupa 1), 28 osoba sa blago povišenim krvnim piriskom i hipertenzijom stepena 1 (grupa 2) i 75 bolesnika sa arterijskom hipertenzijom stepena 2 (grupa 3), prema poslednjim *Joint National Committee* (JNC) 8 preporukama. Ispitivana je funkcija rezervoara, prikazana kao totalni volumen pražnjenja leve pretkomore (TEV) i totalna frakcija pražnjenja leve pretkomore (TEF). Vreme provođenja impulsa je izmereno pulsnim tkivnim Doplerom, uključujući vreme provođenja impulsa u levoj pretkomori i vreme provođenja impulse između dve pretkomore. **Rezultati.** Vrednosti TEF su se progresivno smanjivale od grupe 1, preko grupe 2 do grupe 3 ($p < 0,001$). Disperzija P talasa (55,1 ± 9,8 ms vs. 46,8 ± 3,1 ms vs. 43,1 ± 2,6 ms; $p < 0,01$) i vreme provođenja impulsa unutar leve pretkomore (22,7 ± 11,0 ms vs. 8,4 ± 4,7 ms vs. 5,6 ± 2,4 ms; $p < 0,001$) su bili značajno

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produženi samo u grupi 3. Vreme provođenja impulsa između dve pretkomore značajno se povećavalo od grupe 1, preko grupe 2 do grupe 3 (15,6 ± 3,9 ms vs. 24,6 ± 5,7 ms vs. 50,4 ± 20 ms; $p < 0,05$ i $p < 0,05$). Vrednost kašnjenja impulsa između dve pretkomore od 19,5 ms može odvojiti odrasle osobe sa povišenim krvnim pritiskom od normotenzivnih zdravih osoba sa osetljivošću od 85%, i specifičnošću od 89% [površina ispod *receiver operating characteristic* (ROC) krive 0,911). **Zaključak.** Produženo vreme provođenja impulsa između dve pretkomore procenjeno pulsnim tkivnim Doplerom može biti korisno za identifikovanje osoba sa blago povišenim krvnim pritiskom i arterijskom hipertenzijom prvog stepena.

Ključne reči:
krvni pritisak; hipertenzija; ehokardiografija, doppler; elektrokardiografija; dijagnoza.

Introduction

Elevated blood pressure can easily turn into high blood pressure (hypertension) unless one changes lifestyle habits. Both elevated blood pressure and high blood pressure increase risk of cardiovascular morbidity, mortality and stroke. It is highly prevalent in the general population [1]. The prolonged elevation of blood pressure leads to the structural and functional remodeling of the left atrium (LA). LA dilatation and fibrosis lead to impaired impulse conduction and nonhomogeneous propagation of impulses, both known as electrophysiological characteristics of atrial fibrillation. Enlargement of the LA and impairment of the LA mechanical function are potential indicators of the presence of cardiac diseases and arrhythmias [2, 3]. Atrial conduction time (ACT) reflect electrical and structural remodeling of the atria. Tissue Doppler imaging, a simple, non-invasive and applicable method, enablesthe evaluation of impulse propagation through the atria [4, 5]. Sequential analysis of atrial electromechanical coupling by the Tissue-Doppler imaging enables the analysis of atrial electromechanical coupling between the regions. Previous studies have shown that prolongation of interatrial and intra-atrial electromechanical times promotes atrial fibrillation [6–10]. Adults in early hypertensive disease are asymptomatic and it is of importance to establish early diagnosis and to assess the presence of subtile structural changes in their cardiovascular system [11].

Our hypothesis was that a noninvasive echocardiographic marker of slow sinus impulse propagation (ACT) may identify adults with early hypertensive disease and hypertensive adults.

Methods

One hundred and forty nine adults with normal and elevated blood pressure were studied: 46 normotensive persons (group 1) [blood pressure (BP) range: systolic blood pressure (SBP) < 120 mmHg, diastolic blood pressure (DBP) < 80 mmHg]; 28 adults with elevated blood pressure (SBP range: 120–129 mmHg, DBP < 80 mmHg) and hypertension stage 1 (SBP 130–139 mmHg, DBP 80–89 mmHg) (group 2), and 75 adults with hypertension stage 2 (group 3) (SBP ≥ 140 mmHg and (or) a DBP ≥ 90 mmHg). Blood pressure classification was based on the Joint National Committee-8 (JNC-8) guidelines, using an average of ≥ 2 readings obtained ≥ 2 occasions (officially based). We examined 243 consecutive participants with elevated blood pressure, and hypertension stage 1 and 2 who were in sinus rhythm referred by echocardiography. Patients with hypertension stage 2 were medically treated, i.e. they had pharmacologically regulated hypertension.

Patients with impaired ejection fraction of the left ventricle (LV) less than 50%), valvular heart disease, left or right bundle branch block, electrocardiographic conduction impairment, pericarditis, thyroid dysfunction, anemia, electrolyte disbalance, renal insufficiency, pulmonary disease, poor echocardiographic image as well as patients with documented paroxysms of atrial fibrillation, or those converted to a sinus rhythm pharmacologically or electrically, and who were on antiarrhythmic therapy, were excluded from further analysis. Therefore, the final study population consisted of 149 patients.

Clinical examination included recording of their weight, height, blood pressure and resting heart rate.

Standard 12-lead electrocardiograms (ECGs) were obtained using a recorder (Cardioexress SL 12) set at a 25 mm /s paper speed, and 1 mV/cm standardization. We measured P wave duration manually with calipers and a magnifying glass. The mean P wave duration of 3 complexes was calculated in each lead. P maximum and P minimum were measured in 12 leads of the ECG surface. The difference between the P maximum and the P minimum was calculated and defined as P wave dispersion.

All the examinees were screened by an echocardiographer. Transthoracic echocardiographic examination was performed on the Vivid T8 (GEHealthcare) using phased array transducer of 3.5 and 2.5 MHz. During an echocardiographic examination, one ECG lead was continuously recorded.

M mode measurements and Doppler echocardiographic examination were performed according to the criteria of the American Echocardiographic Association [12, 13]. M mode measurements included: the dimensions of the LA, end-systolic and end-diastolic dimension of the LV and dimension of the LA in the parasternal longitudinal section. The LV ejection fraction (LV EF) was determined according to Simpson's rule. By using the pulsed Doppler in the apical four chambered view was measured the mitral flow, early diastolic (E wave) and late diastolic (A wave) inflow, E/A ratio, isovolumetric relaxation time (IVRT) and deceleration time of the mitral E wave (DT).

The volume of the LA (LAV) was measured from the apical four chamber view cross section, by using the biplane area length method [14-16]. LAV is indexed in relation to the surface of the body and expressed in mL/m^2. The LAV maximum (LAV max) was measured at the end of the ventricular systole, at the beginning of the opening of the mitral valve. LAV presystolic (LAV pre A) was measured in the middle

diastole at the beginning of the atrial systole (P ECG wave form) and LAV minimal (LAV min) at the start of closure of the mitral valve.

The parameters of the LA function were calculated from the LAVmax and LAV min. Reservoir function was presented as total emptying volume (TEV) and total emptying fraction (TEF). TEV was calculated as difference between LAVmax and LAVmin. TEF of the LA was calculated as (LAVmax-LAV min) / LAVmax x 100.

The atrial conduction time was evaluated by the pulsed tissue Doppler. The frequencies of transducers were 3.5 to 4.0 MHz. Adjusting the pulse Doppler signal to a Nyquist limit of 15 to 20 cm/s was done by using the minimum optimal gain. The signal speed of the monitor was set to 50-100 mm/s to optimize the spectral display of myocardial velocity. In the apical four chamber view section of the cursor, pulse tissue surplus was placed on the lateral mitral LV anulus, the septal mitral LV anulus, and the tricuspid anulus of the right ventricle to obtain spectral tissue Doppler image (TDI). Peak early diastolic (Em), and late diastolic (Am) wave were measured out of the surfaces. The same observer performed all echocardiographic measurements.

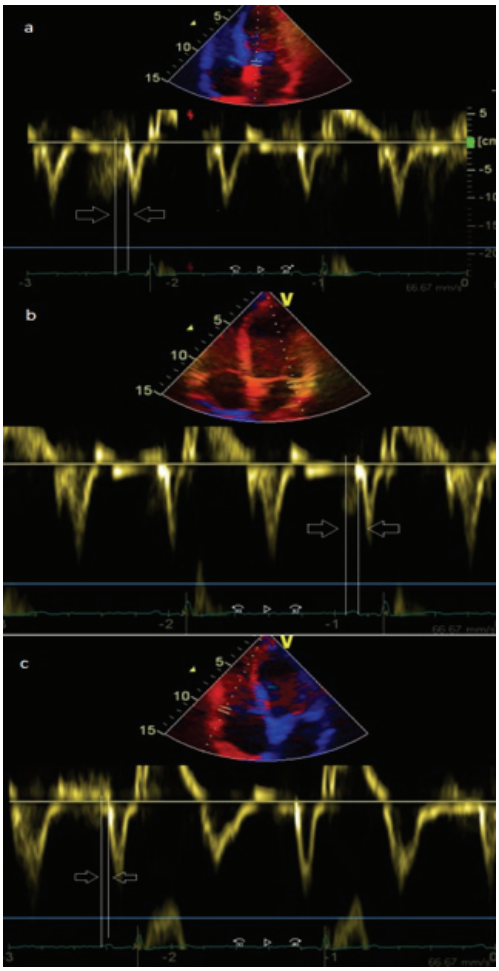

Fig. 1 – Measurement of time interval with tissue Doppler echocardiography, from the onset of P wave on surface of electrocardiogram (ECG) to the beginning of Am wave: a) on mitral septal annulus; b) on mitral lateral annulus; c) on tricuspid annulus.

The time from the beginning of the P wave on the ECG to the beginning of the Am wave of the tissue Doppler signal was accepted as the atrial conduction time (ACT). The difference in ACT of the left mitral lateral annulus and the right ventricle tricuspid annulus was defined as inter-ACT. The difference in ACT of the mitral lateral LV annulus and mitral septal LV annulus was defined as an intra-ACT (Figure 1).

A local Ethics Committee approved the study.

Data analysis

The descriptive statistics, including means and standard deviations of continuous variables, as well as frequencies and percentages of categorical variables were used to characterize the study sample. The differences among groups on continuous and categorical variables were analyzed by the use of one-way analysis of variances and the Pearson χ^2 test, respectively. For atrial conduction time, as the main predictor of interest for the detection of adults with elevated blood pressure vs. normotension, we calculated measures of diagnostic accuracy, including sensitivity, specificity and area under curve (AUC). The R environment for statistical computing (R Core Team, 2016) and IBM SPSS software (version 22) were used to conduct statistical analyses. Significance level (alpha level) was set at 0.05.

Interobserver variability was analyzed using the interclass coefficient (Table 1).

Table 1

Intraobserver variability

Variable	ICC
LAVImax	0.998
LAVImin	0.997
LAVIpreA	0.999
ACT septal (ms)	1.000
ACT lateral (ms)	0.899
ACT tricuspid (ms)	0.877

ICC – interclass coefficient; ACT – atrial conduction time; LAVImax – left atrial volume index maximum; LAVImin – left atrial volume index minimum; LAVIpreA – left atrial volume index presystolic.

Results

There was no diference in terms of age, sex, resting heart rate, LV EF, BMI among all three groups (all $p > 0.05$). Through the study design, systolic blood pressure was significantly higher in the group 2 (all $p < 0.001$). Distolic blood pressure was higher in the group 3 and the group 2 ($p < 0.001$) compared to that in the group 1 (Table 2).

Patients in the group 3 had higher LA diametar, LAVI max, LAVI pre A, LA TEV and LVS Em than those ofthe groups 2 and 1 (all $p < 0.001$). E/A ratio and LVS Em in the group 3 were significantly lower than those of the groups 1 and 2. E/Em velocity and LAVI min increased progressively from the group 1 to the groups 2 and 3 (all $p < 0.001$). LA TEF decreased progressively from the group 1 to the groups 2 and 3 (all $p < 0.01$). Peak mitral E wave was significantly higher in the group 1 compared to the groups 2 and 3 ($p < 0.001$) (Table 3).

Table 2

Baseline characteristics of the study population

Characteristics	Group 1	Group 2	Group 3	p^a	p^b	p^c
Age (years), mean ± SD	51.0 ± 9.3	50.6 ± 10.5	53.5 ± 9.8	0.986	0.401	0.422
Gender, n (%)				0.106	0.047	1.00
male	24 (54)	20 (77)	50 (75)			
female	20 (46)	6 (23)	17 (25)			
BMI (kg/m^2), mean ± SD	26.8 ± 2.1	25.6 ± 1.4	26.2 ± 2.8	0.100	0.239	0.671
SBP (mmHg), mean ± SD	106.8 ± 3.3	135.5 ± 2.1	123.2± 5.8	< 0.001	< 0.001	< 0.001
DBP (mmHg), mean ± SD	72.2 ± 2.6	84.3 ± 2.2	81.8 ± 6.5	< 0.001	< 0.001	0.067
Heart rate (beat/min), mean ± SD	74.4 ±3.5	75.3 ± 3.9	74.1 ± 7.9	0.839	0.965	0.689
LVEF (%), mean ± SD	66.4 ± 3.8	64.4 ± 9.3	64.1 ± 8.1	0.523	0.237	0.978

Group 1 – normotensive adults; Group 2 – adults with elevated blood pressure and hypertension stage 1;
Group 3 – adults with hypertension stage 2.
BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; LVEF – left ventricle ejection fraction; SD – standard deviation; p^a – significance of the difference between groups 1 and 2; p^b – significance of the difference between groups 2 and 3; p^c – significance of the difference between groups 1 and 3.

Table 3

Left ventricle and left atrial structural and functional parameters in the study population

Variables	Group 1 (mean ± SD)	Group 2 (mean ± SD)	Group 3 (mean ± SD)	p^a	p^b	p^c
P-WD (msec)	43.1 ± 2.6	46.8 ± 3.1	55.1 ± 9.8	0.096	< 0.001	< 0.001
E/A ratio	1.4 ± 0.03	1.3 ± 0.14	0.9 ± 0.28	0.109	< 0.001	< 0.001
LA diametar (mm)	3.2 ± 0.39	3.4 ± 0.27	3.8 ± 0.40	0.110	< 0.001	< 0.001
LAVI max. (mL/m^2)	24.7 ± 6.9	28 ± 7.5	40 ± 6.8	0.092	< 0.001	< 0.001
LAVI min. (mL/m^2)	8.7 ± 2.2	11.3 ± 2.8	17.8 ± 5.2	0.030	< 0.001	< 0.001
LAVIpreA (mL/m^2)	11.9 ± 2.6	14.7 ± 3.4	29.4 ± 7.3	0.110	< 0.001	< 0.001
LATEV (mL/m^2)	16.0 ± 2.5	16.7 ± 2.5	22.1 ± 2.5	0.702	< 0.001	<0.001
LATEF (%)	64.8 ± 4.4	59.8 ± 5.2	55.6 ± 7.3	0.004	< 0.001	0.011
LV DT (msec)	142.5 ± 8.1	145.1 ± 12.3	194.6 ± 29.1	0.882	< 0.001	< 0.001
IVRT (msec)	101.9 ± 18.2	110.1 ± 14.3	128.8 ± 15.8	0.105	< 0.001	< 0.001
LVSEm (m/s)	10.5 ± 1.8	9.8 ± 1.3	6.7 ± 1.7	0.267	< 0.001	< 0.001
LVSE/Em	7.2 ± 1.4	9.5 ± 2.5	12.5 ± 2.1	< 0.001	< 0.001	< 0.001
Peak mitral Ewave (m/s)	8.3 ± 0.5	7.5 ± 0.9	7.1 ± 0.9	0.001	< 0.001	0.153
Peak mitral A wave (m/s)	8.6 ± 1.7	9.0 ± 1.6	9.2 ± 1.4	0.577	0.107	0.799

Group 1 – normotensive adults; Group 2 – adults with elevated blood pressure and hypertension stage;
Group 3 – adults with hypertension stage 2.
PWD – P wave dispersion; LA – left atrium; LV – left ventricle; LAVI – left atrial volume index; IVRT – isovolumetric relaxation time; DT – deceleration time; LATEV – left atrium total emptying volume; LATEF – left atrium total emptying fraction; LVSEm – septal tissue Doppler early diastolic wave; E – early diastolic wave; A – late diastolic wave; SD – standard deviaton; min. – minimum; max. – maximum; p^a – significance of the difference between groups 1 and 2; p^b – significance of the difference between groups 2 and 3; p^c – significance of the difference between groups 1 and 3.

Table 4

Atrial conduction time assessed by the tissue Doppler imaging in the study population

Variables	Group 1 (mean ± SD)	Group 2 (mean ± SD)	Group 3 (mean ± SD)	p^a	p^b	p^c
ACT septal (ms)	20.9 ± 5.0	32.6 ± 6.5	58.3 ± 16.7	0.001	< 0.001	< 0.001
ACT lateral (ms)	26.6 ± 5.6	41.2 ± 6.9	81.1 ± 28.3	0.006	< 0.001	< 0.001
ACT tricuspid (ms)	10.9 ± 3.1	16.8 ± 4.9	29.8 ± 8.6	0.001	< 0.001	< 0.001
ACT lateral-ACTseptal (ms)*	5.6 ± 2.4	8.4 ± 4.7	22.7 ± 11.0	0.345	< 0.001	< 0.001
ACT lateral-ACTtricuspid (ms)†	15.6 ± 3.9	24.6 ± 5.7	50.4 ± 20.0	0.034	< 0.001	< 0.001

Group 1 – normotensive adults; Group 2 – adults with elevated blood pressure and hypertension stage;
Group 3 – adults with hypertension stage 2.
ACT – atrial conduction time, interval from the onset of P wave on the surface of electrocardiogram to the beginning of the late diastolic wave (Am wave) assessed by the tissue Doppler imaging; p^a – significance of the difference between groups 1 and 2; p^b – significance of the difference between groups 2 and 3; p^c – significance of the difference between groups 1 and 3; *intraartial conduction time; †interatrial conduction time.

P wave dispersion in the group 3 was significantly higher than that in the groups 1 and 2. Atrial conduction time findings measured by TDI are shown in Table 4.

Atrial conduction time on septal, lateral and tricuspid annulus were significantly increasing from the group 1 to the groups 2 and 3. There was no difference in inter ACT between the group 1 and the group 2. Patients in the group 3 had significantly prolonged intra ACT than those in the groups 1 and 2. Related to the group 1, inter ACT was prolonged in the group 2 ($p = 0.034$) and further prolonged in the group 3 ($p < 0.001$).

Using a cut off level of 19.5 ms, interatrial ACT could separate adults with slightly elevated blood pressure and hypertension stage 1 from normotensive persons with a sensitivity of 85%, and specificity of 89% [area under receiver operating characteristic (ROC) curve (AUC) 0.911] (Figure 2).

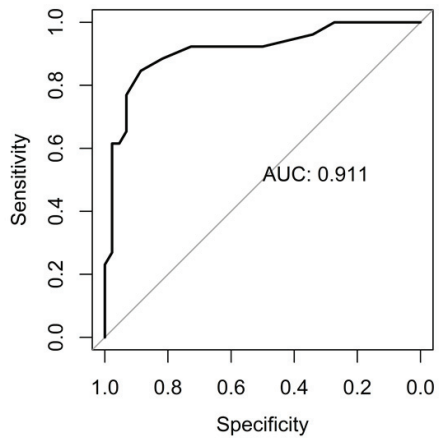

Fig. 2 – The area under receiver operating characteristic (ROC) curve (AUC) of the interatrial conduction time for predicting presence of early hypertensive disease vs. normotension was 0.911 (sensitivity 85%, specificity 89%). Optimum cut-off point was 19.5 ms.

Discussion

The principal finding of this study is that interatrial conduction time is a predictor of the presence of early hypertensive disease in adults. A cut off 19.5 ms for inter ACT had a sensitivity 85% and specificity 89% in identifying early hypertensive disease in adults. According to available sources, this is a new finding, which has not been previously reported.

In our study, we demonstrated that impairment in propagation of sinus impulses is present in hypertension. However, adults with slightly elevated blood pressure had a slowing of sinus impulse propagation. It was demonstrated that ACT on lateral, septal mitral, and tricuspid annulus, inter and intra ACT were prolonged in hypertension compared to normotension [17]. Atrial electromechanical delay and P wave dispersion are longer in hypertensions with elevated pulse pressure and without significant structural remodeling [18]. Inter and intra ACTs are prolonged in hypertension [19], and in early stages of hypertension without significant prolongation of maximum P wave duration on 12 lead ECG. This is an explanation for presence of earlier period of atrial electrical impairment, before the appearance of P wave prolongation on 12 lead ECG [6]. Our study shows that P wave dispersion was similar between normotensive persons and those with slightly elevated blood pressure, while it was significantly prolonged in patients with hypertension stage 2. Slowing atrial conduction velocity and atrial conduction delay can occur due to atrial fibrosis, remodeling and can be a trigger for atrial fibrillation. Atrial conduction delay can be easily discovered with tissue Doppler, and may be an indicator for the presence of arrhythmogenic substrate [16]. In our study we demonstrated significantly prolonged intra ACT, inter ACT and P wave dispersion in patients with hypertension, even when it is medically regulated. We found prolonged intra ACT in patients with early hypertension disease, without increase in P wave dispersion on 12 channel ECG, which is known as electrophysiological marker of inhomogenous propagation of sinus impulses. Our findings suggest that inter ACT conduction time measured by tissue Doppler is the first sign of impaired conduction of sinus impulses in the early stage of hypertension, even in persons with slightly elevated blood presure.

In addition to morphologic remodeling known to be present in hypertensive patients, even slightly elevated blood pressure also cause electrical remodeling of atria which are responsible for slowing conduction of sinus impulses. LA enlargement causes slowing of sinus impulse conduction because of structural and electrophysiological changes in the atrial myocardium.

Our study also showed impairment of the mechanical function of LA in hypertension but in adults with slightly elevated blood pressure as well. Impairment of the LA mechanical function and LA enlargement were common findings in hypertension [18, 19]. Decreased mechanical function of LA has been observed in recently diagnosed hypertensive patients and it was related to an increased LAVI min [20]. The LA volume and active systolic function assessed by real-time three-dimensional echocardiography (RT3DE) were significantly increased in prehypertension [16]. Some sources [21] suggested that the occurrence of paroxysmal atrial fibrillation in hypertensive patients is associated with enhanced LA reservoir and conduit function and worsening booster pump function. In our study population dimensions of the LA were normal. Using pulsed wave Doppler methods [DT, isovolemic relaxation time (IVRT), LV E/A, mitral A wave], we did not find complete impairment of diastolic function of the LA in the group 2, but we found it in the group 3. All the examinees including those with hypertension had normal LA dimensions. By analyzing the LA volume in various phases of the heart cycle, we showed that there was a deterioration of the mechanical function of the LA in the group 2, and significant impairment in the group 3, even though they were pharmacologically regulated. There was continuous trend in the progression of reduction in the atrial reservoir function from slightly elevated blood pressure to hypertension.

Our study demonstrated the presence of disturbance of atrial conduction in adults with slightly elevated blood pressure and significant impairment of interatrial and intraatrial sinus impulse conduction in medically controlled hypertensive patients.

We found that inter ACT measured by tissue Doppler may be useful echocardiographic marker for the identification of adults with slightly elevated blood pressure.

The limitation of this study is that atrial conduction time was not investigated by invasive electrophysiological techniques and compared to echocardiographic examination.

Another limitation was a single operator acquired echocardiographic and electrocardiographic measurements, that unabled us to compare interobsever variability.

Conclusion

Mechanical and electrical function of the LA is impaired in patients with early hypertensive disease and significantly impaired in hypertension. Prolonged atrial conduction time estimated with a tissue Doppler may be useful for distinguishing normotensive persons from those with slightly elevated blood pressure and those with hypertension stage 1.

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Assessment of oral health of the Serbian Armed Forces members

Procena oralnog zdravlja pripadnika Vojske Srbije

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Abstract

Background/Aim. Oral health is an integral part of general health. The state of oral health greatly affects the psychological and physical condition of patients. The aim of study was to determine oral health among the Serbian Armed Forces members. **Methods.** This prospective cross-sectional pilot study was conducted on 648 examinees at the mean age of 34.47 ± 8.14 years who had dental check-ups. All the categories of military personnel aged 18–64 years were divided into five groups. Assessment of oral health was obtained by clinical examination and the personal perception of oral health obtained by fulfilling the questionnaire "Oral Health Questionnaire for Adults" of the World Health Organization. The results obtained by processing questions from the questionnaire were compared with the data of clinical examination. **Results**. The average value of the the Decayed, Missing and Filled Teeth (DMFT) index was 10.55 ± 4.79; the mean value of decayed teeth per participant was 2.00 ± 2.55, and on average, each respondent was missing 3.21 ± 3.35 teeth. Based on data obtained from the questionnaire, 39.4% of the participants smoked cigarettes. The majority of subjects (58.3%) brushed their teeth regularly two or more times a day. Oral health as poor was assessed by 18.9% of the participants. Participants who brush their teeth less than twice a day evaluated their oral health as poor 3.08 times more often compared to those who brush their teeth more than twice a day. **Conclusion**. The self-assessment of poor oral health significantly failed when compared with a high value of DMFT which means that only a small percentage of participants evaluated their oral health objectively.

Key words:
adults; dmf index; military personnel; oral health; serbia; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Oralno zdravlje je sastavni deo opšteg zdravlja. Stanje oralnog zdravlja značajno utiče na psihološko i fizičko stanje pacijenata. Cilj studije bio je da utvrdi stanje oralnog zdravlja pripadnika Oružanih snaga Srbije. **Metode.** Studija preseka obuhvatila je 648 ispitanika, životnog doba 34,47 ± 8,14 godina, koji su imali stomatološki pregled. Sve kategorije vojnih lica starosti od 18 do 64 godine podeljene su na pet grupa. Procena oralnog zdravlja dobijena je kliničkim pregledom i ličnom percepcijom oralnog zdravlja dobijenom ispunjavanjem upitnika „Upitnik za oralnu zdravstvenu zaštitu odraslih" Svetske zdravstvene organizacije. Rezultati dobijeni obradom pitanja iz upitnika upoređeni su sa podacima kliničkog pregleda. **Rezultati.** Prosečna vrednost indeksa karijes, ekstrakcija, plomba (KEP) iznosila je 10,55 ± 4,79; prosečna vrednost karijesnih zuba po ispitaniku bila je 2,00 ± 2,55, a svakom ispitaniku nedostajalo je prosečno 3,21 ± 3,35 zuba. Na osnovu podataka dobijenih iz upitnika, 39,4% ispitanika su bili pušači. Većina (58,3%) ispitanika redovno je prala zube, dva ili više puta dnevno. Oralno zdravlje kao loše procenilo je 18,9% ispitanika. Ispitanici koji su prali zube ređe od dva puta dnevno procenili su svoje oralno zdravlje kao loše 3,08 puta češće u poređenju sa onima koji su prali zube dva i više puta dnevno. **Zaključak**. Samoprocena lošeg oralnog zdravlja značajno podbacuje u poređenju sa visokim vrednostima KEP indeksa, što znači da je samo nizak procenat ispitanika objektivno ocenio svoje oralno zdravlje.

Ključne reči:
odrasle osobe; dmf indeks; vojni kolektiv; usta, zdravlje; srbija; ankete i upitnici.

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Introduction

Oral health is multifaceted and includes several abilities – to speak, smile, smell, taste, touch, chew, swallow, and conveys a range of emotions through facial expressions with confidence and without pain, discomfort, and disease of the craniofacial complex. It is a fundamental component of health and physical and mental well-being. It reflects the physiological, social, and psychological attributes that are essential to the quality of life [1]. Oral illnesses and disorders may have a negative impact on the individuals' life.

Nowadays, numerous studies deal with the oral health condition measured by the the Decayed, Missing and Filled Teeth (DMFT) index, but there is a significantly smaller number of studies dealing with self-assessment of oral health made by patients themselves. Clinicians when examining oral health measure the caries index, such as the number of carious, extracted and filled teeth. These indices may indicate the severity of oral problems. However, the perception of oral health has to be reported by patients themselves [2].

Information on caries prevalence, oral hygiene status and periodontium condition are very important for establishing priorities and determining the type of preventive measures, as well as the provision of necessary services and treatment [3].

It is commonly accepted that oral diseases can have different effects on people and their life quality. Dental diseases cause pain, discomfort, and affect functions such as chewing, speech and smiling. The results of various studies show that dental treatments may improve the life quality. Medical practice has also recognized an increasing importance of oral health assessment made by patients themselves. However, in dental literature there is only a few data concerning oral health condition assessed by patients themselves. Therefore, this is a possible field for future research, which should focus on the self-assessment of life quality; this would be a secondary, or even primary measure in assessing the health condition [4].

Military members represent a pillar of security and safety for the whole country; therefore, their general health is of exceptional importance. The aim of this study was to examine the relationship between the oral condition measured through the DMFT index and the perception of oral health reported by the Serbian Armed Forced (SAF) members themselves.

Methods

The study was conducted as a cross-sectional observational study. It is adapted to the Strengthen Reporting of Observational Studies in Epidemiology (STROBE) [5]. The study was approved by the Ethics Committee of the Military Medical Academy in Belgrade, Serbia (No. 1/ 15 - 17). The study was conducted in the period 2016–2017, in accordance with the Helsinki Declaration [6]. Each subject voluntary participated and was informed about the type of research and the process of data collection. For determination of oral health of SAF members, calculation of adequate sample size, based on population prevalence of carious lesions was needed. According to data presented by Frencken et al. [7], 2017, prevalence of untreated cavitated, dentine carious lesions in Central Europe in 2010 was 47% (0.47). Based on this data, with the test power of 80%, alpha probability of 0.05 and expected error of 5%, the calculated number of participant was at least 383. The study included 655 randomly selected professional members of the Serbian Army. The criteria for entering the study were that the subject was over 18 years old and younger than 64 and a professional member of the SAF. The exclusion criteria from the study were the professional status of a civilian employee in the SAF, presence of systemic diseases and acute symptoms of dental diseases. According to these criteria the study seven persons were excluded. The final sample consisted of 648 subjects. All selected subjects from the sample filled in the questionnaire and were clinically examined.

Clinical examination

All participants were subjected to a basic dental clinical examination in accordance with criteria recommended by the World Health Organization (WHO) [8]. Clinical examination was carried out by two trained and interconnected examiners in dental offices where SAF officers perform their duties. All participants were examined with standard dental diagnostic tools (dental mirrors, dental probe, artificial lighting, dental chair). The parameter used to assess oral health was DMFT index [9]. Clearly visible lesions with dental cavity formed on the surface of teeth were registered as dental caries, while changes in transparency and initial demineralization of the enamel with intact surface, without cavitation, were registered as healthy teeth [10].

All participants were divided into five groups according to age: I – 18–24 years (n = 84), II – 25–34 years (n = 260), III – 35–4 years (n = 211), IV – 45–54 years (n = 91) and V – 55–64 years (n = 2).

Based on the DMFT value and the Petersen [11] categorization (2004), all participants were divided into four groups: very low index value (< 5), low index value (5–8.9), moderate index value (9–13.9) and high index value (> 13.9).

Questionnaire

The Oral Health Questionnaire for Adults of the WHO was used in this study [8]. The questionnaire consisted of 16 questions designed to gather important socio-demographic characteristics, oral health habits (frequency of tooth brushing, the use of aids for maintaining oral hygiene), self-assessment of oral health, habits in nutrition, and smoking habits.

Statistical analysis

In case of continuous data, variables were presented as mean value ± standard deviation, or mean value followed by confidence intervals. Some of the variables were presented as frequency of certain categories, while statistical signifi-

cance of differences was tested with the χ^2 test. All variables were tested for normal distribution by the Kolmogorov-Smirnov test. In according to the result of this test, the statistical significance of differences was tested using the *t* test or Mann-Whitney *U* test (two group comparison). In case of multi-group comparison, the Kruskal-Wallis test (*post hoc* Mann-Whitney test) was applied.

Calculations of relative risk ratios and their 95% confidence intervals were conducted to determine association between potential risk factors and outcomes (fair/poor oral health). For that purpose, the most promising independent variables were incorporated into binary logistic regression analyses (univariate analysis).

Differences between groups were considered significant at $p < 0.05$. Complete statistical analysis of the data was conducted with the statistical software package, SPSS Statistics 18 (Chicago, Illinois, USA).

Results

The average age of 648 participants (558 men and 90 women) was 34.47 ± 8.14 years. Female subjects were younger than men which was statistically significant (t = 8.13; $p < 0.001$). Nearly two-thirds (63.7%) of participants had secondary school education. There was no statistically significant difference in school education between men and women. More than half of the subjects (64.2%) were married.

More than a third of participants (39.4%) were smokers. Participants brushed their teeth two or more times a day (58.3%). Almost all participants (97.7%) used a toothbrush for tooth brushing; however, the dental floss was used only by 25.2% and a mouthwash solution by 29% of the participants. Regardless of the number of extracted teeth, 81.6% of the subjects had over 20 of their teeth in jaws, and 5.7% had mobile prostheses.

Concerning general health condition, 94% of participants were healthy, while 39 (6%) of them reported the existence of a chronic illness – hypertension was leading (33.3%), followed by disc herniation (15.4%).

Half (54.3%) of the study group were regularly visiting dentists, every 6 months. The most common reason for visiting a dentist was the pain and problem with teeth and mouth. The difficulty with chewing and the feeling of inconvenience due to aesthetic appearance of the teeth were the most statistically significant problems that have occurred in the last 12 months in the examined group.

The average DMFT value of the whole group was 10.55 ± 4.79. Men on average had a higher number of caries and extracted teeth compared to women (Figure 1). There was no statistically significant difference in DMFT between genders, but this difference was found between the genders in caries (z = 3.308, p = 0.001) and extracted teeth (z = 3.151, p = 0.002) that were more often observed in men, while women had statistically larger number of filled teeth (z = 2.702, p = 0.007).

Based on the DMFT value, 247 (38.1%) of the subjects had moderate index value (9–13.9), while the very low index value (< 5) had 70 (10.8%) of the participants, while low index value (5–8.9) had 151 (23.3) and very high index value (> 13–9) had 180 (27.4) of the examineers.

DMFT value was increased with age (Table 1). The smallest DMFT value had participants with faculty education, those who brush their teeth more than 2 times a day, use a mouthwash solution, visit the dentist for consultations, and nonsmokers. Except the group 5 (n = 2), there were significant differences (at least $p < 0.05$) among all other group pairs in the values of DMFT (Table 1).

When asked how they felt about their teeth condition, 18.8% of the examinaers group assessed their condition as bad and very bad. There was a statistically significant difference between men and women concerning self-assessment of their teeth (χ^2 = 6.02; p = 0.014). Men in a significantly higher percentage (20.4%) experienced their teeth condition as bad and very bad compared to women (8.9%). When we compared the DMFT values, with perception of oral health, we found that the youngest population, those who wash their teeth more than twice a day, regularly visit the dentist for consultation, and do not consume cigarettes, had good perception of oral health (Table 2).

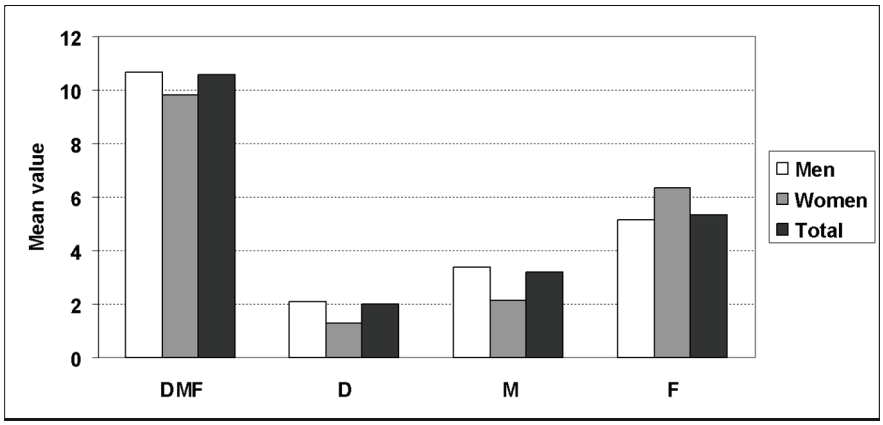

Fig. 1 – Mean values of the Decayed-D, Missing-M and Filled-F Teeth (DMFT) index regarding gender. DMFT in total (mean 10.55; SD 4.79); DMFT men (mean 10.67); DMFT women (mean 9.83); D (decayed teeth in total) – mean 2; SD 2.55; men (mean 2.11); women (mean 1.30); M (missing teeth in total) – mean 3.21; SD 3.35; men (mean 3.38); women (mean 2.16); F (filled teeth in total) – mean 5.33, SD 3.72; men (mean 5.16); women (mean 6.32). SD – standard deviation.

Table 1
Oral health status using the Decayed, Missing and Filled Teeth (DMFT) index according to participants' characteristics

Parameters	DMFT		Probability
	mean	95% CI	
Age at examination (years)			
18–24 (n = 84)	7.76	6.74 – 8.9	
25–34 (n = 260)	9.88	9.32 – 10.49	
35–44 (n = 211)	11.45	10.80 – 12.03	$\chi^2 = 61.98$; $p < 0.001$*
45–54 (n = 91)	12.87	12.00 – 13.82	
55–64 (n = 2)	14.50	11.00 – 18.00	
Education			
primary school	10.50	8.63 – 12.38	
secondary school	10.76	10.31 – 11.23	$\chi^2 = 2.50$; $p = 0.285$
faculty or more	10.18	9.53 – 10.8	
Brushing frequency			
< 2 times/day	11.18	10.60 – 11.78	$z = 2.875$; $p = 0.004$
> 2 times/day	10.10	9.59 – 10.61	
Use to clean teeth			
oral floss	10.15	9.50 – 10.82	$z = 0.844$; $p = 0.377$
fluoride mouth rinses	9.76	9.18 – 10.41	
Usual reason for dental visit			
relief of pain	11.36	10.82 – 11.90	$z = 3.552$; $p < 0.001$
consultation/advice	9.23	8.37 – 10.18	
Tobacco smoking			
yes	11.05	10.49 – 11.69	$z = 1.909$; $p = 0.056$
no	10.23	9.93–10.71	

***except for group aged between 55 and 64 years (n = 2), there are significant differences (at least $p < 0.05$) among all others pairs of groups.**
n = number of participants; CI – confidence interval.

Table 2
Perception of oral health (teeth) status by participants (both sexes)

Parameters	Excellent/very good/good/average		Bad/very bad	
	%	95% CI	%	95% CI
Age at examination (years)				
18–24	91.7***	84.5–96.4	8.3	3.6–15.5
25–34	85.0	80.4–89.2	15.0	10.8–19.6
35–44	79.1	73.9–84.8	20.9	15.2–26.1
45–54	65.9	56.0–75.8	34.1	24.2–44.0
55–64	50.0	0.0–100.0	50.0	0.0–100.0
Education				
primary school	62.5	25.0–87.5	37.5	12.5–75.0
secondary school	78.7	74.6–82.6	21.3	17.4–25.4
faculty or more	86.3*	81.9–90.7	13.7	9.3–18.1
Brushing frequency times/day				
< 2	71.1	65.2 76.7	28.9	23.3–34.8
> 2	88.4***	85.2–91.5	11.6	8.5–14.8
Use to clean teeth				
oral floss	89.0^{ns}	84.0–93.3	11.0	6.7–16.0
fluoride mouth rinses	85.6	80.3–90.4	14.4	9.6–19.7
Usual reason for dental visit				
relief of pain	74.9	69.8–80.0	25.1	20.0–30.2
consultation/advice	89.1**	83.2–95.0	10.9	5.0–16.8
Tobacco smoking				
yes	74.1	68.6–79.2	25.9	20.8–31.4
no	85.8***	82.7–89.3	14.2	10.7–17.3

***$p < 0.05$; ** $p < 0.01$; ***$p < 0.001$ *vs* corresponding subcategory/ies; CI – confidence interval.**
All comparisons are made within (excellent/very good/good/average) parameters.

Table 3
Factors associated with perception of fair/poor oral health (univariate model)

Parameters	RR	95% CI		*p*
		lower	upper	
Age at examination (years)				
18–24	1.0	–	–	–
25–34	1.941	0.834	4.520	0.124
35–44	2.898	1.249	6.727	0.013
45–54	5.683	2.341	13.797	< 0.001
55–64	11.000	0.619	195.521	0.102
Education				
primary school	3.794	0.863	16.676	0.078
secondary school	1.712	1.096	2.674	0.018
faculty or more	1.0	–	–	–
Brushing frequency				
< 2 times/day	3.084	2.047	4.647	< 0.001
> 2 times/day	1.0	–	–	–
Use to clean teeth				
oral floss				
no	2.183	1.277	3.732	0.004
yes	1.0	–	–	–
fluoride mouth rinses				
no	1.552	0.974	2.473	0.064
yes	1.0	–	–	–
Usual reason for dental visit				
relief of pain	2.741	1.384	5.425	0.004
consultation/advice	1.0	–	–	–
Tobacco use				
yes	2.101	1.411	3.129	< 0.001
no	1.0	–	–	–

RR – relative risk; CI – confidence interval.

Univariate logistic regression analysis showed that subjects aged 45 to 54 years compared to the youngest category of subjects, 5.68 times more often evaluated their oral health as bad, while those with secondary school, compared to those with faculty diploma 1.71 times more often evaluated their oral health as bad (Table 3). Participants who brush their teeth less than twice a day 3.08 times more often evaluated their oral health as bad compared to those who brush their teeth more than twice a day. The subjects who usually visit dentists to remove pain 2.74 times more often evaluated their oral health as bad than those who visit dentists for consultations and advice. Finally, participants who do not use dental floss compared to those who use it, 2.18 times more often evaluated their oral health as bad (Table 3).

Clinical examination of our participants found that 27.8% had a very high DMFT value (> 13.9), while only 18.9% of them self-assessed their oral health as bad. Comparing subjects with a very high DMFT value with subjects who assessed their oral health as bad, there was a statistically significant difference in the self-assessment of bad oral health and high values of DMFT ($\chi^2 = 14.02$; $p < 0.001$).

Discussion

In Serbia, there is still no data on oral health of military population. This is the first study in the SAF that describes the applicability of the WHO oral health questionnaire in the military population and compare it with clinical finding. Oral health has two dimensions: one is its physical status in terms of number of teeth, dental caries and periodontal status, and the other one is how an individual experiences his/her oral health. Both dimensions are necessary for the overall characterization of oral health [12]. Therefore, our study had a task to determine the DMFT index, factors that affect oral health and the self-assessment of oral health by individuals. The use of indices that measure the impact of oral health on life quality is becoming more and more necessary in dental practice, because based on the obtained information the needs for treatment are determined, the decision on the type of dental treatment is made and the effectiveness of the applied therapeutic procedures is assessed. Oral health greatly affects the quality of life of individuals, both in psychological, functional and aesthetic terms [13].

The aim of our study was to determine the oral health condition expressed through the DMFT index and to determine its relationship with the perception of oral health condition, satisfaction with oral health and necessary treatment among the SAF members. We made a comparison between the clinically measured oral health condition and the self-assessment of oral health by the SAF members. In our study, the average value of the DMFT index for professional members of the SAF was 10.55 ± 4.79. This is slightly more than the DMFT value in the Iranian

Armed Forces (9.67) [14], Jordan (8.69) [15], and Malaysia (8.15) [16]. The average value of the DMFT in the youngest age group was 7.76 (6.74–8.9), which is similar to the values of young Croatian soldiers aged 19 (7.32) [17].

Unlike our results, the average number of teeth extracted in members of the Croatian Armed Forces was significantly higher, 2.3 in recruits and 5.1 in professional military members [18]. On the other hand, the average number of teeth extracted in the Danish Armed Forces was 0.02 to 0.5, which was considerably less than our results showed [19]. Difference in the results can be explained by very young profile of Danish participants. This confirms the age influence on the number of extracted teeth. The greater number of teeth extracted in the Croatian Armed Forces compared to our Armed Forces can be explained by the fact that, in our study, there were 90 women who had less extracted teeth compared to men. In our study, on average, women lacked 2.16 teeth, while this number was significantly higher in men (3.35).

However, in our study, we noticed that the average number of carious teeth was smaller than the number of extracted teeth. This can be explained by the fact that in our country there is still not enough attention paid to preservation of teeth and that the subjects decide more often to have the tooth extracted, rather than to treat. In addition, our soldiers are often engaged in terrains, military exercises and have extraordinary engagements where there are no conditions for restorative and endodontic treatment; therefore, they decide on tooth removal. In addition, other factors that affect this outcome should not be neglected, such as fear of dental intervention, dental pain as well as the low level of awareness of the importance of oral health. This is confirmed by the fact that in 42.4% of participants in our study visited dentist only when pain and similar problems arise.

As for self-assessment of oral health, this study found that most of the subjects evaluated their health as good, similar to the results of studies done in Israel [20], Qatar [21], Australia [2] and Nigeria [22], but contrary to some other studies [23, 24].

Bad or very bad tooth condition was estimated by 18.9% of our army members, which is much more than results reported in the Israel Armed Forces (7.2%) [20], and similar to the results in Australia (16.2%) [2]. These results probably relate to the fact that in our culture, dental consultations and regular visits are rare, and most of the subjects visit a dentist to remove pain. In our study, as many as 42.4% of participants, as a reason for the last visit to the dentist, mentioned pain and problem with their teeth. This is probably connected to high number of extracted teeth in our study. Pain is the leading reason for the use of dental services in other regions as well [25]. Pain, according to Kim et al. [24], may lead to physical as well as functional limitation, which impacts quality of life of the affected persons invariably, which is significantly associated with poor ratings of oral health.

Findings reported in developing countries and poorly developed countries confirm that low level of education and high use of cigarettes are associated with poor oral health [26]. Also, income inequality has potential to affect both functional and social dimensions of oral health, possibly through a psychosocial pathway [27].

The results of our study also confirm the link between age, education, tooth brushing and smoking frequency with both oral health dimensions – clinical (DMFT value) and subjective oral health perceptions. The DMFT index increases with age, lower education level, tooth brushing frequency less than twice a day and smoking. The results of our study are consistent with studies in other countries. We found that 39.4% of the subjects consume cigarettes occasionally or daily, which is a significantly higher percentage compared to the Iranian Armed Forces (22.1%) [15], and similar to other European countries, Greece and Italy [28].

The study of our military population has showed that smoking was associated with poor oral health perception, as demonstrated in the study in Northern Finland [29]. A study in India confirmed that young people have better perception of oral health than older ones [30], and the results of our study also confirmed that persons aged 45–55 had a 5.68 higher risk of having a poor perception of oral health compared to the youngest age group.

In addition to oral health self-assessment of military subjects, we set criteria for assessing oral condition according to Petersen [11]. As a parameter for clinically poor oral status, we took the DMFT index value higher than 13.9. In our study, 27.78% of the subjects had the DMFT index value greater than 13.9, or clinically poor oral status. This was significantly different from the results obtained from the questionnaire, where only 18.9% of the subjects assessed their tooth condition as bad or very bad. This can be explained by the fact that people in the underdeveloped and developing countries still do not give enough attention to oral health, because they do not have a sufficiently developed awareness of its importance. It is necessary to inform the entire population about the importance of oral health and its impact on general health. This would be best achieved through preventive measures, primarily by motivation and training on the proper maintenance of oral hygiene, regular dental examinations, as well as constant monitoring and remotivation of the subjects. In this way, the number of extracted teeth would be reduced, and thus the self-assessment of oral health, as well as clinical status of the subjects, would be improved. Our study has opened up a question for future studies, to further explore interaction of other parameters with the self-assessment of oral health, as well as the clinical situation.

Conclusion

The results of our study show the absence of compliance between oral health measured by the DMFT index and oral health perception of military participants.

Therefore, we consider that further studies are needed to determine possibilities of improving synchronization of clinical findings and the self-assessment of oral health. It seems that measures of oral health perception should be somehow included when examining condition of oral health. Concerning the army personnel, we propose implementation of education on the importance of oral health through lectures, posters and electronic presentations, and introduction of a mandatory dental systematic examination of all mem-

bers of the SAF once a year, as well as sending skilled dentists to barracks where there is no organized dental service to train army personnel on proper oral hygiene maintenance, and motivate and educate them about the importance of oral health.

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Effect of Vitamin D on proteinuria, lipid status, glycoregulation and C-reactive protein in patients with type-2 diabetes mellitus

Efekat vitamina D na proteinuriju, lipidni status, glikoregulaciju i C-reaktivni protein kod bolesnika sa dijabetes melitusom tip 2

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Abstract

Background/Aim. Vitamin D insufficiency/deficiency is often present in patients with type-2 diabetes mellitus (DM) and could present a risk factor for rapid progression of diabetic nephropathy and for higher incidence of cardiovascular events. The aim of this study was to examine the influence of vitamin D supplementation on proteinuria, cholesterol, triglycerides, C-reactive protein (CRP) and hemoglobin A1c in patients with type-2 DM and vitamin D insufficiency/deficiency. **Methods.** This prospective, cohort study included 90 patients with type-2 DM and vitamin D insufficiency/deficiency divided into 3 equal groups: with normal proteinura, with microproteinuria and with macroproteinuria. Therapy included six months of supplementation with cholecalciferol drops: first two months with 20,000 IU twice weekly, than if level of vitamin D was below normal the same dose was given next four months. If the level of vitamin D was normal 5,000 IU was given twice weekly. At the begining and at the end of the study the levels of urea, creatinine, fasting blood glucose, calcium, phosphorus, cholesterol, triglycerides, CRP, hemoglobin A1c, intact parathyroid hormone, 24-hour urine protein and creatinine clearance were determined. Levels of calcium, phosphorus and vitamin D were also checked 2 months after beginning of therapy due to possible correction of cholecalciferol dose. **Results.** The lowest level of vitamin D before therapy was found in patients with macroproteinuria, while at the end of the study the significantly higher level of vitamin D was found in all three groups. After 6 months of therapy a significant decrease of 24-hour urine protein, cholesterol, triglycerides, hemoglobin A1c in all three groups, and CRP in patients with normal proteinuria and microproteinuria were found. Significantly negative correlation between vitamin D and 24-hour urine protein, cholesterol and CRP was found in patients with macroproteinuria. Also, significantly negative correlation was found between vitamin D and hemoglobin A1c, in patients with normal proteinuria, vitamin D and CRP in patients with microproteinuria. **Conclusion.** A preventive use of high-dose cholecalciferol supplementation in patients with type-2 DM (with or without proteinuria) decreases cholesterol, triglycerides, proteinuria, CRP and hemoglobin A1c.

Key words:
c-reactive protein; cholesterol; diabetes mellitus, type 2; diabetic nephropathies; glycated hemoglobin a; vitamin d; treatment outcome; proteinuria; triglycerides.

Apstrakt

Uvod/Cilj. Nedostatak vitamina D je često prisutan kod bolesnika sa dijabetes melitusom (DM) tip 2 i može biti faktor rizika od brže progresije dijabetesne nefropatije i veće incidencije kardiovaskularnih događaja. Cilj studije bio je da se ispita uticaj supstitucije vitamina D na proteinuriju, holesterol, trigliceride, C-reaktivni protein (CRP) i hemoglobin A1c kod bolesnika sa DM tip 2 i nedostatkom vitamina D. **Metode.** Prospektivnom, kohortnom studijom obuhvaćeno je 90 bolesnika sa DM tip 2 i nedostatkom (insuficijencija/deficijencija) vitamina D svrstanih u tri grupe po 30 bole-

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snika: I – sa normalnom proteinurijom, II – sa mikroproteinurijom i III – sa makroproteinurijom. Sprovedena je šestomesečna nadoknada vitamina D holekalciferol kapima: tokom prva dva meseca sa 20 000 i.j. dva puta nedeljno, a zatim, je kod bolesnika kod kojih je nivo vitamina D ostao snižen nastavljeno sa istom dozom još četiri meseca. Kod bolesnika kod kojih se nivo vitamina D normalizovao, nastavljeno je sa 5 000 i.j. dva puta nedeljno. Na početku i na kraju ispitivanja meren je nivo uree, kreatinina, jutarnje glikemije, kalcijuma, fosfora, holesterola, triglicerida, CRP, hemoglobina A1c, intaktnog paratireoidnog hormona, 24-časovne proteinurije i klirensa kreatinina. Zbog eventualne korekcije doze holekalciferola vrednosti kalcijuma, fosfora i vitamina D proverene su i dva meseca posle započinjanja supstitucije. **Rezultati.** Najniži nivo vitamina D pre terapije imali su bolesnici u grupi sa makroproteinurijom, dok je na kraju ispitivanja utvrđen statistički značajno povišen nivo vitamina D, u sve tri grupe. Nakon šestomesečne primene vitamina D, postignuto je statistički značajno sniženje nivoa 24-časovne proteinurije, holesterola, triglicerida i hemoglobin A1c u sve tri ispitivane grupe, a CRP u grupi sa normalnom proteinurijom i mikroproteinurijom. Statistički značajna negativna korelacija između vitamina D i 24-časovne proteinurije, holesterola i CRP dokazana je u grupi sa makroproteinurijom. Statistički značajna negativna korelacija dokazana je između vitamina D i HBA1c u grupi sa normalnom proteinurijom i vitamina D i CRP u grupi sa miroproteinurijom. **Zaključak.** Supstitucija vitamina D visokim dozama holekalciferola i njegova preventivna primena kod bolesnika sa DM tip 2 (sa ili bez proteinurije) snižava holesterol, trigliceride, proteinuriju, CRP i hemoglobin A1c.

Ključne reči:
c-reaktivni protein; holesterol; dijabetes melitus, tip-2; dijabetičke nefropatije; hemoglobin a, glikozilovan; vitamin d; lečenje, ishod; proteinurija; trigliceridi.

Introduction

There were 382 million people with diabetes mellitus (DM) worldwide in 2013, and it is estimated that number will rise to 585 million in 2035 [1]. It was estimated that more than 700 million people in 2015 had DM or glucose intolerance, but half of it was unrecognized [2]. Approximately one third of the patients with DM will develop diabetes nephropathy (DN) and chronic kidney disease (CKD) [1]. The DM and DN are the main causes of end stage renal disease (ESRD) in the USA, with prevalence of 800 million people [3]. The DN is chronic microvascular complication of DM, presenting as clinical syndrome, manifesting with persistent albuminuria [urine albumin/creatinine ratio (UACR) > 300 mg/g], arterial hypertension, decreasing of glomerular filtration rate and increased cardiovascular events [4]. Considering high direct and indirect costs for treating ESRD patients with renal transplantation or hemodialysis, continuous research are conducting to prevent or slow progression of DN.

It is already proved that changed life habits like regular physical exercise, reduction of body weight in obese patients, reduced intake of salt, proteins and alcohol, smoking cessation, tight control of blood pressure and glucose level, slow down DN progression [4]. Certain drugs can have renoprotective effect: angiotensin converting enzyme inhibitors (ACEI) [5], angiotensin II receptor blockers (ARBs) [5], alsikiren [6], sodium-glucose cotransporter 2 (SGLT2) inhibitors [7], pentoxifylline [8], nonsteroidal mineralocorticoid receptor antagonist- finerenone [9], fenofibrate [10], allopurinol [11], spirinolactone [12] and hydrochlorothiazide [12].

Vitamin D is essential hormone obtained from food (10%–20%) and skin synthesis. Apart from its primary role on calcium and phosphorus homeostasis, it is believed that vitamin D has certain renoprotective effect, antifibrotic, anti-inflammatory effect, inhibits renin angiotensin aldosterone system (RAAS), role in maintaining cardiomyocyte health and insulin sensitivity and role in reducing albuminuria in patients with CKD and DM [13–17].

Studies have confirmed low serum 25-hydroxivitamin D [25(OH)D] levels in patient with CKD [13, 14]. Low vitamin D level seems to be associated with impaired glucose metabolism including DM [18]. Risk factors for vitamin D deficiency in patient with DN remain unclear [18]. The prevalence of vitamin D deficiency is 25% in lean patients and 35% in obese patients [19].

Proteinuria seems to be the most important target to treat in order to prevent cardiovascular events in patients with DN and CKD [13, 14, 17, 20, 21]. Paricalcitol and cholecalciferol can decrease proteinuria in patients with CKD or DM [22–25]. It is assumed that high dose of cholecalciferol like 40,000 international units (IU) weekly could decrease albuminuria and urine transforming growth factor beta-1 (TGF-β1) in patients with DM and Vitamin D insufficiency/deficiency [23].

If we take all in consideration, we can conclude that vitamin D supplementation in patients with vitamin D insufficiency/deficiency could have renoprotective effect and potentially slow progression of DN.

Considering that measuring vitamin D level in blood is easy feasible and its supplementation is not expensive, we decided to conduct the study in patients with type-2 DM and vitamin D insufficiency/deficiency. The aim of this study was to assess the effect of vitamin D on 24-hour urine protein, cholesterol, triglycerides, C-reactive protein (CRP), fasting blood glucose (FBG) and hemoglobin A1c (HBA1c) in patients with type-2 DM and vitamin D insufficiency/deficiency. Those parameters were chosen as possible predictors of progression of DN.

Methods

This 6-month prospective, cohort study included 90 patients patients with type-2 DM and vitamin D insufficiency/deficiency.

Inclusion criteria were: males and females between 18 and 75 years diagnosed with type-2 diabetes mellitus and vitamin D insufficiency (50–75 nmol/L) or deficiency (< 50 nmol/L), medical therapy for type-2 DM during three months

before screening in stable dose (also during study), creatinine clearance > 60 mL/min/1.73 m^2, therapy with ACEI or ARBs at least three months before screening in stable dose during that period (also during study).

Exclusion criteria: glomerulonephritis, connective tissue diseases, serum calcium (corrected for albumin) > 2.45 mmol/L, serum phosphorus > 1.65 mmol/L, congestive heart failure, previous myocardial infarction, poorly regulated arterial hypertension, malignant disease, liver cirrhosis, hepatitis B or hepatitis C infection, HIV, currently enrolled in another trial, women who are pregnant/nursing and previous treatment with vitamin D during six months before screening.

Patients were divided into three equal groups according to initial 24-hour urine protein: I group – patients with normal proteinuria (< 150 mg/24 h); II group – patients with microproteinuria (150–500 mg/24 h); III group – patients with macroproteinuria (> 500 mg/24 h).

Treatment consisted of cholecalciferol drops (Vigantol® 20,000 IU/mL oral drops, solution – Cholecalciferol; Merck KgaA, Germany) in period of six months. During first two months patients received cholecalciferol 20,000 IU twice weekly. After two months patients with normal level of vitamin D received cholecalciferol 5,000 IU twice weekly, and patients with low level of vitamin D received cholecalciferol 20,000 IU twice weekly next four months.

The following variables were analyzed in patients: gender, age, body mass, height, body mass index, urea, creatinine, FBG, calcium, phosphorus, total cholesterol, triglycerides, CRP, HBA1c, intact parathyroid hormone (iPTH) and 24-hour urine protein and creatinine clearance [using CKD-Epidemiology Collaboration (EPI) formula [26]].

All parameters are measured at screening and after six months of vitamin D therapy, except calcium, phosphorus and vitamin D, which are measured also after two months of therapy.

The research was approved by Ethics Committee of Military Medical Academy, Belgrade, Serbia (date of approval 1/21/2016). All patients signed the inform consent.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics 19.0 computer program (IBM, USA, 2011). All continuous variables were described in the form of the median [interquartile range (IQR): range between 25th and 75th percentile], because the data distribution was not normal (Shapiro-Wilk test; $p < 0.05$). The categorical variables were expressed as percentages and examined using the χ^2 test. Relationship between variables was tested by Spearmen's coefficient correlation. Cohen's criteria for correlation are follows: $r < 0.29$ is small correlation, $r = 0.30–0.49$ is moderate correlation and $r > 0.50$ strong correlation. Intra-group comparisons of continuous variables were performed by the non-parametric Wilcoxon signed rank test (two groups – before and after vitamin D therapy). Inter-group comparisons of continuous variables were performed by the non-parametric Kruskal Wallis test (three groups according to proteinuria). Comparisons of nonparametric variables between 2 groups were performed by the Mann-Whitney *U* test. The normality distribution of data was tested with the Shapiro-Wilk test (subject number in the group less than 50). All the analyses were evaluated at the level of statistical significance of $p < 0.05$.

Results

A total of 90 patients with type-2 DM and vitamin D insufficiency/deficiency group were divided in three equal groups according to the basal proteinuria levels: the first group with normal proteinuria, second with microproteinuria, and third with macroproteinuria. All patients were treated with cholecalciferol drops during patients 6 months. Out of 48 (53.33%) male and 42 (46.67%) female patients; 22 (24.44%) patients had vitamin D insufficiency, and 68 (75.55%) patients had vitamin D deficiency. Basic sociodemographic and anthropometric patient characteristics are presented in Table 1.

Average duration of type-2 DM in group with normal proteinuria was 10.00 years (5.75–14.25), in group with microproteinuria also 10.00 years (5.75–15.25) and in group with macroproteinuria 15.00 years (10.00–20.00).

Type-2 DM was treated with oral hypoglycemic drugs in 16 (53.33%) patients in the first group, 13 (43.33%) patients in the second group and 11 (36.67%) patients in the third group (χ^2; $p = 0.425$), 13 (43.33%) patients received insulin therapy in the first group, 15 patients (50.00%) in the second group and 17 patients (56.66%) in the third group (χ^2; $p = 0.587$), while 1 (3.33%) patient received combination therapy in the first group, 2 (6.66%) patients in the second group and also 2 (6.66%) patients in the third group (χ^2; $p = 0.809$).

Table 1

Demographic characteristics of type-2 diabetes mellitus patients according to 24-hour urine protein

Parameter	24-hour urine protein (mg/24 h)			*p*
	normal (< 150)	microproteinuria (150–500)	macroproteinuria (> 500)	
Gender, n (%)				
male	16 (53.3)	17 (56.7)	15 (50.0)	0.875*
female	14 (46.7)	13 (43.3)	15 (50.0)	
Age (years), median (IQR)	62.00 (58.00–69.00)	62.00 (52.50–69.00)	66.00 (62.25–69.50)	0.281**
Heigh (m), median (IQR)	1.70 (1.05–1.77)	1.73 (1.70–1.83)	1.71 (1.68–1.80)	0.035**
Weight (kg), median (IQR)	80.00 (74.25–85.00)	89.00 (79.88–91.25)	87.50 (79.00–91.00)	0.010**
BMI (kg/m^2), median (IQR)	27.50 (25.62–28.70)	27.45 (26.52–30.12)	28.40 (26.31–91.62)	0.120**

BMI – body mass index; *χ^2-test; **Kruskal Wallis test; IQR – interquartile range: 27–75 percentiles.

Table 2

Vitamin D level in the patients with type-2 diabetes mellitus, before and six months after vitamin D supplementation (S)

24-hourour urine protein	Vitamin D (nmol/L)		*p*
	before S	after S	
Normal proteinuria, median (IQR)	42.53 (30.40–46.25)	79.65 (69.09–92.12)	< 0.001**
Microproteinuria, median (IQR)	47.03 (33.18–53.88)	86.65 (70.50–92.85)	< 0.001**
Macroproteinuria, median (IQR)	28.49 (22.40–47.67)	69.22 (54.74–78.04)	< 0.001**
p	0.006*	0.009*	

***– Kruskal Wallis test; **– Wilcoxon Signed Ranks test; IQR- interquartile range: 25–75 percentiles.**

Table 3

Parameters of inflammation,glycoregulation and renal function in relation to 24-hour urine protein before and six months after vitamin D supplementation (S)

Parameter	24-hour urine protein (mg/24 h)			*p*
	normal (< 150)	microproteinuria (150–500)	macroproteinuria (> 500)	
CRP (mg/L), median (IQR)				
before S	1.48 (0.99–3.08)	1.60 (0.95–2.22)	1.52 (1.01–2.25)	0.786*
after S	1.42 (0.87–2.96)	1.16 (0.80–2.15)	1.22 (0.95–2.16)	0.809*
p	< 0.001**	0.001**	0.943**	
FBG (mmol/L), median (IQR)				
before S	8.60 (6.85–10.02)	7.50 (6.67–9.35)	9.40 (7.90–10.60)	0.036*
after S	7.90 (6.68–8.93)	7.95 (5.70–8.23)	8.05 (7.13–8.95)	0.366*
p	0.001**	0.020**	0.001**	
Hemoglobin A_{1C} (%), median (IQR)				
before S	6.97 (6.60–7.80)	7.15 (6.57–8.42)	7.80 (6.72–8.70)	0.192*
after S	6.80 (6.20–7.70)	6.95 (6.40–7.60)	7.10 (6.80–8.62)	0.069*
p	0.001**	0.001**	0.016**	
Creatinine (μmol/L), median (IQR)				
before S	76.50 (66.75–90.25)	82.00 (71.00–96.15)	86.00 (74.75–106.00)	0.149*
after S	73.00 (64.00–82.50)	83.50 (70.50–94.25)	82.00 (71.50–104.75)	0.136*
p-value	0.273**	0.090**	0.022**	
Creatinine clearance (mL/min), median (IQR)				
before S	84.30 (64.05–91.55)	68.60 (61.00–94.25)	63.20 (60.57–89.97)	0.101*
after S	86.30 (71.15–95.45)	74.15 (63.82–90.70)	68.80 (62.00–90.07)	0.143*
p	0.286**	0.049**	0.040**	
Urea (mmol/L), median (IQR)				
before S	5.95 (5.10–7.13)	6.90 (5.47–8.20)	8.40 (5.37–9.72)	0.004*
after S	5.85 (5.15–6.57)	6.20 (5.20–7.95)	8.15 (5.47–9.22)	0.003*
p	0.032**	0.147**	0.484**	

***Kruskal Wallis test; **Wilcoxou Signed Ranks test; IQR – interquartile range: 27–75 percentiles.**
CRP – C-reactive protein; FBG – fasting blood glucose.

The lowest level of vitamin D, before therapy, was found in the patients with macroproteinuria, on average 28.49 nmol/L (22.40–47.67). After six months of supplementation a significantly increased vitamin D level was found in patients in all three groups (Table 2).

After six months of vitamin D supplementation, significantly decreased CRP level was found in the group with normal proteinuria ($p < 0.001$) and the group with microproteinuria ($p = 0.001$). All three groups had significantly decreased levels of HBA1c and FBG (Table 3).

We performed a correlation between vitamin D level and levels of CRP, FBG and HBA1c after six months of cholecalciferol therapy and we found a moderately negative correlation between increased vitamin D level and decreased CRP level in the group with microproteinuria ($r = -0.368$; $p = 0.046$), and in the group with macroproteinuria ($r = -0.375$; $p = 0.041$). We found a moderately negative correlation ($r = -0.342$; $p = 0.064$) between increased vitamin D level and decreased HBA1c only in group with normal proteinuria.

We found the significantly decreased serum creatinine level, after therapy with cholecalciferol, only in the group with macroproteinuria ($p = 0.022$). Increased creatinine clearance level was significant in the group with microproteinuria, 68.60 mL/min (61.00–94.25) at the beginning of the study, after therapy 74.15 mL/min (63.82–90.70) ($p = 0.049$), and in the group with macroproteinuria where we have increase from 63.20 mL/min (60.57–89.97) to 68.80 mL/min (62.00–90.07) ($p = 0.040$) (Table 3).

We did not find calcium level over 2.45 mmol/L and *p* level over 1.65 mmol/L. The iPTH was significantly decreased after therapy only in the group with normal proteinuria ($p = 0.003$), while in the group with microproteinuria level of iPTH was significantly increased from 5.05 pmol/L (4.00–6.15) to 5.10 pmol/L (3.35–6.22) ($p = 0.021$) (Table 4).

Table 4

Parameters of calcium and phosphate homeostasis, and lipid profile in relation to 24 h-proteinuria before and six months after vitamin D supplementation (S)

Parameter median	24-hour urine protein (mg/24 h)			*p*-values
	normal (< 150)	microproteinuria (150–500)	macroproteinuria (> 500)	
Calcium (mmol/L), median (IQR)				
before S	2.39 (2.31–2.42)	2.38 (2.29–2.40)	2.3 (2.26–2.37)	0.002*
after S	2.38 (2.32–2.42)	2.34 (2.30–2.40)	2.35 (2.28–2.40)	0.401*
p	0.628**	0.354**	0.002**	
Phosphorus (mmol/L), median (IQR)				
before S	1.10 (1.01–1.19)	1.10 (0.99–1.17)	.16 (1.02–1.30)	0.123*
after S	1.06 (0.91–1.19)	1.07 (0.98–1.19)	1.10 (1.00–1.29)	0.149*
p	0.165**	0.684**	0.348**	
IPTH (pmol/L), median (IQR)				
before S	3.40 (2.18–5.05)	5.05 (4.00–6.15)	7.10 (6.48–9.20)	< 0.001*
after S	3.25 (2.10–4.50)	5.10 (3.35–6.22)	7.02 (5.05–9.70)	< 0.001*
p	0.003**	0.021**	0.363**	

***Kruskal Wallis test; **Wilcoxon Signed Ranks test; IQR – interquartile range: 27–75 percentiles.**
iPTH – intact parathyroid hormone.

Table 5

Parameters of proteinuria and lipid profile in relation to 24-hour urine protein before and six months after vitamin D supplementation (S)

Parameter median	24-hour urine protein (mg/24 h)			*p*-values
	normal (< 150)	microproteinuria (150–500)	macroproteinuria (> 500)	
Total cholesterol (mmol/L), median (IQR)				
before S	5.01 (4.36–5.32)	5.52 (4.84–6.32)	5.66 (4.73–6.36)	0.032*
after S	4.45 (3.90–5.10)	4.66 (84.14–5.30)	4.79 (4.39–5.77)	0.333*
p	< 0.001**	< 0.001**	< 0.001**	
Triglyceride (mmol/L), median (IQR)				
before S	1.81 (1.31–2.41)	2.16 (1.61–2.80)	1.81 (1.58–2.90)	0.194*
after S	1.44 (1.10–1.77)	1.43 (1.16–1.71)	1.49 (1.40–2.22)	0.116*
p	< 0.001**	< 0.001**	< 0.001**	
24-hour urine protein (mg/24 h), median (IQR)				
before S	0.071 (0.046–0.101)	0.221 (0.182–0.273)	0.967 (0.707–4.993)	< 0.001*
after S	0.060 (0.026–0.085)	0.136 (0.100–0.200)	0.664 (0.319–5.140)	< 0.01*
p	0.003**	0.001**	0.015**	

***Kruskal Wallis test; **Wilcoxon Signed Ranks test; IQR – interquartile range: 27–75 percentiles.**

Significantly decreased levels of total cholesterol and triglycerides, after six months of vitamin D supplementation, were achieved in all three groups ($p < 0.001$) (Table 5).

We found significantly decreased 24-hour urine protein in all three groups, after six months of vitamin D supplementation, in the group with normal proteinuria ($p = 0.003$), in the group with microproteinuria ($p < 0.001$) and in the group with macroproteinuria ($p = 0.015$) (Table 5).

We performed correlation between vitamin D blood level and levels of total cholesterol, triglycerides and 24-hour urine protein, after six months therapy of cholecalciferol, we found strong negative correlation ($r = - 0.570$; $p < 0.001$) only in the group with macroproteinuria, between increased vitamin D level and decreased total cholesterol level, and between increased vitamin D level and decreased 24-hour urine protein ($r = -0.685$; $p < 0.001$) at the end of the study (Figures 1 and 2).

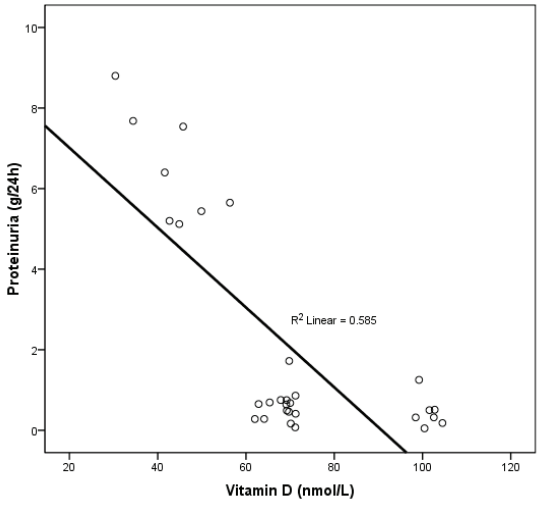

Fig. 1 – Correlation between vitamin D level and 24-hour urine protein in the group with macroproteinuria six months after vitamin D supplementation (Sperman's rho -0.685; $p < 0.001$).

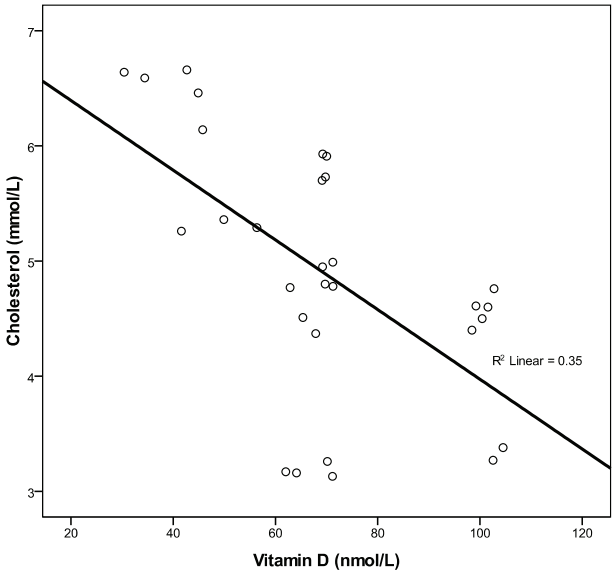

Fig. 2 – Correlation between vitamin D level and total cholesterol level in the group with macroproteinuria six months after vitamin D supplementation (Spearman's rho -0.570; $p < 0.001$).

Discussion

We conducted prospective, cohort study about effect of the six months therapy with cholecalciferol on 24-hour urine protein, lipid status, glicoregulation and parameters of inflammation in the patients with type-2 DM and vitamin D insufficiency/deficiency. We chose cholecalciferol because it is cheap comparing to others vitamin D drugs.

Based on other experience on vitamin D supplementation, we treated patients with higher dose of cholecalciferol, 20,000 IU twice weekly, until normalization of vitamin D level [23, 27–29]. There were no adverse events particularly meaning hypercalcemia or hyperphosphatemia.

After six-month therapy, level of vitamin D was significantly higher in all three groups. The lowest level of vitamin D, before therapy, was found in the patients with macroproteinuria. These patients had highest level of serum creatinine and lowest level of creatinine clearance at the beginning of the study, which is in correlation with data from other authors that vitamin D deficiency is more often in the patients with type-2 DM with macroalbuminuria and in CKD [13, 18, 24, 30].

In the patients with normal 24-hour urine protein, significantly decreased iPTH level was found. Since all patients had normal iPTH level, it is not clear if this decrease is of clinical importance. Although if we consider positive effect of vitamin D on bone turnover by increased osteoclast activation, which leads to increased bone volume, trabecular thickness and osteoid surface [13, 24], probably this iPTH decrease that we found may be clinically important [31].

We measured HBA1c and FBG as parameters for glycoregulation. All patients had a significantly decreased levels of HBA1c and FBG after six months of the therapy with cholecalciferol. We found moderately negative correlation of HBA1c ($r = -0.342$; $p = 0.064$) between increased vitamin D level and decreased HBA1c level in the patients with normal proteinuria. Better glycoregulation after vitamin D supplementation was confirmed by other authors [27, 31, 32]. That effect is achieved by increased insulin sensitivity through stimulation of expression of insulin receptors in skeletal muscles and through activation of peroxisome proliferator activator receptor δ (PPAR δ), RAAS (known as inhibitor of insulin action on peripheral tissues) inhibition [27, 31, 32]. Vitamin D also increases insulin releasing by stimulating intracellular level of calcium in pancreatic beta cells [13, 31]. Vitamin D probably has direct effect on beta cell function by binding to vitamin D receptor (VDR) expressed on beta cells. It is proved that mice without functional VDR have damaged glucose stimulated insulin secretion [13–15, 33].

Chronic inflammation plays crucial role in development of DM and DN [4, 13, 34, 35] and vitamin D can directly or indirectly diminish that effect [14, 31, 36]. Anti-inflammatory effect is achieved by reduced releasing of proinflammatory cytokines (TNF-α, IL-6, IL-12, IL-8, IL- 1β, IFN-γ), blocking of dendritic cell differentiation, inhibition of lymphocytes proliferation, inhibition of foam cells generation, decreased macrophages cholesterol intake and improved development of regulatory T lymphocytes or increased releasing of anti-inflammatory cytokines, like IL-10 [4, 13, 31, 33, 35, 36]. We chose CRP as biomarker of inflammation. After six months of supplementation with vitamin D we found significantly decreased CRP level in the patients with normal proteinuria and microproteinuria with moderately negative correlation between increased vitamin D level and decreased CRP level in the patients with microproteinuria ($r = -0.368$; $p = 0.046$) and macroproteinuria ($r = -0.375$; $p = 0.041$).

Proteinuria is the main target to treat in order to prevent and slow down DN and decrease incidence of cardiovascular events [20, 21, 24]. Vitamin D decreases proteinuria by RAAS inhibition and reduces renal fibrosis by reduction of TGF-β/SMAD pathway [15–17]. Vitamin D, apart of renal RASS inhibition, reduces renin expression in heart thereby decreasing arterial blood pressure [20, 21]. Vitamin D, by slowing down the fibrosis, slows down progression of left ventricle hypertrophy and development of heart failure, lowers brain natriuretic peptide level, reduces gene expression important for atherosclerosis/vascular growth factors [20, 21]. All our patients had significantly decreased proteinuria after six-month therapy. Patients with macroproteinuria had strong negative correlation ($r = -0.685$; $p < 0.001$) between increased vitamin D level and decreased 24-hour urine protein at the end of the study.

We found different data about influence of vitamin D on lipid status in literature [13, 29, 37]. All our patients had significantly decreased levels of total cholesterol and triglycerides after therapy. Patients with macroproteinuria have strong negative correlation ($r = -0.570$; $p < 0.001$) between increased vitamin D and decreased total cholesterol.

We can conclude that after six months of supplementation with vitamin D and correction of vitamin D insufficiency/deficiency all our patients had significantly decreased levels of proteinuria, HBA1c, CRP, total cholesterol and triglycerides. Significantly negative correlation was found between increased vitamin D level and decreased 24-hour urine protein, total cholesterol and CRP in the patients with ma-

croproteinuria. These patients had lowest level of creatinine clearance at the beginning, but at the end of the study we found significantly increased creatine clearance. Based on that we can confirm that vitamin D had renoprotective effect especially in the patients with macroproteinuria and initial CKD. According to our finding of significantly decreased 24-hour urine protein, levels of total cholesterol, triglycerides, HBA1c and CRP in other two groups, but without significant correlation, except in HBA1c in the patients with normal proteinuria and in CRP in the patients with microproteinuria, we can conclude that vitamin D has renoprotective role in type-2 DM in all patients. Based on our results we can assume that vitamin D, apart of renoprotective effect in type-2 DM, could prevent cardiovascular events, which was not studied during our research but could be the goal for future studies.

Limitation of the study and future tasks

Considering limited number of patients in all groups, it would be possible to draw conclusion that a higher number of patients and longer follow up would provide us more specific results. We could investigate effect of vitamin D on proinflammatory cytokines which we have not done in our study. Future research could include new urinary and serum biomarkers marked by other authors as early biomarkers of development and progression of DN.

Conclusion

Vitamin D supplementation in higher dose than conventional and its prolonged preventive use in patients with type-2 DM, has renoprotective effect resulting in decreased 24-hour urine protein, total cholesterol, triglycerides, HBA1c and CRP, especially in the patients with macroproteinuria. If we consider that positive effect is achieved in the patients with normal proteinuria and microproteinuria it is possible that we should treat all patients with type-2 DM with vitamin D supplementation, but we need further trial to confirm that.

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Prehypertension and hypertension prevalence and risk factors among adult population in Republic of Serbia: A cross-sectional study

Prevalencija i faktori rizika od prehipertenzije i hipertenzije kod odrasle populacije u Republici Srbiji: studija preseka

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Abstract

Background/Aim. Prehypertension and hypertension are an important public health problem worldwide and although they can be modified, they are often a risk for cardiovascular diseases. The aim of this study was to determine the prevalence of prehypertension and hypertension and associated risk factors in the adult population (15+ years) of Serbia. **Methods.** The cross-sectional study covered 14,623 adult respondents, but 14,422 volunteered to measure their blood pressure according to a pre-defined protocol. A stratified two-stage national representative random sampling approach was used for the selection of the survey sample. **Results**. In 2013, 17.7% of Serbian population, aged 15 and over, was normotensive, every third (33.1%) person had prehypertension, and every second (49.3%) had hypertension. The standardized prevalence of prehypertension and hypertension was 40.6% and 34.5%, respectively; 57.8% of the hypertensive population were receiving medical treatment. Among those receiving medical treatment, 35.2% (36.4% males and 33.2% females) had a blood pressure within the normal range. According to the results of multivariate logistic regression analysis, independently significant risk factors for hypertension compared to persons with normotension were older age (50 and more) ($p < 0.001$), overweight ($p < 0.001$) and obesity ($p < 0.001$), moderate ($p < 0.001$) and large ($p < 0.001$) waist circumference in both sexes, and nonurban place of residence in females ($p = 0.006$). In females, independently significant risk factors for prehypertension compared to persons with normotension were older age (50 and more) ($p < 0.001$), overweight ($p < 0.001$) and obesity ($p < 0.001$), moderate ($p < 0.001$) and large ($p < 0.001$) waist circumference, but high level of physical activity was significantly protective ($p = 0.014$). In males, overweight ($p < 0.001$) and obesity ($p < 0.001$) were independently significant risk factors for prehypertension. **Conclusion.** Serbia belongs to countries with a high prevalence of prehypertension and hypertension. Our results emphasize the need for a new public health strategy for the prevention, detection and treatment of prehypertension and hypertension.

Key words:
adults; age factors; cardiovascular diseases; hypertension; obesity; overweight; prehypertension; risk factors; prevalence; sex factors.

Apstrakt

Uvod/Cilj. Prehipertenzija i hipertenzija su važni javnozdravstveni problemi širom sveta. Mada se na njih može uticati, oni su skoro uvek faktori rizika od kardiovaskularnih bolesti. Cilj istraživanja bio je da se utvrde prevalencija i faktori rizika prehipertenzije i hipertenzije kod odrasle populacije (15+ godina) u Srbiji. **Metode.** Studijom preseka obuhvaćeno je 14 623 odraslih ispitanika, od kojih je 14 422 pristalo na merenje krvnog pritiska u kućnim uslovima. Korišćen je stratifikovani dvoetapni reprezentativni slučajni uzorak stanovništva Srbije. **Rezultati.** U 2013, 17,7% odraslih osoba, uzrasta 15+ godina bilo je normotenzivno, svaka treća (33,1%) osoba imala je prehipertenziju, a svaka druga (49,3%) hipertenziju. Standardizovana prevalencija prehipertenzije bila je 40,6%, hipertenzije 34,5%, a 57,8% hipertenzivnih osoba bilo je na antihipertenzivnoj terapiji. Među osobama koje su bile na medikamentnoj terapiji 35,2% (36,4% muškaraca i 33,2% žena) imalo je krvni pritisak unutar normalnih vrednosti. Prema rezultatima multivarijantne logističke regresione analize, značajni nezavisni faktori rizika od hipertenzije, u odnosu na osobe sa normotenzijom, bili

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su životno doba (50+ godina) ($p < 0,001$), prekomerna težina ($p < 0,001$) i gojaznost ($p < 0,001$), umereno veliki ($p < 0,001$) i velik ($p < 0,001$) obim struka kod osoba oba pola, a kod žena i mesto boravka van grada ($p = 0,006$). Kod žena, značajni nezavisni faktori rizika od prehipertenzije, u poređenju sa osobama sa normotenzijom, bili su starije životno doba (50 + godina) ($p < 0,001$), prekomerna težina ($p < 0,001$) i gojaznost ($p < 0,001$), umereno veliki ($p < 0,001$) i veliki ($p < 0,001$) obim struka, dok je visok stepen fizičke aktivnosti bio značajan protektivni faktor ($p = 0,014$). Kod muškaraca, prekomerna težina ($p < 0,001$) i gojaznost ($p < 0,001$) bili su značajni nezavisni faktori rizika od prehipertenzije. **Zaključak.** Srbija pripada zemljama sa visokom prevalencijom prehipertenzije i hipertenzije. Naši rezultati naglašavaju potrebu za novom javnozdravstvenom strategijom za prevenciju, otkrivanje i lečenje osoba sa prehipertenzijom i hipertenzijom.

Ključne reči:
odrasle osobe; životno doba, faktor; kardiovaskularne bolesti; hipertenzija; gojaznost; telesna masa, prekomerna; prehipertenzija; faktori rizika; prevalenca; pol, faktor.

Introduction

Prehypertension (PreHTN) and hypertension (HTN) are an important public health problem worldwide and although they can be modified, they are often a risk for cardiovascular diseases (CVD), cerebrovascular diseases (stroke) and kidney diseases in the terminal phase [1, 2]. It is estimated that approximately 40% of adult population of the world have diagnosed HTN, which makes an average of 9.4 million people who die annually [1]. HTN is responsible for approximately half of the deaths from CVD and stroke. PreHTN elevates the risks of CVD (RR = 1.55), coronary heart disease (RR = 1.50) and stroke (RR = 1.71) [3].

In the adult population aged 25 and over, the age-standardized prevalence of HTN is the highest in underdeveloped countries and developing countries (Africa – 46%), while the lowest is in developed countries of the world (North America – 35%). The majority of people with undiagnosed, untreated and uncontrolled HTN lives in low- and middle-income countries with insufficiently developed health systems [1].

PreHTN prevalence and HTN prevalence are attributed to the growth and aging of the population and risk behaviors such as malnutrition, overweight, harmful use of alcohol, smoking tobacco, insufficient physical activity, high cholesterol, diabetes and long-term stress exposure [4–6].

However, little is known about the epidemiology of PreHTN and HTN in the adult population of Serbia. The aim of the study was to determine the prevalence of PreHTN and HTN and associated risk factors in the adult population (15+ years) of Serbia.

Methods

Data for this cross-sectional study were obtained from the National Survey of the Population of Serbia in 2013 that was carried out by the Ministry of Health of Serbia and the Institute of Public Health of Serbia "Dr Milan Jovanović Batut". The research was carried in the period October 7th, 2013 to December 30th, 2013. The study population included adults ≥ 15 years old, permanent residents of the Republic of Serbia. Exclusion criteria were: age below 15 years, persons who lived in collective households and/or institutions (institutions of social protection, nursing homes, prisons, and psychiatric institutions), residents of Kosovo and Metohija region (under the UN Mission), persons who were mentally unable to participate in the survey and examinees for whom there were no data for the requested variables.

Sampling design

This cross-sectional study was performed in line with recommendations of EUROSTAT, The European Health Interview Survey wave 2, and Methodological Manual [7, 8]. For obtaining the sample of households and examinees, a stratified two-stage national representative random sampling approach was used. In Serbia, 4 administrative areas were identified: Vojvodina, Belgrade, Šumadija and Western Serbia and Eastern and South Serbia. A further stratum classification was urban vs. other area of residence. In the first stage of 2-stage sampling, census circles were selected (total, 670 census circles: Belgrade – 162, Vojvodina – 187, Šumadija and Western Serbia – 179 and Eastern and South Serbia – 142) by probability proportional sampling. In the second stage, households were selected by a linear method of sampling, with a random beginning and an equal selection interval, by random sampling without replacement. In the second stage, households were selected by a linear method of sampling, with a random beginning and an equal selection interval, by random sampling without replacement. The 2011 Serbian population census framework was used for the selection of clusters. From every census circle 10 households were selected (total, 6,700 households). Out of 6,700 households planned, a sample of 6,500 households was accepted to be include in this study (the household response rate – 97.0%). Out of 16,474 registered members of households aged 15 and over, 14,623 agreed to be interviewed (response rate of 88.8%). Out of the adult household members, 14,422 adult respondents whom blood pressure (BP) were measured at home were included in our study (response rate of 98.6%).

The research was approved by the Ethics Committee of The Faculty of Medicine, University of Belgrade (29/IV-16) and the Ethics Committee of the Institute for Public Health of Serbia "Dr Milan Jovanović Batut" (1567/1).

Instruments and variables

The research of health of the population in Serbia was conducted by collecting data with three questionnaires (household questionnaire, personal health questionnaire and

self-completing questionnaire) for population above 15 years. The questionnaires were written according to the recommendations of Eurostat Working Group on Public Health Statistics of the European Commission [7]. Variables included in analysis from those questionnaires were: gender (male/female), age (≤ 49, 50–59 and 60 and more years), place of residence (town/the other; a town is a territorial unit established by the law, which is an economic, administrative, geographic and cultural center and has more than 100,000 inhabitants; other territorial units, according to the stated law and administrative-legal criteria, do not fulfill these conditions) and duration of daily physical activity – walking and/or riding a bicycle (low: 10–29 min/daily; moderate: 30–59 min/daily; high: 60 and more min/daily) [8].

Measurements

After the interview with the households, previously trained persons carried out objective measurements according to defined standardized procedures. A digital BP monitor with suitable cuffs was used for measuring BP. BP was measured by automatic upper arm BP monitor, three times with pauses of at least one min. For the categorization of the level of BP, the average value of all three measurements was used and information of treated HTN over the last 4 weeks. According to the classification of the World Health Organization (WHO) [9], the examinees without antihypertensive therapy over the last 4 months were classified according to values of BP into the following categories: normal blood pressure (systolic BP – SBP and diastolic BP – DBP: < 120 and < 80 mmHg); PreHTN (SBP/DBP: 120–139 and/or 80–89 mmHg); and HTN (SBP/DBP: ≥ 140 and/or ≥ 90 mmHg). All persons who used antihypertensive therapy over the last 4 weeks were included in the category of hypertensive individuals. Among the subjects with HTN, 45.9% (1,532) of men and 68.3% (2,571) of women was on antihypertensive treatment. In population who knew that they had HTN, only 85.3% of females and 75.2% of males used antihypertensive therapy over the last 4 weeks.

Anthropometric measurements were carried out according to standard international procedures. Body height was measured with the portable height measuring rod, body mass with the electronic scale for medical usage with decimal scale, and the waist circumference (WC) with the nonelastic strip for measuring, which is 205 cm long. Body mass index (BMI), as the measure of weight [10], which was obtained as the ratio of body mass and the squared body height (m^2), was classified according to the criteria of WHO: underweight – BMI < 18.5 kg/m^2, normal weight – BMI 18.5–24.9 kg/m^2, overweight (pre-obesity) – BMI 25.0–29.9 kg/m^2, obesity – BMI ≥ 30.0 kg/m^2.

WC was measured at the midpoint between the lower border of the rib cage and the iliac crest by using a flexible inch tape. The following cut off values of WC were used to assess the abdominal obesity for women: normal < 80 cm, moderate 80–87 cm, large ≥ 88 cm; and for men: normal < 94 cm, moderate 94–101 cm, large ≥ 102 cm [11].

Statistical analysis

Prevalence rates with appropriate 95% confidence intervals (CI) were estimated for 3 categories of BP according to age, place of residence, BMI, WC and physical activity, but separately for males and females participants. The prevalence of normotensive, PreHTN and HTN subjects were age and sex standardized according to the 2010 world population using the direct method [12].

In statistical analysis of data, Pearson χ^2 test, univariate logistic regression analysis (ULRA) and multivariate logistic regression analysis (MLRA) were used. χ^2 test was used to find statistically significant differences according to sex and between BP categories according to categories of age, place of residence, BMI, WC and physical activity, but separately for males and females participants.

Association between categories of BP and health related factors were analyzed with ULRA and MLRA. Variables that according to ULRA had p value ≤ 0.1 were included into the model of MLRA. The dependent variables formed two different multivariable models – PreHTN and HTN, each of them vs. normal BP as referent category, separately for males and females. Independent variables were: age, place of residence, BMI, WC and physical activity. They were reported with odds ratios and their 95% CI, along with probability p. The computer program SPSS for Windows, version 20.0 (US Government of Commercial Computer Software, Chicago, USA, 2012) was used in statistical analysis of the data. Statistical hypotheses were tested on the level of statistical significance from 0.05.

Results

The cross-sectional study included 14,422 participants (6,652 – 46.1% of men and 7,770 – 53.9% of women) (response rate 98.6%). The largest percentage of our sample of the population consisted of people under the age of 50 (49.1% of males and 46.0% of females), from urban place of residence (55.1% of males and 57.4% of females), with overweight and obesity (62.8% of males and 54.0% of females) and with moderately and large WC (58.5% of males and 70.5% of females) (Table 1). About 40% of men and about 50% of women did not perform physical activity sufficiently (i.e. the physical activity they performed was shorter than 30 min per day). People with HTN, for both sexes, were more often an older, from nonurban place of residence, with overweight and obesity, with large WC and low physical activity in relation to normotensive and PreHTN individuals.

Among people with hypertension (7,104), only 57.8% used antihypertensive therapy over the last 4 weeks. Among those receiving medical treatment, only 35.2% (36.4% males and 33.2% females) had a BP within the normal range. Users of medical treatment for HTN were more often females (62.7%), elderly people (60 and older – 68.9%), people from urban areas (56.0%), with overweight and obesity (78.3%), with moderate and large WC (85.9%) and with low physical activity (less than 30 min per day) (52.4%).

Table 1

Blood pressure (BP) categories according to baseline characteristics of study participants by gender

Characteristics	Males (n = 6,652), n (%)				Females (n = 7,770), n (%)			
	normal BP (n = 671)	prehypertension (n = 2,643)	hypertension (n = 3,338)	all (n = 6,652)	normal BP n = 1,879)	prehypertension (n = 2,125)	hypertension (n = 3,766)	all (n = 7,770)
Age (years)								
< 50	498 (74.2)	1,849 (70.0)	917 (27.5)	3,264 (49.1)	1,620 (86.2)	1,395 (65.6)	558 (14.8)	3573 (46.0)
50–59	61 (9.1)	391 (14.8)	725 (21.7)	1,177 (17.7)	165 (8.8)	383 (18.0)	831 (22.1)	1,379 (17.7)
≥ 60	112 (16.7)	403 (15.2)	1,696 (50.8)	2,211 (33.2)	94 (5.0)	347 (16.3)	2,377 (63.1)	2,818 (36.3)
Place of residence								
urban	389 (58.0)	1,489 (56.3)	1,789 (53.6)	3,667 (55.1)	1,153 (61.4)	1,252 (58.9)	2,052 (54.5)	4,457 (57.4)
other	282 (42.0)	1,154 (43.7)	1,549 (46.4)	2,985 (44.9)	726 (38.6)	873 (41.1)	1,714 (45.5)	3,313 (42.6)
Body mass index (kg/m^2)								
underweight (< 18.50)	40 (6.1)	47 (1.8)	22 (0.7)	109 (1.7)	162 (8.7)	83 (4.0)	58 (1.6)	303 (4.0)
normal weight (18.5024.99)	367 (55.9)	1,124 (42.9)	815 (25.3)	2,306 (35.5)	1,253 (67.4)	1,069 (51.4)	831 (23.3)	3,153 (42.0)
overweight (25.00–29.99)	203 (30.9)	1,082 (41.3)	1,436 (44.6)	2,721 (41.9)	336 (18.1)	621 (29.9)	1,324 (37.1)	2,281 (30.4)
obesity (≥ 30.00)	47 (7.2)	364 (13.9)	948 (29.4)	1,359 (20.9)	108 (5.8)	305 (14.7)	1,357 (38.0)	1,770 (23.6)
Waist circumference (men/women in cm)								
normal (< 94/< 80)	427 (65.3)	1,345 (52.2)	894 (28.0)	2,192 (29.5)	1,010 (55.7)	769 (37.6)	413 (11.5)	2,192 (29.5)
moderate (94–101/ 80–87)	131 (20)	613 (23.8)	807 (25.3)	1,408 (18.9)	421 (23.2)	460 (22.5)	527 (14.7)	1,408 (18.9)
large (> 102/ > 88)	96 (14.7)	621 (24.1)	1,492 (46.7)	3,841 (51.6)	383 (21.1)	817 (39.9)	2,641 (73.8)	3,841 (51.6)
Physical activity – total time (min/per day)								
low (< 30)	215 (33.3)	924 (36.8)	1,251 (41.0)	3,414 (48.5)	766 (42.3)	918 (45.8)	1,730 (53.7)	3,414 (48.5)
moderate (30–59)	222 (34.4)	802 (32.0)	967 (31.7)	2,185 (31.0)	592 (32.7)	672 (33.5)	921 (28.6)	2,185 (31.0)
high (60+)	208 (32.2)	784 (31.2)	830 (27.2)	1,440 (20.5)	454 (25.1)	416 (20.7)	570 (17.7)	1,440 (20.5)

SBP – systolic BP; DBP – diastolic BP; normal BP (SBP < 120 mm Hg and DBP < 80 mm Hg); prehypertension (SBP = 120–139 mm Hg and/or DBP = 80–89 mm Hg); hypertension (SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg, or current treatment with antihypertensive medications).

Table 2
Crude and standardized prevalence (%) of blood pressure (BP) categories according to age and sex in adult (15+ years) population of the Republic of Serbia, 2013

Parameters	Males (n = 6,652) % (95% CI)			Females (n = 7,770) % (95% CI)			Both sexes (n = 14,422) % (95% CI)		
	normal BP (n = 671)	prehypertension (n = 2,643)	hypertension (n = 3,338)	normal BP (n = 1,879)	prehypertension (n = 2,125)	hypertension (n = 3,766)	normal BP (n = 2,550)	prehypertension (n = 4,768)	hypertension (n = 7,104)
Age (years)									
< 50*	15.3 (14.0–16.5)	56.7 (54.9–58.4)	28.1 (26.5–29.7)	45.3 (43.7–47.0)	39.0 (37.4–40.7)	15.6 (14.4–16.8)	31.0 (29.9–32.1)	47.5 (46.2–48.7)	21.6 (20.6–22.6)
50–59*	5.2 (3.9–6.5)	33.2 (30.5–35.9)	61.6 (58.8–64.4)	12.0 (10.2–13.7)	27.8 (25.4–30.2)	60.3 (57.6–62.9)	8.8 (7.7–10.0)	30.3 (28.5–32.1)	60.9 (58.9–62.8)
≥ 60*	5.1 (4.1–6.0)	18.2 (16.6–19.9)	76.7 (74.9–78.5)	3.3 (2.7–4.0)	12.3 (11.1–13.6)	84.4 (83.0–85.7)	4.1 (3.5–4.7)	14.9 (13.9–15.9)	81.0 (79.9–82.1)
Total (15+ years)	10.1 (9.3–10.9)	39.7 (38.6–40.9)	50.2 (48.9–51.4)	24.2 (23.2–25.2)	27.4 (26.3–28.4)	48.5 (47.3–49.6)	17.7 (17.0–18.3)	33.1 (32.3–33.9)	49.3 (48.4–50.1)
Standardized prevalence**	12.9 (12.5–13.3)	48.5 (48.0–48.8)	38.6 (38.2–39.2)	36.0 (35.5–36.5)	33.4 (33.0–33.8)	30.6 (30.1–31.1)	24.9 (27.7–25.1)	40.6 (40.3–40.9)	34.5 (34.0–35.0)

***p* value for χ^2 test between genders for each age category < 0.001; **Age-standardized prevalence according to the 2010 world population;
CI – confidence interval; SBP – systolic BP; DBP – diastolic BP; normal BP (SBP < 120 mmHg and DBP < 80 mmHg); prehypertension (SBP = 120–139 mmHg and/or DBP = 80–89 mmHg); hypertension (SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg, or current treatment with antihypertensive medications).

Table 3
Prevalence (%) of blood pressure (BP) categories in both gender according to place of residence, body mass index (BMI), waist circumference (WC) and physical activity among adult (15+ years) population of the Republic of Serbia, 2013

Parameter	Men (n = 6,652) % (95% CI)				Women (n = 7,770) % (95% CI)				
	normal BP (n = 671)	prehypertension (n = 2,643)	hypertension (n = 3,338)	*p* value* (men)	normal BP (n = 1,879)	prehypertension (n = 2,125)	hypertension (n = 3,766)	*p* value* (women)	*p* value* (between genders)
Place of residence									
urban	11.0 (9.9–12.1)	41.4 (39.7–43.1)	47.6 (45.9–49.3)	0.031	27.7 (26.3–29.1)	28.8 (27.4–30.3)	43.5 (41.9–45.1)	< 0.001	< 0.001
other	9.9 (8.7–11.1)	39.8 (37.9–41.7)	50.3 (48.4–52.3)		23.4 (21.8–25.0)	28.3 (26.6–30.0)	48.3 (46.5–50.2)		
BMI (kg/m^2)									
underweight (< 18.50)	36.7 (27.5–45.9)	43.1 (33.6–52.6)	20.2 (12.5–27.9)	< 0.001	57.4 (51.4–63.3)	27.9 (22.5–33.4)	14.7 (10.4–19.0)	< 0.001	0.004
normal weight (18.50–24.99)	15.9 (14.4–17.5)	48.7 (46.7–50.8)	35.3 (33.3–37.3)		41.0 (39.1–42.8)	34.3 (32.5–36.1)	24.7 (23.1–26.3)		< 0.001
overweight (25.00–29.99)	7.5 (6.4–8.5)	39.8 (37.9–41.6)	52.8 (50.9–54.7)		14.8 (13.2–16.3)	28.1 (26.1–30.1)	57.1 (54.9–59.3)		< 0.001
obesity (≥ 30.00)	3.5 (2.4–4.5)	26.8 (24.4–29.2)	69.8 (67.3–72.2)		6.3 (5.0–7.6)	18.4 (16.4–20.4)	75.3 (73.1–77.5)		< 0.001
WC (men/women in cm)									
normal (< 94/< 80)	16.4 (14.9–17.9)	51.0 (49.0–53.0)	32.6 (30.8–34.5)	< 0.001	47.4 (45.2–49.6)	35.4 (33.3–37.5)	17.1 (15.5–18.8)	< 0.001	< 0.001
moderate (94–101/ 80–87)	8.8 (7.3–10.3)	39.7 (37.1–42.2)	51.5 (48.9–54.1)		31.0 (28.5–33.6)	32.8 (30.2–35.4)	36.2 (33.5–38.8)		< 0.001
large (> 102/ > 88)	4.4 (3.5–5.3)	28.7 (26.7–30.7)	67.0 (64.9–69.0)		10.6 (9.5–11.7)	22.7 (21.2–24.2)	66.7 (65.0–68.3)		< 0.001
Physical activity - total time (min/per day)									
low (< 30)	9.0 (7.8–10.3)	39.0 (37.0–41.0)	52.0 (49.9–54.0)	< 0.001	22.5 (21.0–23.9)	27.2 (25.7–28.8)	50.3 (48.5–52.1)	< 0.001	< 0.001
moderate (30–59)	11.2 (9.7–12.6)	40.6 (38.3–42.8)	48.2 (46.0–50.5)		27.4 (25.4–29.3)	30.6 (28.5–32.6)	42.1 (39.9–44.2)		< 0.001
high (60+)	11.6 (10.1–13.2)	43.1 (40.8–45.4)	45.3 (42.9–47.6)		31.8 (29.3–34.3)	28.8 (26.4–31.2)	39.4 (36.8–42.0)		< 0.001

***p* value for χ^2 test; CI – confidence interval; SBP – systolic BP; DBP – diastolic BP; normal BP (SBP < 120 mmHg and DBP < 80 mm Hg); prehypertension (SBP = 120–139 mmHg and/or DBP = 80–89 mmHg); hypertension (SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg, or current treatment with antihypertensive medications).**

Table 4

Independent risk factors for hypertension and prehypertension compared to persons with normotension among adult (15+ years) population of the Republic of Serbia, 2013: multivariate logistic regression analysis

Parameter	Men, OR (95% CI)		Women, OR (95% CI)	
	prehypertension	hypertension	prehypertension	hypertension
Age (years)				
< 50		1	1	1
50–59		5.458 (4.013–7.423)*	2.279 (1.846–2.813)*	10.437 (8.369–13.017)*
60+		7.596 (5.959–9.682)*	3.432 (2.611–4.511)*	47.394 (36.346–61.801)*
Body mass index				
underweight and normal weight	1	1	1	1
overweight	1.810 (1.499–2.185)*	2.205 (1.720–2.825)*	1.722 (1.414–2.098)*	2.637 (2.063–3.371)*
obesity	2.679 (1.931–3.717)*	5.082 (3.288–7.855)*	2.224 (1.641–3.013)*	8.048 (5.778–11.209)*
Waist circumference				
normal		1	1	1
moderate		1.716 (1.318–2.235)*	1.093 (0.915–1.307)	1.696 (1.327–2.169)*
large		2.449 (1.741–3.444)*	1.411 (1.136–1.752)*	2.403 (1.818–3.176)*
Nonurban place of residence				1.291 (1.075–1.551)*
Physical activity				
low			1	
moderate			1.002 (0.857–1.172)	
high			0.801 (0.671–.956)*	

***p*-value < 0.01 according to multivariate logistic regression analysis; OR – odds ratio; CI – confidence interval.**

In the Serbian population, aged 15 and over, in 2013, 17.7% of population was normotensive, every third (33.1%) person had PreHTN, and every second (49.3%) person had HTN (Table 2). Men had significantly higher prevalence of PreHTN, as well as HTN than women for all age groups and total, except for HTN for age group 60+ years where prevalence was higher in women. In both sexes and in total, the prevalence of HTN increased with age, while PreHTN prevalence decreased. In Serbia, the standardized prevalence of PreHTN was 40.6% (men 48.5% and women 33.4%) and 34.5% for HTN (men 38.6% and women 30.6%).

In urban population, as well as in nonurban one, prevalence of PreHTN and HTN was significantly higher in males than in females (Table 3). Among both sexes, prevalence of PreHTN was higher in urban population (41.4% of males, and 28.8% of females), while HTN prevalence was significantly higher in nonurban population (50.3% of males, and 48.3% of females).

Males had significantly higher prevalence of PreHTN and HTN for all BMI categories, except for overweight and obesity, where prevalence of HTN was higher in females. Prevalence of normotension and PreHTN decreased, while HTN increased with the increase in BMI, for both sexes. The highest prevalence of HTN was in obese category for both sexes (69.8% of males, and 75.3% of females).

Males had significantly higher prevalence of PreHTN and HTN for all WC categories. Prevalence of normotension and PreHTN significantly decreased, while HTN increased with the increase in WC, for both sexes. The highest prevalence of PreHTN was in normal WC category (51.0% of males, and 35.4% of females), while HTN prevalence was highest in large WC (67.0% of males, and 66.7% of females).

The prevalence of PreHTN and HTN was significantly higher in males for all physical activity categories. Prevalence of normotension increased while the prevalence of HTN decreased with the increase in physical activity levels, in both sexes. Prevalence of PreHTN was highest in high physical activity category for males (43.1%) and in moderate physical activity category for females (30.6%). The highest prevalence of HTN was among males (52.0%) and females (50.3%) with low physical activity.

Variables that according to ULRA had p value ≤ 0.1 were included into the model of MLRA. For normotension and HTN these variables were age, place of residence, BMI, WC, and physical activity, in both sexes. According to the results of MLRA, independently significant risk factors for HTN compared to persons with normotension were older age (50 and more), overweight and obesity, moderate and large WC in both sexes, and nonurban place of residence in females (Table 4).

Variables for normotension and PreHTN included into the model of MLRA were BMI and WC for males, and age, BMI, WC and physical activity in females. In females, independently significant risk factors for PreHTN were older age (50 and more), overweight and obesity, moderate and large WC, but high level of physical activity was significantly protective. In males, overweight and obesity were independently significant risk factors for PreHTN (Table 4).

Discussion

In the adult population of Serbia, aged 15 and over, in 2013, high prevalence of PreHTN (33.1%; standardized prevalence 40.6%) and HTN (49.3%; standardized prevalence 34.5%) were identifed. Both age-standardized prevalences were higher in men (48.5% and 38.6%, respectively) than in women (33.4% and 30.6%, respectively). Among the subjects with HTN, only 45.9% of men and 68.3% of women (57.8% of all) was on antihypertensive treatment. Among those receiving medical treatment, only 35.2% (36.4% of males and 33.2% of females) had BP within the normal range. In the United States in 2004 prevalence of antihypertensive medication use among HTN adults, 18 years and more, was 77.3%, more often among women (82.5%) and individulas 60 years and more (83.6 %) [13]. Among adult Chinese 35–75 years of age, 30.1% take medication for HTN in the period 2014–2017 [14]. In Serbia and in other developing countries prevalence of PreHTN and HTN is high and rates of awareness, treatment, and control are low [15]. Therefore, epidemiological situation of HTN in our country is alarming; there is a huge hidden burden of undiagnosed HTN subjects and that requires urgent attention. If we add those with PreHTN, the magnitude of the problem is much higher.

Worldwide age-standardized prevalence of HTN in adults, 20 years and above, in 2010, was 31.1% (31.9% in men and 30.1% in women), and it was lower in high-income countries (28.5% in both sexes, 31.6% in men and 25.3% in women) than in low- and middle-income countries (31.5% in both sexes, 31.7% in men and 31.2% in women) [16]. It was estimated that 1.4 billion people had HTN on the global level. Aproximately ¾ of individuals with HTN lived in low- and middle-income countries. In the same year, the highest prevalence for men was in Europe and Central Asia (38.8%) and for women in sub-Saharan Africa (36.3%), but the lowest for men was in South Asia and for women in high-income economies (26.9%). It is estimated that HTN prevalence has decreased in high-income countries and increased in low- and middle-income countries between 2000 and 2010 [16]. In low- and middle-income countries, aging and urbanisation with unhealthy lifestyle may play a role in the epidemic of HTN [16–18].

In some investigations, prevalence of PreHTN was also analized. For example, in the National Health and Nutrition Examination Survey the prevalence of PreHTN in disease-free US adults, over the age of 20 years, was 36.3% and was higher in men (44.8%) than in women (27.3%) [19]. Also, prevalence increased with age (31.2% for ages 20–39 years, 42.3% for ages 40–59 years and 44.2% for ages 60–69 years). But, in our study, in both sexes and in total, the prevalence of PreHTN decreased. There is growing evidence that individuals with PreHTN would have a significantly higher risk of developing HTN and CVDs within a few years [20].

In our research, as in most other studies, the prevalence of PreHTN and HTN was higher in males than in females [16, 21, 22]. The differences in the frequency of high BP between the sexes can be partly explained by biological factors that include sex hormones, chromosomal differences,

and other biological gender differences that protect women from HTN until women reach menopause and after that gender differences in HTN become smaller or nonexistent [23, 24].

In our study, according to MLRA results, independently significant risk factors for HTN compared to persons with normotension were older age (50 and more), overweight and obesity, moderate and large WC in both sexes, and nonurban place of residence in females. In females, independently significant risk factors for PreHTN were the same as for men with HTN and high level of physical activity was significantly protective factor. In males, only overweight and obesity were independently significant risk factors for PreHTN.

These results are very important because in a small number of studies independent risk factor for HTN was analyzed according to sex. Age has been recognized as a risk factor for HTN and some researchers believe that high prevalence of HTN among older people might be due to changes that occur in the blood vessels [25].

The results of this study are similar with the findings of the studies of Hu et al. [21], Dua et al. [26], Tripathy et al. [27] and Erem et al. [28] who noticed that overweight and obesity lead to PreHTN and HTN in both sexes. In addition, the prevalence of HTN appears to increase even with a relatively small increase in body weight [29]. Similarly, among postmenopausal women, the risk of developing high BP is doubled with a high BMI or high WC [30]. Deng et al. [31] and Hu et al. [21], also found that BMI and WC were positively related to the prevalence of HTN. The prevalence of PreHTN and HTN increased with an increase in BMI. The prevalence of PreHTN decreased in parallel to an increase in the prevalence of HTN. This was particularly noted in subjects up to 44 years of age. In relation to individual nutrition indicators, the WC and BMI combination proved to be superior in predicting risk factors for HTN [32].

In our study, men with PreHTN and HTN had higher prevalence of overweight (39.8% and 52.8%, respectively) and obesity (26.8 and 69.8%, respectively) than women (28.1% and 57.1%, respectively for overweight, and 18.4% and 75.3%, respectively for obesity). The prevalence of overweight and obesity in both sexes increased with an increase in BP and was the highest in people with HTN. Improved living standard has led to an increase in obesity throughout the world, especially during childhood. In the United States, 70.9% of adult men and 61.9% of women are overweight, and nearly one-third of the adult population, 31.6% of men and 33.9% of women are obese [32]. In Sub-Saharan Africa, the highest prevalence of obesity was recorded among women in South Africa (42.0%).

In our study, only in PreHTN women, high level of physical activity (60 and more min per day) was an independently significant protective factor for PreHTN development, but not for HTN. Adults who are insufficiently physically active have 20%−30% increased risk of all-cause mortality compared to those who do at least 150 min of moderate-intensity physical activity per week, or equivalent (as recommended by the WHO) [33]. In 2010, globally, 23.3% of adults (19.8% of men and 26.8% of women) were insufficiently physically active (less than 150 min of moderate-intensity physical activity per week or equivalent) [33]. Prevalence of physical inactivity is higher in high-income countries and amounts to 32.7% (27.7% of men and 37.6% of women). Physical inactivity is higher in women than in men and is more frequent in older age groups. Daily exercise of at least 30 min of physical activity can reduce BP and prevent the development of pre-HTN and HTN [33]. The mechanisms by which physical activity may reduce BP and prevent the development of PreHTN and HTN are unclear. Same animal studies suggest that aerobic exercise may prevent increases in BP by increasing insulin sensitivity and autonomic nervous system function [34], while resistance training may prevent increases in BP through beneficial alterations in vasoconstriction regulation [35].

Exploring the regional differences and an in-depth analysis of the urban and rural variations in prevalence may provide important insight into the underlying determinants of the increasing PreHTN and HTN. In most studies, living in urban areas was positively associated with HTN [36]. Urbanization influences lifestyle patterns, leading to a decrease in physical activity, changes in food consumption, and increased stress [36]. However, a small number of recent studies showed that the HTN prevalence was higher in rural areas than in urban ones. In Turkish adult population aged 20 years or more, the overall (age-standardized) prevalence of HTN was 24.9%, and was higher in rural (28.4%) than in urban areas (23.9%) [37]. Women were more likely to had HTN in rural areas than in urban areas ($p < 0.05$). In our study, similar to these results, nonurban place of residence in females was independent risk factor for HTN. In our females, prevalence of HTN was 50.3% in nonurban and 47.6% in urban place. Possible explanation for our results could be emerging urbanization in rural areas. This suggests that analyzing HTN risk factors according to gender may provide greater insight in understanding the variations in HTN development between urban and rural areas.

Noncommunicable diseases represent an upcoming epidemic for developing countries, including Serbia. In the light of this trend, the current study casts a significant light on the prevalence of HTN in our country.

Limitations of this study include: cross-sectional study design which implies that no causal conclusion about the relationship between health behavior and anthropometric parameters variables and BP values can be made; the data based on self-reporting for physical activity could lead to recall bias, which may have prevented us from accurately estimating the association between physical activity and BP categories. Some risk factors, which are important in risk assessment, were not taken into accounts, such as psychosocial factors, social class, smoking, nutrition habits and others.

Conclusion

Serbia belongs to countries with a high prevalence of prehypertension and hypertension and the prevalence of undiagnosed and untreated hypertension is high in the adult population of Serbia. Our results emphasize the need for a new public health strategy for the prevention, detection and

treatment of prehypertension and hypertension. Moreover, regular physical activity and weight reduction through education and awareness among people are the key to reducing the load of prehypertension, hypertension and cardiovascular diseases. The results of this research can help decision makers in the health system to establish interventions that will more effectively control prehypertension and hypertension in our country.

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Clinical significance of diagnostic algorithm in detection of mild hemostasis disorders in women with menorrhagia

Klinički značaj dijagnostičkog algoritma u detekciji blažih poremećaja hemostaze kod pacijentkinja sa menoragijom

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Abstract

Background/Aim. Coagulation disorders could be a cause of menorrhagia in women of reproductive age. The aim of the study was to estimate frequency of coagulation disorders and design an appropriate algorithm for detection of coagulation disorders. **Methods.** We investigated coagulation in 115 women (36.1 ± 9.6 years) with anamnestic data of menorrhagia, verified using semiquantitative method - Pictorial Bleeding Assessment Chart (PBAC) with score ≥ 100. **Results.** Menorrhagia was objectively verified in sixty-four women (55.7%) and in comparison with those with normal menstruation they had higher PBAC score of menstrual cycle [median (Md) = 150.0 vs. Md = 50.0; $p < 0.001$] but not its duration (7.2 ± 2.1 days vs. 7.3 ± 1.9 days; $p > 0.05$). Coagulation defects was found in 12 (10.4%) women - decreased F IX: Ac in 4 (3.5%), decreased F VII: Ac in 1 (0.9%), decreased F X: Ac in 1 (0.9%), decreased F XI: Ac in 1 woman (0.9%), while 5 (4.3%) women matched criteria for mild von Willebrand disease (VWD) type 1. Women with coagulation disorders had prolonged prothrombin time (PT) [Md = 13.1 s, range: 12.2–14.8 s vs. Md = 12.5 s, range 10.6–18.3 s; $p = 0.032$]. Anemia was diagnosed in 61 (53.0%) women. The strongest predictor of the hemostasis disorder was menorrhagia (Quotient of probability 0.018), then anemia presence (12.43), PT (2.35), menstrual cycle duration (1.16) and the PBAC score (0.98). **Conclusion.** The results of the study indicate the need to form a diagnostic algorithm for hemostasis disorders, primarily VWD. Sophisticated analysis of hemostasis is required, especially if predictive factors of statistical models are detected: objectively verified menorrhagia, anemia, prolonged menstrual cycle, PBAC score > 100 and extended PT.

Key words:
von willebrand disease; hemostasis, disorders; anemia.

Apstrakt

Uvod/Cilj. Poremećaji koagulacije mogu da budu uzrok menoragije kod žena u reproduktivnom periodu. Cilj istraživanja bio je utvrđivanje učestalosti poremećaja koagulacije kod žena sa menoragijom i kreiranje odgovarajućeg algoritma za detektovanje poremećaja koagulacije. **Metode.** Ispitivani su parametri koagulacije kod 115 žena (36,1 ± 9,6 godina) sa anamnestičkim podatkom o postojanju menoragije koja je verifikovana primenom semi-kvantitativne metode - Pictorial Bleeding Assessment Chart (PBAC) sa skorom ≥ 100. **Rezultati.** Menoragija je bila objektivno verifikovana kod 55,7% ispitanica. Pacijentkinje sa menoragijom imale su viši PBAC skor [medijana (Md) = 150,0 vs. Md = 50,0; $p < 0,001$], ali ne i dužinu menstrualnog ciklusa (7,2 ± 2,1 dana vs. 7,3 ± 1,9 dana; $p > 0,05$). Poremećaji koagulacije bili su detektovani kod 12 (10,4%) ispitanica – snižene vrednosti faktora (F) IX: Ac kod 4 (3,5%), F VII: Ac kod 1 (0,9%), F X: Ac kod 1 (0,9%), F XI: Ac kod 1 (0,9%), a 5 (4,3%) pacijentkinja ispunjavalo je kriterijume blage forme von Willebrandove bolesti (VWB) tip 1. Ispitanice sa poremećajima koagulacije su imale produženo protrombinsko vreme (PT) [Md = 13,1 s (12,2–14,8 s) vs. Md = 12,5 s (10,6–18,3) s; $p = 0,032$]. Anemija je dijagnostikovana kod 61 (53,0%) pacijentkinje. Najjači prediktor poremećaja hemostaze bilo je postojanje objektivno verifikovane menoragije (količnik verovatnoće 0.018), a zatim prisustvo anemije (12.43), PT (2.35), dužina menstrualnog ciklusa (1.16) i vrednost PBAC skora (0.98). **Zaključak.** Rezultati istraživanja ukazuju na potrebu formiranja dijagnostičkog algoritma poremećaja hemostaze. Sofisticirane i skupe laboratorijske analize za dijagnozu poremećaja hemostaze bilo bi racionalno sprovesti kod pacijentkinja koje imaju menoraguju verifikovanu objektivnim metodama, PBAC skor > 100, produžen menstrualni ciklus, anemiju i produženo PT.

Ključne reči:
von Willebrand-ova bolest; hemostaza, poremećaji; anemija.

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Introduction

Menorrgahia is fairly common problem among women of reproductive age. According to *World Health Organization* (WHO) 18 million women in the world, aged 30 to 55, have this disorder [1]. Objectively, menorrhagia is defined as menstrual blood loss exceeding 80 mL *per* menstruation or heavy menstrual bleeding that lasts for more than 7 days [2]. Diagnosis is often made subjectively by patient self-report of excessively heavy menstrual bleeding, but correlation between anamnestic and objectively verified menorrhagia is poor. Data from literature suggested that approximately 10% of reproductive-aged women had objective evidence of menorrhagia, but studies based on self-reported information suggested that approximately 30% of women of reproductive age were afflicted with heavy menstrual bleeding [3, 4]. Menorrhagia may result from anatomic, endocrinologic, iatrogenic and organic causes [5–8]. Underlying bleeding disorders belong to the group of organic causes of menorrhagia and only have been recognized during the last two decades as a significant etiopathogenetic factor for menorrhagia formation. Frequency of hemostasis disorders in women with menorrhagia is in the range of 10% to 20% [9, 10]. The reported prevalence of von Willebrands disease (vWD) as the most frequent among them is 13%, based on a systematic review [11]. The considerable proportion of women with menorrhagia is found to have single coagulation factor deficiencies such as factor (F) XI deficiency (1–4%), carriers of hemophilia A and hemophilia B observed in approximately 1–4% of females with menorrhagia and less common deficiencies of factors I, II, V, VII, X, XI, XIII [12–15]. Coagulation abnormalities have a major impact on health-related quality of life, work impairment and health-care costs [16]. Anemia is associated with menorrhagia and coagulation abnormalities in women of reproductive age**.** At least 20% of women with heavy menstrual bleeding experience anemia [17].

The aim of the study was to estimate prevalence of coagulation disorders in females with menorrhagia as well as frequency of menorrhagia and its characteristics and design an appropriate algorithm for patients and define required laboratory tests for them.

Methods

Patients

This clinical-laboratory study included population of 115 women aged 36.1 ± 9.6 years (range 15 to 58 years). The main including criterion was anamnestic information about the existence of heavier and/or prolonged menstrual cycles. Excluding criteria were: the existence of endocrine diseases and diseases of genital and urethral tract which could be the cause of menorrhagia, treatment with antiplatelet and anticoagulant drugs within 2 weeks prior to the present study, known bleeding disorder, pregnancy. Informed consent was obtained from all patients. Menorrhagia was verified using semiquantitative method – Pictorial Bleeding Assessment Chart (PBAC) with score greater than 100 (which was equivalent to greater than 80 mL amount of blood loss measuring with alkaline hematin analysis of sanitary towels) [5, 6]. Complete blood count (CBC), iron, total ron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), bleeding time (BT) and coagulation analyses as well as ABO blood group typing were performed.

Assays

Following coagulation tests were repeated on two occasions, before day 7 of the menstrual cycle on platelet poor plasma (fresh blood containing 3.2% sodiumcitrate anticoagulant centrifuged with 2500 G rpm for 15 minutes) on the ACL 9000: activated partial tromboplastin time (aPTT) (aPTT-SP liquid, Hemosil, Instrumentation Laboratory Company-Lexington USA), protrombin time (PT), (PT-Fibrinogen Recombinant, Hemosil, Instrumentation Laboratory Company-Lexington USA), *International normalized ratio* (INR), fibrinogen (PT-Fibrinogen Recombinant, Hemosil, Instrumentation Laboratory Company-Lexington USA), D-dimer (D-dimer, Hemosil, Instrumentation Laboratory Company-Lexington USA), factor clotting activity (F II, F V, F VII, F VIII, F IX, F X, F XI) (Factor deficient plasma, Hemosil, Instrumentation Laboratory Company-Lexington USA), von Willebrand factor antigen (vWFAg) (von Willebrand Factor Antigen, Hemosil, Instrumentation Laboratory Company-Lexington USA), von Willebrand factor activity (vWFAc) (von Willebrand Factor Activity, Hemosil, Instrumentation Laboratory Company-Lexington USA).

Statistical analysis and assessment

Statistical analysis was performed by SPSS 13.0. Mean and standard deviation were used to describe the variables. ANOVA test and *t*-test were used to analyze quantitative variables. The Fisher exact test and chi-square (χ^2) test were carried out for qualitative variables. With the help of direct logistic regression, the predictive value of the model including some of the parameters tested was examined.

Results

The frequency and characteristics of menorrhagia in the study population

Sixty four (55.7%) women of the total number of patients (115) with anamnestically data of menorrhagia, had objectively verified menorrhagia using semiquantitive method – Pictorial Bleeding Assessment Chart (PBAC) with score > 100 (equivalent > 80 mL blood). Characteristics of menorrhagia and hematologic tests results of patients with and patients without menorrhagia are shown in Table 1.

Coagulation tests results of patients with menorrhagia and patients without menorrhagia are shown in Table 2.

Table 1

Characteristics of menorrhagia and hematologic tests results of patients with and without menorrhagia

Parameters	All patients (mean ± SD or Md, min-max) (n = 115)	Women with menorrhagia (mean ± SD or Md, min-max) (n = 64)	Women without menorrhagia (mean ± SD or Md, min-max) (n = 51)	*p*
Age (years)	36.1 ± 9.6	38.0 (15–55)	36.0 (17–58)	> 0.05*
Score (points)	100.0 (26–778)	150.0 (100–778)	50.0 (26–95)	< 0.001*
Duration of menstrual bleeding (days)	7.0 (4–28)	7.0 (6–28)	7.0 (4–12)	> 0.05*
Iron level (imol/L)	9.8 (min 2.1, max 43.6)	8.6 (2.1–36.2)	8.1(5.2–43.6)	> 0.05*
RBC ($\times 10^{12}$/L)	4.28 (2.27–5.50)	4.34 (3.30–5.50)	4.73 (2.27–5.40)	> 0.05*
Hemoglobin (g/L)	114.1 ± 22.2	114.9 ± 19.1	113.1 ± 25.9	> 0.05**
MCV (fL)	81.8 ± 10.1	83.1 ± 9.8	81.3 ± 10.6	> 0.05*
Hematocrit (%)	0.350 (0.126–0.462)	0.360 (0.22–0.462)	0.359 (0.126–0.440)	> 0.05*
Platelets ($\times 10^{9}$/L)	283.9 ± 92.9	290.1 ± 86.9	275.3 ± 100.9	> 0.05**

RBC – red blood cells; MCV – mean cell volume; Md – median.
***Mann-Whitney *U*-test; **Student *t*-test.**

Table 2

Coagulation tests results of patients with menorrhagia and patients without menorrhagia

Parameter	Women with menorrhagia (mean ± SD or Md, min-max) (n = 64)	Women without menorrhagia (mean ± SD or Md, min-max) (n = 51)	*p*
Bleeding time (s)	120 (60–270)	90 (60–180)	> 0.05
aPTT (s)	27.9 (22.4–46.2)	28.6 (21.3–49.1)	> 0.05*
PT (s)	12.6 (10.8–15.0)	12.5 (10.6–18.3)	> 0.05*
Fibrinogen (g/L)	3.26 ± 0.82	3.50 ± 0.85	> 0.05**
F II (%)	84.4 (50.9–149.0)	92.5 (52.0–182.0)	> 0.05*
F V (%)	98.5 (50.0–213.0)	110.0 (50.0–241.0)	> 0.05*
F VII (%)	78.0 ± 28.3	100.5 ± 35.0	> 0.001**
F VIII (%)	132.0 (39.0–525.0)	124.0 (22.0–596.0)	> 0.05*
F IX (%)	79.6 (27.0–772.0)	79.2 (45.0–472.0)	> 0.05*
vWFAc (%)	98.5 (26.2–182.0)	96.6 (38.5–279.0)	> 0.05*
vWFAg (%)	111.5 (30.0–535.0)	95.8 (32.0–348.0)	> 0.05*

aPTT – activated partial thromboplastin time; PT – prothrombin time; F – factor; vWFAc – von Willebrand factor activity; vWFAg – von Willebrand factor antigen; Md – median.
***Mann-Whitney *U*-test; **Student *t*-test.**

The frequency and characteristics of coagulation disorders in the study population

In the examined population, coagulation defects were found in 12 (10.4%) women – decreased F IX: Ac in 4 (3.5%), decreased F VII: Ac in 1 (0.9%), decreased F X: Ac in 1 (0.9%), 1 woman (0.9%) was a hemophilia C carrier, while 5 women (4.3%) matched criteria for mild VWD type 1. Groups of patients with and without hemostatic disorders did not differ significantly with respect to the studied parameters (age, length of menstrual cycle, PBAC score, hematology, most of the coagulation factors) as expected. Patients with some of hemostasis disorders registered had prolonged PT [median (Md) = 13.1 s (12.2–14.8 s) vs. Md = 12.5 s (10.6-18.3 s); $p = 0.032$]. After adjustment for the presence of F VII and F X deficiency this finding was persistent for the entire group.

Connection between menorrhagia and coagulation disorders

Chi-square (χ^2) test of independence (with correction by Yeats) showed significant association between the existence of menorrhagia and the existence of hemostasis disorders [c^2 (1, n = 115) = 5.506, $p = 0.019$, fi = -0.247, Cramer`s V = 0.247]. Among patients with menorrhagia, 17.2% of them have hemostasis disorder, while the number was significantly lower among patients who had no verified menorrhagia (1 of 51).

The frequency of anemia in the study population

Anemia was diagnosed in 61 (53.0%) women. Taking into account the average values of hematological parameters, microcytic, hypochromic, hiposideremic anemia was present in all patients.

Table 3
Prediction of the existence of hemostasis disorder in patients who state a history of the existence of menorrhagia (5 parameters)

Parameters	Á	Standard error	Wald	Degrees of freedom	*p*	Quotient of probability	95% confidence interval for quotient of probability	
							lower limit	upper limit
Menorrhagia	-4.024	1.470	7.495	1	0.006	0.018	0.001	0.319
Anemia	2.520	0.965	6.816	1	0.009	12.427	1.874	82.404
PBAC score	-0.015	0.009	3.197	1	0.050	0.985	0.968	1.001
PT	0.856	0.422	4.102	1	0.043	2.353	1.028	5.384
Cycle duration	0.148	0.076	3.821	1	0.050	1.160	1.000	1.345
Constant	-13.196	5.460	5.742	1	0.016	0.000		

PBAC – Pictorial Bleeding Assessment Chart; PT – prothrombin time.

Predictive factors for the existence of hemostasis disorders in the study population

We investigated the predictive ability of the analyzed parameters in the detection of coagulation disorders. The model included five parameters: objectively verified menorrhagia, the presence of anemia, menstrual cycle duration, the value of PBAC score, menstrual cycle and PT.

Prediction of the existence of coagulation disorder in patients who stated a history of the existence of menorrhagia is shown in Table 3.

The strongest predictor of the coagulation disorder was objectively verified menorrhagia, which quotient of probability was 0.018, then anemia presence (12.43), PT (2.35), menstrual cycle duration (1.16) and the PBAC score (0.98).

Discussion

In more than half of patients (55.7%) who self-reported abundant and/or prolonged menstrual bleeding, menorrhagia was really diagnosed. Therefore, there is a need to apply an objective method for estimating intensity of menstrual bleeding. The most spread one is a semi-quantitative method of comparative analysis of used sanitary material with standard tables and calculation of the PBAC score [6]. Obtained results are in the best correlation with the "golden standard" method of menstrual bleeding intensity estimation by determining alkaline hematin [5]. PBAC score of menstrual cycle (its intensity), but not its duration, was higher in women with menorrhagia. Thus, intensity but not duration of menstrual cycle led to greater blood loss in women with menorrhagia.

Patients with and without menorrhagia did not differ among themselves regarding examined factors of coagulation except for F VII. After adjustment for the presence of F VII deficiency this finding was persistent for the entire group. The existence of states and disorders which could influence activity of F VII were excluded: sepsis [18], malignity [19], transplantation of bone marrow [20, 21], transitory deficit after surgery [22] procreation of antibodies on F VII [23–26], influence of circadian rhythm on activity F VII [27, 28]. Considering a short half-life of F VII, it seems that at patients with menorrhagia it was possible to deplete this vitamin K dependent glycoprotein during prolonged bleeding. Still, these results demand examination of more patients and additional tests.

Every tenth patient who stated a history of the existence of menorrhagia, had some of coagulation disorders (12 of 115 patients). The most frequent disorder is mild VWD type 1. A similar prevalence of specific hemostasis disorders was also obtained in representative studies [29]. On our territory data on coagulation disorders in women with menorrhagia are scarce and the current study is one of the first conducted in Serbia. In women with menorrhagia we detected mild VWD type 1 and mild forms of the deficit of individual factors, which incidence is not significantly different from other researches. Among patients with menorrhagia, 17.2% of them had hemostasis disorder. Kadir et al. [10], in one of the most representative studies, have stated that about 17% of patients had such disorder. The proportion of women with VWD is 6.25%. Meta-analysis by Shankar et al. [11] that included a total of 11 studies with 988 women, showed that the prevalence of VWD was in the range of 5% to 24%. The proportion of women with a deficit of individual coagulation factors was 6% for F IX and 2% for F VII, F X and F XI. Some reports showed that incidence of deficit of individual factors in women with menorrhagia was in the range of 1% to 4%, an average of 2.5% [14, 15].

Our research showed that PT had a significant role in predicting coagulation disorder. We found out that patients with a registered coagulation disorder had significantly higher values of PT in comparison with patients with normal hemostasis and almost all of them had menorrhagia. The study of Hutspardol et al. [30] showed the similar average value of PT in the group of patients with menorrhagia.

Anemia was diagnosed in over a half of the patients included into the study (53%). The survey by Philipp et al. [14] showed that 58% of patients with menorrhagia had anemia and in 4% of them substitution therapy of blood transfusions was applied.

We investigated the predictive ability of the analyzed parameters in the detection of coagulation disorders. There are numerous attempts to determine the importance of specific symptoms and signs in terms of predicting the existence of coagulation disorders. The consensus of international expert panel for the diagnosis and treatment of VWD and other disorders of hemostasis in women with menorrhagia, for the

prediction of hemostatic disorders in women with menorrhagia rely on symptoms and signs of clinical hemorrhage [31]. Toseto et al. [32] and Rodeghiero et al. [33] valorized some of the most common clinical manifestations of hemorrhagic syndrome for prediction of VWD. To consolidate multiple parameters in the prediction of hemostasis disorders in our research a direct logistic regression was conducted and the predictive model was postulated that, in addition to menorrhagia, emphasized the presence of anemia, duration of the menstrual cycle, the value of PBAC score and PT. The strongest predictor of the existence of coagulation disorders was presence of objectively verified menorrhagia (ratio of the probability 0.018), then the presence of anemia (12.43), PT (2.35), the menstrual cycle lenght (1.16) and the PBAC score value (0. 98). This practically means that the chance of existence of coagulation disorders in a group of patients who stated the history of menorrhagia was 55.56 times higher in those with objectively verified menorrhagia, 12.43 times higher if they had anemia, with each increase in PT for 1 second probability of having a coagulation disorder increased 2.35 times, for each day of prolonged menstrual cycles it increased 1.16 times and with each additional point in the score of menstrual cycle it increased 1.02 time. These five parameters could also represent the strongest predictors for the presence of coagulation disorders.

Although inclusion of more patients and completing the study with additional investigations primarily related to platelet function is mandatory, the results of our study can be seen as a contribution to form a diagnostic algorithm for disorders of hemostasis, primarily VWD. Firstly, it is necessary to estimate the abundance of menstrual cycles (PBAC), and then, in the second step, it is important on the basis of simple anamnesis (duration of menstrual cycle) and standard laboratory analyses (laboratory parameters for anemia and PT) to extract the group of patients in whom it is rational to implement a set of expensive diagnostic procedures. Sophisticated analysis of hemostasis is required in highly specialized centers, especially if predictive factors of statistical models were detected: objectively verified menorrhagia, anemia, prolonged menstrual cycle, PBAC score > 100 and extended PT.

This gradual approach would allow a rational, comprehensive and timely diagnosis of mild forms of hemostasis disorders often present in patients with menorrhagia which are less likely to think about, because they have a subclinical manifestation and inapparent flow. A special clinical significance they get only in life-threatening situations, such as trauma or surgery, when untimely detection of these disorders can lead even to fatal consequences.

Conclusion

Our results can contribute to the development of a diagnostic algorithm for disorders of hemostasis, primarily VWD. Sophisticated analysis of hemostasis is required, especially if predictive factors of statistical models were detected: objectively verified menorrhagia, anemia, prolonged menstrual cycle, PBAC score > 100 and extended PT.

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The operative risk stratification models in cardiac surgery: EuroSCORE II model – risk groups categorization

Modeli za stratifikaciju operativnog rizika u kardiohirurgiji: kategorizacija grupa rizika za *EuroSCORE* II model

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Abstract

Background/Aim. The treshold that defines a low, moderate or high-risk patients is not uniformly determined for the European System for Cardiac Operative Risk Evaluation (EuroSCORE II) by literature at present. The aim of this study was to suggest risk groups categorization within EuroSCORE II risk statification model. **Methods.** A 7,641 consecutive patients were scored preoperatively using EuroSCORE II. The end point for the study was in-hospital mortality accross the risk group categories. Patients with EuroSCORE II values of ≤ 2.50, > 2.50–6.50%, and > 6.50% were defined to be at low, moderate, and high perioperative risk, respectively. Discriminative power of the model was tested by calculating the area under the receiver operating characteristic curve (AUC). The calibration of the model was assessed by Hosmer-Lemeshow statistics, and with observed/expected (O/E) mortality ratio. **Results.** In-hospital mortality observed in our sample was 3.85% (295 out of 7,641 patients). The EuroSCORE II discriminative power was acceptable (AUCs > 0.70) for the low and high risk groups, while it failed to confirm good discrimination in the moderate risk group. Hosmer-Lemeshow statistics confirmed good calibration across risk group categories. The O/E mortality ratio failed to confirm good calibration in the low and high risk group (slight, but significant underprediction ratio of 1.24; 95% confidence interval 1.05–1.43), but confirmed good calibration in all three subcategories of the high risk group. **Conclusion.** The results of this study showed an acceptable overall performance of the EuroSCORE II in terms of discrimination and accuracy of model predictions for perioperative mortality across risk group categories. Validation of EuroSCORE II performances across risk group categories needs to be further studied for a continuous improvement of patients' risk stratification before planned cardiac surgery.

Key words:
cardiac surgical procedures; hospital mortality; risk factors; risk assessment; models, theoretical; europe.

Apstrakt

Uvod/Cilj. Granične vrednosti evropskog sistema za evaluaciju operativnog rizika u kardiohirurgiji (EuroSCORE II) koje definišu grupe rizika (niska, umerena, visoka) nisu trenutno uniformno određene u literaturi. Cilj naše studije bio je da se predloži kategorizacija grupa rizika u *EuroSCORE* II modelu. **Metode.** Preoperativno je kod 7 641 uzastopnih bolesnika procenjen preoperativni rizik upotrebom *EuroSCORE* II. Primarni cilj studije bio je određivanje bolesničkog mortaliteta prema pripadnosti određenoj kategoriji grupe rizika. Bolesnici sa vrednostima *EuroSCORE* II ≤ 2,50, > 2,50–6,50% i > 6,50% smešteni su u kategoriju niskog, umerenog ili visokog perioperativnog rizika, respektivno. Diskriminaciona snaga modela testirana je izračunavanjem površine ispod *the receiver operating characteristic* (ROC) krive (AUC). Kalibracija modela je bila procenjena upotrebom Hosmer-Lemeshow testa, kao i sa odnosom između zabeleženog i očekivanog (O/E) mortaliteta. **Rezultati.** Zabeleženi bolnički mortalitet u našem uzorku je bio 3.86% (295 od 7 641 bolesnika). Diskriminatorna snaga *EuroSCORE* II je bila prihvatljiva za grupu niskog i visokog operativnog rizika (AUCs > 0,70), dok dobra diskriminacija nije potvrđena u grupi umerenog rizika. Hosmer-Lemeshow testom potvrđena je dobra kalibracija u svim kategorijama grupa rizika. Odnos između zabeleženog i očekivanog (O/E) mortaliteta nije potvrdio dobru kalibraciju u grupi niskog i visokog rizika (blago, ali signifikantno potcenjivanje mortaliteta, O/E odnos od 1.24; 95% interval pouzdanosti 1,05–1,43), ali je potvrdio dobru kalibraciju u sve tri podkategorije grupe visokog operativnog rizika. **Zaključak.** Rezultati ove studije pokazuju prihvatljive sveukupne performance *EuroSCORE* II modela u smislu diskriminacije i tačnosti predviđanja perioperativnog mortaliteta u definisanim kategorijama grupa rizika. Potrebna je dodatna provera performansi *EuroSCORE*

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II u definisanim kategorijama grupa rizika zbog kontinuiranog poboljšanja stratifikacije rizika bolesnika pre planirane kardiohirurške intervencije.

Ključne reči:
hirurgija, kardijalna, procedure; mortalitet, bolnički; faktori rizika; rizik, procena; modeli, teorijski; evropa.

Introduction

Although there has been an important progress in preoperative screening, surgical techniques, myocardial protection, and intensive care unit (ICU) treatment, open-heart surgery still carries a certain risk of mortality and morbidity. Being the most useful tool for the improvement of patients' selection and counseling, scoring systems have been developed over the last two decades, and used to predict perioperative risk in cardiac surgery. Therefore, risk adjusted perioperative mortality rate following cardiac surgery has been widely adopted as an indicator of quality of care as well as for comparison of outcomes among institutions and surgeons (in the United Kingdom). Predicted probability of occurrence of postoperative death has also enabled stratification of patients in different clinical risk groups (low, moderate, high) [1], and, subsequently, made it possible to target high-risk surgical patients in need of new therapeutic interventions [2, 3]. Being the most widely used worldwide, the Society for Thoracic Surgeons (STS) Predicted Risk of Mortality (PROM) score, and the European System for Cardiac Operative Risk Evaluation (EuroSCORE II) have recently been adopted by guidelines [4]. The EuroSCORE study group [5] in presentation of original additive EuroSCORE model, has stratificated risk groups as low (score 0–2), moderate (3–5) and high (6 ≥) perioperative risk. Although both versions (additive and logistic) of the old EuroSCORE have retained very good discriminatory power, old models no longer accurately predict operative mortality due to an overestimation of the adult cardiac patients surgical risk (poor calibration) in the range of two to three fold [6, 7]. Therefore, the aged EuroSCORE has recently been updated and renewed into EuroSCORE II [6]. However, there are only a few reports [8–10] in which authors tried to determine risk group categories based on the score values of EuroSCORE II model. Our arbitrary determined risk group boundaries are based on predicted risk values which should represent a real world scenario, and should have a more clinically meaningful power than previously reported arithmetic quartile grouping (with similar number of patients), resulting in a very low score values for moderate, and especially for high risk patients [8, 9]. Therefore, the aim of our study was to suggest more real risk group categorization using EuroSCORE II model.

Methods

EuroSCORE II data were prospectively calculated (online calculator (http://www.euroscore.org) [11], and stored in the institutional database for a series of 7,641 consecutive patients who underwent adult (≥ 18 years of age) cardiac surgery at „Dedinje" Cardiovascular Institute in Belgrade, Serbia, from 1st January 2012 to 31st December 2015. Due to a low number of patients with a postinfarction ventricular septal defect (VSD) included in the developmental database of EuroSCORE II, no risk coefficient was assigned to postinfarction VSD closure procedure any more [6]. Therefore, patients with postinfarction VSD were excluded from our study, as well as from several subsequent EuroSCORE II validation studies [12, 13]. Only the first procedure for each patient was entered into the registry, while reinterventions for any cause in the same admission as the primary operation were coded as a complication. The primary end point for the study was in-hospital mortality (any-cause postoperative death occuring during the index hospitalization, in the hospital in which operation took the place) accross the arbitrary determined risk group categories. Patients with EuroSCORE II values of ≤ 2.50, > 2.50–6.50%, and > 6.50% were defined to be at low, moderate, and high perioperative risk, respectively. High risk patients were further divided into three subcategories – higher, very high and extremely high perioperative risk, with EuroSCORE II values of > 6.50–13.50%, > 13.50–20.00%, and > 20.00%, respectively. The Institutional Ethics Committee approved the study and requirement for informed written consent was waived due to the fact that patients' identities were masked.

Statistical analyses were performed using the statistical package SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). Categorical variables were expressed as percentages, and continuous variables were expressed as mean ± standard deviation (SD). Statistical analyses were performed by the Fisher's exact test or χ^2 test for categorical variables and by *t*-test for continuous variables. A *p*-value of less than 0.05 was considered significant.

The performance of the EuroSCORE II was analyzed focusing on discrimination power and calibration. Discrimination measures the capacity of the model to recognize the individuals of a cohort who will suffer an event (in this case perioperative death) and those who will not, thus distinguishing low-risk from high-risk patients. Discrimination can be assessed by the area under the receiver operative characteristic (ROC) curve (AUC). The AUC is a percentage of randomly drawn pairs (meaning one death and one survivor patient-pairs) for which it is true that a patient who died had a higher risk score than a patient who survived. The discriminative power is thought to be excellent if the AUC is > 0.80, very good if it is > 0.75 and good (acceptable) if it is > 0.70 [14].

Calibration refers to the agreement between observed events and predicted probability of occurrence of these events. The Hosmer-Lemeshow (H-L) goodness-of-fit test has been the most popular test to validate calibration, measuring the differences between observed and expected out-

comes over deciles of risk. A well-calibrated model gives corresponding *p*-value > 0.05 [15]. We also evaluated EuroSCORE II calibration using the observed to expected (O/E) mortality ratio. Ideally, this ratio equals one (the observed mortality equals expected mortality, thus the predictive model is perfectly calibrated). A value above one means that model underestimates mortality, a value below one means that model overestimates mortality. If the 95% confidence interval (CI) of the O/E mortality ratio includes the value of 1.0, the model is well calibrated [15].

Results

A total of 7,641 patients fulfilled the study criteria (patients < 18 years of age and patients with postinfarction VSD were excluded). The baseline patients characteristics and operative details (EuroSCORE II risk factors) for our study population are presented in Table 1. There were no missing data reffering to variables necessary for EuroSCORE models risk calculation. Definitions of all EuroSCORE II variables are available on the web-site: http://www.euroscore.org [11]. The following subgroup procedures were performed: coronary artery bypass grafting (CABG) surgery [n = 4,044 (52.9%)]; valve(s) surgery (surgery of one or more valves) [n = 1732 (22.7%)]; combined cases (CABG and valve(s) surgery) (n = 1,182 (15.5%)]; aortic (thoracic aorta) surgery [n = 545 (7.1%)] and other major cardiac procedures [n = 138 (1.8%)].

Discriminatory and calibration abilities of EuroSCORE II for the whole sample and across basic (low, moderate, high) risk group categories are summarised in Table 2.

The in-hospital mortality observed in our sample was 3.86% (295 out of 7,641 patients), while EuroSCORE II predicted mortality was 3.62%. Discriminative power of the EuroSCORE II model was determined by calculation of the AUCs. Very good discrimination was confirmed (all AUCs > 0.75; for whole cohort and for all subgroups procedures which were performed – CABG, valve(s), combined, aortic, other). The EuroSCORE II discriminative power was acceptable (AUCs > 0.70) for the low and high risk groups, while it failed to confirm good discrimination in the moderate risk group. In the low risk group, only subgroup of valve(s) surgery showed good discrimination, as well as two subgroups (aortic, others) in the moderate risk group. Surprisingly, almost all results in the high risk category confirmed acceptable discrimination [mostly, AUCs > 0.70; close to borderline in the CABG subgroup (AUC = 0.69); failed in the subgroup – other]. Although H-L statistics confirmed overall good calibration in all risk group categories (overall and in all subgroups), it failed to confirm good calibration of EuroSCORE II model for the whole cohort and for subgroups of the CABG and aortic surgery (Table 2). However, the O/E mortality ratio confirmed good calibration for the whole sample, and for all subgroups of performed cardiac procedures, excluding aortic surgery (significant underestimation of mortality; O/E mortality ratio = 1.63; 95% CI 1.25–2.01). In risk group categories, the O/E mortality ratio confirmed good calibration in the moderate risk group (including all subgroups), but it failed to confirm good calibration in the low and high risk groups (whole risk group sample and aortic surgery), as well as for CABG patients in the low risk group (Table 2).

Table 1

Patients characteristics and operative details (European System for Cardiac Operative Risk II - EuroSCORE II risk factors)

Variable	Number (%) of patients	Variable	Number (%) of patients
Age (years), mean ± SD	63.0 ± 10.5	Left ventricle function	
Gender (female)	2,269 (29.7)	good	2,890 (37.8)
Renal impairment		moderate	3,585 (45.9)
normal	3,989 (52.2)	poor	745 (9.8)
moderate	2,905 (38.0)	very poor	421 (5.5)
severe	706 (9.2)	Recent myocardial infarction	1,003 (13.1)
dialysis	41 (0.5)	Pulmonary hypertension	
Extracardiac arteriopathy	1,382 (17.4)	moderate	2,198 (28.8)
Poor mobility	54 (0.7)	severe	643 (8.4)
Previous cardiac surgery	283 (3.7)	Urgency	
Chronic lung disease	387 (5.1)	Elective	5,712 (74.8)
Active endocarditis	94 (1.2)	Urgent	1,403 (18.4)
Critical preoperative care	73 (1.0)	Emergency	518 (6.8)
Diabetics on insulin	792 (10.4)	Salvage	8 (0.1)
NYHA class		Weight of the intervention	
I	1,085 (14.2)	isolated CABG	4,044 (52.9)
II	4,303 (56.3)	single non-CABG	1,485 (19.4)
III	2,151 (28.2)	two procedures	1,502 (19.7)
IV	102 (1.3)	three procedures	610 (8.0)
CCS Class IV	481 (6.3)	surgery on thoracic aorta	545 (7.1)

NYHA – New York Heart Association; CCS – Canadian Cardiovascular Society; CABG – coronary artery bypass grafting.

Table 2
Calibration and discrimination of European System for Cardiac Operative Risk II (EuroSCORE II) across risk group categories and for the whole sample

Risk categories – according to EuroSCORE II value	Observed mort., % (n)	Expected mort., % (n)	O / E ratio	(95% CI)	H-L *p*	AUC (95% CI)
Low (n = 4,573; 59.8%), (0. 50–2.50%)						
all patients (n = 4,573)	0.92 (42)	1.32 (60)	0.70	(0.49–0.91)	0.61	0.72 (0.64–0.80)
CABG (n = 2,937)	0.65 (19)	1.28 (38)	0.50	(0.28–0.72)	0.78	0.68 (0.55–0.82)
valve(s) (n = 1,176)	0.85 (10)	1.26 (15)	0.67	(0.26–1.08)	0.35	0.75 (0.58–0.92)
combined (n = 312)	2.24 (7)	1.76 (5)	1.27	(0.33–2.44)	0.90	0.59 (0.36–0.81)
aortic (n = 53)	9.40 (5)	2.10 (1)	5.00	(1.62–9.38)	0.24	0.47 (0.22–0.72)
others (n = 95)	1.05 (1)	1.20 (1)	1.00	(-0.78–2.78)	0.40	0.39 (0.29–0.49)
Moderate (n = 2,055; 26.9%), (> 2.50–6.50%)						
all patients (n = 2,055)	4.18 (86)	3.97 (82)	1.05	(0.83–1.27)	0.76	0.62 (0.56–0.68)
CABG (n = 879)	4.89 (43)	3.81 (33)	1.30	(0.91–1.69)	0.40	0.62 (0.54–0.70)
valve(s) (n = 370)	2.97 (11)	3.87 (14)	0.79	(0.33–1.25)	0.37	0.49 (0.34–0.64)
combined (n = 510)	3.92 (20)	4.14 (21)	0.95	(0.43–1.47)	0.72	0.65 (0.52–0.78)
aortic (n = 262)	4.20 (11)	4.28 (11)	1.00	(0.41–1.59)	0.50	0.72 (0.58–0.85)
others (n = 34)	2.94 (1)	3.94 (1)	1.00	(-0.96–2.96)	0.60	0.79 (0.65–0.93)
High (n = 1,013; 13.3%), (> 6. 50%)						
all patients (n = 1,013)	16.49 (167)	13.3 (135)	1.24	(1.05 – 1.43)	0.06	0.71 (0.67–0.75)
CABG (n = 228)	14.47 (33)	11.7 (27)	1.22	(0.80 – 1.64)	0.22	0.69 (0.59–0.79)
valve(s) (n = 186)	16.13 (30)	14.0 (26)	1.15	(0.74 – 1.56)	0.74	0.71 (0.61–0.81)
combined (n = 360)	13.30 (48)	14.1 (51)	0.94	(0.67 – 1.21)	0.40	0.75 (0.67–0.83)
aortic (n = 230)	23.48 (54)	13.2 (30)	1.80	(1.32 – 2.28)	0.11	0.72 (0.65–0.80)
others (n = 9)	22.20 (2)	13.4 (1)	2.00	(-0.77 – 4.77)	0.13	0.57 (0.00–1.00)
Whole sample						
all patients (n = 7,641)	3.86 (295)	3.62 (277)	1.06	(0.96 – 1.18)	0.001	0.84 (0.82–0.86)
CABG (n = 4,044)	2.35 (95)	2.42 (98)	0.97	(0.78 – 1.16)	0.001	0.84 (0.80–0.88)
valve(s) (n = 1,732)	2.94 (51)	3.19 (55)	0.93	(0.68 – 1.18)	0.06	0.85 (0.80–0.90)
combined (n = 1,182)	6.35 (75)	6.53 (77)	0.97	(0.86 – 1.08)	0.13	0.76 (0.70–0.82)
aortic (n = 545)	12.84 (70)	7.83 (43)	1.63	(1.25 – 2.01)	0.04	0.77 (0.71–0.84)
others (n = 138)	2.90 (4)	2.66 (4)	1.00	(0.02 – 1.98)	0.65	0.78 (0.00–1.00)

mort. – mortality; O – observed; E – expected; CI – confidence interval; AUC – area under curve; CABG – coronary artery bypass grafting; H-L – Hosmer-Lemeshow test.

Table 3
Calibration and discrimination of European System for Cardiac Operative Risk II (EuroSCORE II) across high risk group subcategories

High risk subcategories according to EuroSCORE II value (n =1013)	Observed mortality % (n)	Expected mortality % (n)	O / E ratio	(95% CI)	H-L *p*	AUC (95% CI)
Higher (n = 711; 70.2%), (>6.50–13.50%)						
all patients (n = 711)	11.25 (80)	9.11 (65)	1.23	(0.96–1.50)	0.67	0.66 (0.60–0.72)
CABG (n = 182)	11.0 (20)	9.0 (16)	1.25	(0.70–1.80)	0.14	0.65 (0.52–0.79)
valve(s) (n = 122)	10.7 (13)	8.95 (11)	1.18	(0.54–1.82)	0.97	0.63 (0.47–0.79)
combined (n = 242)	7.85 (19)	9.3 (23)	0.83	(0.46–1.20)	0.35	0.66 (0.53–0.79)
aortic (n = 160)	16.9 (27)	9.1 (15)	1.80	(1.12–2.48)	0.38	0.72 (0.62–0.82)
others (n = 5)	20.0 (1)	8.4 (0.4)	N/A	N/A	0.36	0.25 (0.00–1.00)
Very high (n = 167; 16.5%), (> 13. 50 – 20. 00 %)						
all patients (n = 167)	19.76 (33)	16.37 (27)	1.22	(0.80–1.64)	0.01	0.52 (0.41–0.63)
CABG (n = 26)	26.9 (7)	16.6 (4)	1.75	(0.45–3.05)	0.40	0.33 (0.04–0.62)
valve(s) (n = 35)	17.1 (6)	16 (6)	1.0	(0.20–1.80)	0.47	0.54 (0.29–0.79)
combined (n = 62)	9.7(6)	16.3 (10)	0.6	(0.12–1.08)	0.23	0.54 (0.30–0.78)
aortic (n = 41)	34.1 (14)	16.7 (7)	2.0	(0.95–3.05)	0.09	0.53 (0.35–0.72)
others (n = 3)	0.0 (0)	16 (0.48)	N/A	N/A	N/A	N/A
Extremely high (n = 135; 13.3%), (> 20. 00 %)						
all patients (n = 135)	40.0 (54)	31.7 (43)	1.24	(0.93–1.59)	0.18	0.69 (0.60–0.78)
CABG (n = 20)	30.0 (6)	29.9 (6)	1.0	(0.20–1.80)	0.22	0.48 (0.18–0.77)
valve(s) (n = 29)	37.9 (11)	32.8 (10)	1.10	(0.55–1.75)	0.18	0.76 (0.58–0.94)
combined (n = 56)	41.1 (23)	32.1 (18)	1.28	(0.76–1.80)	0.14	0.71 (0.57–0.85)
aortic (n = 29)	44.8 (13)	31.0 (9)	1.44	(1.13–1.75)	0.42	0.69 (0.49–0.88)
others (n = 1)	100.0 (1)	30.6(0.3)	N/A	N/A	N/A	N/A

N/A – not applicable; O – observed; E – expected; CI – confidence interval; H-L – Hosmer-Lemeshow; CABG – coronary artery bypass grafting.

Discriminatory and calibration abilities of EuroSCORE II across high risk group subcategories are summarised in Table 3.

In the high risk group subcategories, the best discrimination was confirmed in extremely high risk group [close to borderline for the whole group and for aortic surgery (AUCs = 0.69); acceptable (AUCs > 0.70) in valves(s) and combined surgery; while it failed in CABG surgery]. In other two subcategories (higher and very high operative risk), good discrimination was recorded only for aortic surgery in higher risk group (AUC = 0.72) – Table 3.

The H-L statistics confirmed good calibration in all high risk group subcategories (higher, very high and extremely high) for all tested procedures, except for category – all patients in subcategory of very high operative risk (H-L p = 0.01) (Table 3). In the high risk group subcategories, the O/E mortality ratio failed to confirm good calibration only for aortic surgery in the higher and extremely high risk groups [O/E ratio of 1.80 (95% CI 1.12–2.48) and O/E ratio of 1.44 (95% CI 1.13–1.75), respectively] (Table 3).

Discussion

Risk estimation is one of the most powerful tools for the improvement of the standard of care and correct allocation of clinical and economic resources [16]. Owing to perioperative risk stratification models, predicted probability of occurrence of perioperative death has enabled stratification of patients in different clinical risk groups (low, moderate, high), and, subsequently, made it possible to plan the optimal schedule for cardiac surgery, moderate the postoperative workload in ICU and rationally allocated hospital resources [1]. It has been confirmed that the additive EuroSCORE model significantly correlated with cost of cardiac surgery [17], and that ICU and postoperative stay were significantly prolonged across increasing EuroSCORE II risk group categories (subsequently enhancing the cost of open heart surgery) [18]. Therefore, it appears that stratification in clinical risk group categories should be an integral part of the cardiac surgical practice, belonging to risk assessment, decision-making, and informed consent.

Validation of risk stratification abilities of the old, additive EuroSCORE has been conducted and presented in the basic manuscript [5] by the EuroSCORE study group. Validation processing confirmed good calibration for the medium risk group (score 3–5; O/E mortality ratio of 1.04; 95% CI 0.89–1.19), as well as for the high risk group (score ≥ 6; O/E mortality ratio of 0.99; 95% CI 0.91–1.07). For the low risk group (score 0–2; O/E mortality ratio of 0.62; 95% CI 0.42–0.82) model significantly overestimated mortality (O/E mortality ratios and 95% CIs were calculated using the data from quoted manuscript). The AUCs and H-L test p-values were not presented for risk groups.

The treshold that defines a low, moderate, high/very high-risk patients is not uniformly determined for the EuroSCORE II by literature at present. Several groups atempted to present and clarify this topic. Paparella et al. [10] categorised almost 6,200 patients into five risk groups (low ≤ 1.5%, mild 1.6–5.0%, moderate 5.1–10.0%, high 10.1–20.0%, and very high > 20%). However, that categorisation (supported by formation of a hierarchical tree, and subsequent statistical analysis) has been conducted using observed mortality, rather than predicted mortality. In our opinion, categorisation of the risk groups should be performed according to EuroSCORE II predicted mortality, and than, O/E mortality ratio and statistical analysis should be performed. Therefore, that study is not valid for EuroSCORE II risk group categorization. Velicki et al. [8] divided cohort of 1,247 patient in quartiles, resulting in a fact that all patients with EuroSCORE II predicted risk of more than 2.35% (4th quartile), were categorised as high-risk patients. Bai et al. [9] have also devided their sample (4,507 patients) in quartiles, resulting in the high-risk group (4th quartile), with EuroSCORE II value of more than 1.64%. We do believe that it is unacceptable to categorize all patients with EuroSCORE II of more than 1.64%, or even of more than 2.35%, as high-risk patients. Even with such low risk group borderlines, EuroSCORE II underestimated mortality for "high-risk group" in both papers. Two other groups reported risk group stratification, presenting EuroSCORE II values, too, but categorisation was conducted using old EuroSCORE models. Di Dedda et al. [13] presented a cohort of 1,090 patients, divided in quintiles of distribution, but risk stratification was created according to the old logistic EuroSCORE values. In their patient population, for the very high risk patients (observed mortality 11%), EuroSCORE II predicted mortality was 6.5% (significant underestimation). Kalender et al. [19] reported octagenarians (105 patients) who underwent isolated coronary artery surgery, but the old additive EuroSCORE was used for risk group categorisation. The discriminative power of EuroSCORE II model was not shown for risk group categories in any of aforementioned papers. The perioperative mortality related to cardiac surgery has decreased due to improved surgical techniques and perioperative patients menagement, despite sicker and more complex (baseline patients' characteristics, case mix, etc.) patients who are undergoing surgery. Although the EuroSCORE II values are generally lower (compared with additive EuroSCORE values, except for the very high risk category) for the tested group of patients [18], we decided to determine borderlines for risk groups categorisation in such a way to stay close to the basic manuscript [5] by the EuroSCORE study group, as follows: low risk category ≤ 2.5% (basic manuscript 0–2%), moderate risk category > 2.5–6.5% (basic manuscript 3–5) and high risk category > 6.5% (basic manuscript ≥ 6). Arangalage et al. [20] were the only ones who searched for correspondence borderlines values for high risk patients between old logistic EuroSCORE and for EuroSCORE II, and they proposed a threshold of ≥ 7% of EuroSCORE II for high risk patients, which is very close to our suggested borderline value for EuroSCORE II high risk patients.

We confirmed acceptable discriminative power of EuroSCORE II in the low risk group (AUC – 0.72) and the high risk group (AUC – 0.71). In the high risk group subcategories, only for extremely high risk subcategory, discrimination was borderline acceptable (AUC – 0.69). Good discrimina-

tion was also confirmed for some subgroups of performed surgical procedures across risk group categories as well as across high risk group subcategories (Tables 2 and 3). The explanation for reduced discriminative power is statistically simple. When patients are stratified according to the risk score, and than only one strata is analyzed, the regressors and their coefficients within the stratum are different from those which allocated them to that risk group in the first place [21]. Therefore, we should not be surprised if discrimination drops to a lower level within the stratum [21]. Furthermore, a minimum of 100 (and preferably 200) events (perioperative deaths) should be included in the sample size so that model performance can be adequately assessed [22]. The Hosmer-Lemeshow statistics confirmed good calibration in all risk group categories and subcategories of the high risk category, and for all subgroups of performed cardiac procedures. It failed to confirm good calibration only for the whole sample (all patients) in the subcategory of very high risk patients (H-L $p = 0.01$). According to O/E mortality ratio, for the low risk group model significantly overestimated mortality for the whole sample and CABG surgery subgroup, while it significantly underestimated mortality for the aortic surgery subgroup. In the moderate risk group, prediction was good for the whole sample, as well as for all subgroups of performed cardiac surgery. In the high risk group model, mortality was slightly, but significantly underpredicted for the whole sample (O/E mortality ratio – 1.24; 95% CI 1.05–1.43). On the contrary, further analysis of high risk group subcategories confirmed good calibration for category – all patients, in all three subcategories. Therefore, our results are not in accordance with previous statements that EuroSCORE II significantly underestimates mortality in the high risk group category [2, 8, 9, 13]. In the high risk group category, our study is in keeping with results of Barili et al. [7], who showed an optimal EuroSCORE II calibration until 30%-predicted mortality.

Limitations of the study

The limitation of our study is its single-center design, and, therefore, results may not represent national and international practice and outcomes. Although our cohort recruited more than 7,600 patients, another limitation has been sample size, which generated relatively small specimens, including limited number of tested events (in this case perioperative deaths) for more precise subgroup analysis.

Conclusion

The results of this study show an acceptable overall performance of EuroSCORE II in terms of discrimination and accuracy of the model predictions for perioperative mortality across risk group categories (except overprediction of mortality in the low risk group, O/E mortality ratio). Validation of EuroSCORE II performances across risk group categories needs to be further studied for a continuous improvement of patients' risk stratification before planned cardiac surgery.

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Presence of *Tannerella forsythia* in patients with chronic periodontal disease and atherosclerosis

Prisustvo bakterije *Tannerella forsythia* kod bolesnika sa hroničnom periodontalnom bolešću i arterosklerozom

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Abstract

Background/Aim. Periodontal disease is an inflammatory disease that occur in the tissues surrounding the teeth in response to bacterial biofilm accumulation (dental plaque). Among others, *Tannerela forsythia* (*Tf*) was recognized as one of the most significant and specific bacterial species in periodontal pocket („red complex" bacteria). Atherosclerosis is a progressive narrowing of arteries that may lead to occlusion, as a consequence of lipid deposition. It underlies coronary heart disease (80%), as well as myocardial and cerebral infarctions. Increasing evidence over the past decade suggests a link between periodontal disease and atherosclerosis, where *Tf* can enter the systemic circulation directly or indirectly, and be present in atherosclerotic blood vessels. Therefore, the aim of this study was to detect the presence of *Tf* in atheromatous plaques obtained from different blood vessels in patients with chronic periodontitis. **Methods.** Ninety patients (male 61, female 29) with periodontal disease and atherosclerosis [recruited for either carotid artery stenosis requiring endartectomy or percutaneous transluminal coronary angioplasty (PTCA)] were included in this study. Clinical periodontal examinations consisted of plaque index (PI) (according to Silness Löu), gingival index (GI) (according to Löe Silnes), sulcus bleeding index (according to Mühleman-Son) and periodontal probing depth (PPD). Presence of *Tf* in periodontal pockets and atherosclerotic vessels was detected using polymerase chain reaction (PCR) method with positive control *Tf* ATCC 43037. **Results.** *Tf* was present in subgingival plaques of 68 (75.6%) of the patients, while its presence in atheromatous plaques were registered in 42 (53.3%) of the patients. It was significantly present in coronary blood vessels (41.7%), followed by carotid arteries (35.4%) and *a. abdominalis* aneurism (12.5%), *a. mamaria* (8.5%) and *a. femoralis* (2.1%) while in *a. iliaca Tf* was not detected at all. **Conclusion.** The present study suggests strong relationship between periodontal inflammation and atherogenesis; therefore, it should be considered as potential risk factor for atherosclerosis. Accordingly, it would be necessary to control periodontal disease in order to reduce mortality and morbidity associated with atherosclerosis.

Key words:
periapical periodontitits; tannerella forsythia; atherosclerosis; risk factors; comorbidity.

Apstrakt

Uvod/Cilj. Parodontopatija je zapaljensko oboljenje potpornog aparata zuba, koje se javlja kao odgovor na prisustvo dentalnog plaka. Između ostalih, *Tannerella forsythia* (*Tf*) je jedna od značajnih i veoma specifičnih bakterija koja se nalazi u periodontalnom džepu i pripada bakterijama „crvenog kompleksa". Ateroskleroza je progresivno sužavanje arterija koje može, konačno, dovesti do njihovog potpunog začepljenja, kao rezultat nakupljanja masti. Smatra se vodećim uzrokom koronarne srčane bolesti (80%), kao i infarkta miokarda i moždanog udara. Sve više je objavljenih

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radova na temu potencijalne povezanosti parodontopatija i kardiovaskulranih oboljenja, gde se smatra da bakterije prisutne u periodontalnom džepu, između ostalih *Tf*, mogu prodreti u sistemsku cirkulaciju direktnim ili indirektnim putem. Cilj ove studije bio je utvrđivanje prisutnosti *Tf* u aterosklerotskim plakovima različitih krvnih sudova osoba obolelih od hronične parodontopatije. **Metode.** U ovu studiju je bilo uključeno 90 pacijenata (61 muškarac i 29 žena) sa dijagnozom parodontopatije i ateroskleroze koji su bili podvrgnuti endarterektomiji ili perkutanoj transluminalnoj koronarnoj angioplastici. Klinički pregled za parodontopatiju sastojao se od utvrđivanja plak indeksa (po Silness Löu), gingivalnog indeksa (po Löe Silnes), indeksa krvarenja iz sulkusa (po Mühleman-Son) i dubine periodontalnog džepa. Prisustvo *Tf* u periodontalnom džepu i aterosklerozom izmenjenim krvnim sudovima detektovano je metodom lančane reakcije polimeraze [*polymerase chain reaction* (PCR)] uz *Tf* ATCC 43037 kao pozitivne kontrole. **Rezultati.** *Tf* je bila prisutna u subgingivalnom plaku kod 68 (76%) pacijenata, dok je njeno prisustvo u aterosklerotskim plakovima zabeleženo kod njih 42 (53,3%). Značajno prisustvo *Tf* je zabeleženo u koronarnim arterijama (41,7%), karotidnim arterijama (35,4%), zatim u aneurizmama *a. abdominalis* (12,5%), *a. mamaria* (8,5%) i *a. femoralis* (2,1%), dok u *a. iliaca* *Tf* nije bila prisutna. **Zaključak.** Ovom studijom utvrđena je jaka povezanost parodontopatije i aterogeneze, te se smatra da bi se parodontopatija trebala uvrstiti u potencijalne faktore rizika od nastanka ateroskleroze. Takođe, bilo bi neophodno da se kontroliše parodontopatija kako bi se smanjio rizik od oboljevanja i smrtnosti povezanih sa aterosklerozom.

Ključne reči:
periodontitis, periapikalni; tannerella forsythia; ateroskleroza; faktori rizika; komorbiditet.

Introduction

Periodontal disease is affecting up to 90% of the worldwide population [1]. It is an inflammatory disease that occurs in the tissues surrounding the teeth in response to bacterial biofilm accumulation (dental plaque). It is well known that more than 500 virulent microbial species from dental biofilm are mainly responsible for periodontal disease. Although several bacterial species are currently recognized as causally associated with periodontal disease, subgingival colonization is not sufficient for the disease to occur [2]. It is well known that abnormal host response to periodontal disease with specific genetic predisposition and detrimental environment exposures are likely important determinants of suspectibility, e.g. age, poor oral hygiene, cigarette smoking, pregnancy, obesity, stress and systemic conditions such as diabetes mellitus, osteoporosis and rheumatoid arthritis [3].

Atherosclerosis is a progressive narrowing of arteries that may lead to occlusion as a consequence of lipid deposition [2]. It underlies coronary heart disease (80%), as well as myocardial and cerebral infarctions, therefore having a big socio-economic importance [4]. Onset of atherosclerosis is thought to be due to endothelium function disturbances, platelet activation, and oxidative changes in plasma lipoproteins. Several possible biological mechanisms, including common genetic variants may explain the link between cardiovascular diseases and periodontitis [5]. It is believed that there are multiple mechanisms underlying these links, with inflammatory, infectious, immune, and genetic components [4]. The link between periodontal disease and atherosclerosis was first established around 30 years ago when De Stefano et al. [6] reported an increased risk of atherosclerotic plaque formation in a group of patients with periodontitis (25 % higher) based on 14 years of research including 9,760 individuals aged between 25 to 74 years. Recent attention has been directed towards the potential contribution of chronic inflammatory processes that may amplify vascular inflammation in atherosclerosis, and periodontal disease is recognized as a chronic inflammatory disease [4, 7, 8]. Today, the American Hearth Association defines this contribution with level A evidence.

Tannerela forsythia (*Tf*) is one of the most significant and specific bacterial species in periodontal pocket („red complex“ bacteria). Increasing evidence, over the past decade, suggests a link between periodontal disease and atherosclerosis, where *Tf*, mainly present in diseased periodontal pockets, can enter the systemic circulation directly, and may be present in distant organs, such as aterosclerotic blood vessels. Lypopolysaccharides and other products from *Tf* cell breakdown may stimulate inflammatory cytokines, upregulate endothelial adhesion molecules and induce a prothrombotic enviroment, that can enhanced risk of an atherosclerossis [9]. The causal relationship between periodontal disease and atherosclerosis can be detected through bacterial presence at the diseased sites detected by real-time polymerase chain reaction (PCR) [10].

Therefore, the aim of the present study was to detect the presence of *Tf* in subgingival plaques and atheromatous plaques obtained from different blood vessels.

Methods

Patients

Ninety patients, 61 males and 20 females, aged 28-94 years (mean age: 59.2 ± 12.7 years), with periodontal disease and atherosclerosis were recruited in this study. Patients were treated in the Clinic for Vascular and Endovascular Surgery, Clinical Center of Serbia in Belgrade, the Institute for Cardiovascular Diseases „Dedinje“ in Belgrade, the Clinic of Dental Medicine, Military Medical Academy in Belgrade and Clinic of Dental Medicine, Faculty of Medicine in Kosovska Mitrovica for either carotid artery stenosis requiring endarterectomy or percutaneous transluminal coronary angioplasty (PTCA), or *a. abdominalis* aneurisms, thrombosis and occlusions of *a. femoralis* and *a. iliaca.* Patients were divided according to the localization into six groups: group 1: carotid arteries (n = 29); group 2: *a. abdominalis* aneuris-

mas (n = 10); group 3: *a. femoralis* (n = 10); group 4: *a. iliaca* (n = 4); group 5: *a. coronaria* (n = 29); group 6: *a. mammaria* (n = 8).

Patients were recruited only if they were with at least four periodontal pockets. Periodontitis was diagnosed if a patient exhibited clinical attachment level (CAL) > 1 mm and periodontal pocket depth (PPD) > 3 mm, at least at three sites in two different quadrants. According to CAL, patients with diagnosed periodontitis were classified into three subgroups: patients with moderate chronic periodontitis (CP) (CAL = 3–4 mm) and severe CP (CAL ≥ 5 mm). Periodontitis was defined as localized or generalized depending on the number of affected sites [11].

Exclusion criteria were: systemic diseases, antibiotic intake and periodontal treatment in the previous three months. The medical and dental history of each subject was obtained by interview. Smoking and alcohol use were detected by self-report. Patients fulfilling the inclusion criteria were informed of the study and signed informed consent form that was approved by the Ethics Committee of the Faculty of Medicine in Kosovska Mitrovica, Serbia.

Subgingival and atheromatous plaque sample

On the same day of the surgical intervention, a complete periodontal examination was performed by a single periodontist. Clinical examinations included plaque index (PI) (according to Silness Löu), gingival index (GI) (according to Löe Silnes), sulcus bleeding index (according to Mühleman-Son) and periodontal probing depth (PPD). Subgingival plaque samples were collected using the paper point technique (Periopaper, Amityville, Pro Flow, NY, USA) from the bottom of two out of four present periodontal pockets. Each sample site was isolated with coton rolls, scalled carefully supragingivaly, and air dried. A sterile paper point was inserted into the apical extent of each selected pocket, kept for 60 seconds, and transfered immediatelly to a sterile Eppendorf tube and kept in the refrigerator on -70°C until the analysis. Samples from the arteries were taken during the surgery (endarterectomy, PTCA) and transfered immediatelly to a sterile Eppendorf tube with Tris-EDTA, as transport medium, and kept in the refrigerator on -20° C until the analysis by PCR method.

PCR analysis

Presence of *Tf* in periodontal pockets and atherosclerotic vessels was detected using PCR with positive control – *Tf* American Type Culture Collection (ATCC) 43037. The negative control was sterile distilled water instead of template DNA. The positive control consisted of DNA from pure cultures of *Tf* ATCC 43037. Colonies obtained from cultures were suspended in sterile water, centrifuged and subjected to DNA extraction.

Statistical analysis

Continuous variables were presented as means ± standard deviations (SD) or median and range and categorical variables were expressed as absolute and relative frequencies. The normality of the data distribution was confirmed by the Shapiro-Wilk tests. The Mann-Whitney *U*-test and Kruskal-Wallis test were used to compare differences between two groups and for three or more groups, respectively. Categorical data between study groups were analyzed using the chi-squared (χ^2) test. Results were considered statistically significant when *p*-values were less than 0.05. Statistical analysis was performed with the Statistical Package for the Social Science Program (version 22, SPSS Inc., Chicago, IL, USA).

Results

In subgingival plaque samples, *Tf* was detected in 68 (75.3%) of the patients, while in atherosclerotic plaque samples, *Tf* was present in 48 (53.3%) of the patients (Table 1).

Table 1
Presence of *Tannerella forsythia* in subgingival and atherosclerotic plaque samples of patients with periodontal disease and atherosclerosis

Presence of *Tannerella forsythia*	Patients, n (%)
Subgingival plaque samples	
yes	68 (75.6)
no	22 (24.4)
Atherosclerotic plaque samples	
yes	48 (53.3)
no	42 (46.7)

In subgingival and atheromatous plaque samples of patients with atherosclerotic carotid arteries, *Tf* was detected in 79.3% and 58.6% of the patients, respectively. In case of patients with *a. abdominalis* aneurisms, *Tf* was present in sublingval and aterosclerotic plaque samples of 80% and 60% of the patients, respectively. In patients with atherosclerotic *a. femoralis*, *Tf* was present in subgingival plaques of 70% of the patients, while in atheromatous plaques of this blood vessel only in 10% of the patients. At the same time, *Tf* was present in subgingival plaques in 75% of patients with atherosclerosis of *a. iliaca*, while no *Tf* was present in the *a. iliaca* plaque samples. On the contrary, *Tf* was isolated in subgingival plaques of 79.3% of patients with atherosclerotic coronary arteries, and also in atherosclerotic plaque samples of 69% of these patients. In atheroslerotic and subgingival plaque samples of patients with atherosclerotic mammary arteries, *Tf* was found in both cases in 50% of the patients (Table 2).

Among patients with atheromatous plaque positive on *Tf*, 8.3% were with moderate and 91.7% with severe gingival inflammation, which was statistically significant difference in relation to patients with no presence of the bacterium in atheromatous plaques. Further, among patients with positive atheromatous plaque on *Tf*, 22.9% and 77.1% had moderate and severe form of periodontal disease, respectively, which was also statistically significant difference in relation to patients with no presence of *Tf* in atheromatous plaques (Table 3).

Table 2

Presence of *Tannerella forsythia* in subgingival and atherosclerotic plaque samples of patients with periodontal disease and atherosclerosis in relation to different blood vessels

Blood vessel	Patients, n (%)		Total number of patients
	subgingival plaque	atherosclerotic plaque	
A. carotis	23 (79.3)	17 (58.6)	29
A. abdominalis aneurism	8 (80.0)	6 (60.0)	10
A. femoralis	7 (70.0)	1 (10.0)	10
A. illiaca	3 (75.0)	0 (0)	4
A. coronaria	23 (79.3)	20 (69.0)	29
A. mammaria	4 (50.0)	4 (50.0)	8

Table 3

Clinical degree of periodontal disease, gingival inflammation and bleeding on probing (BOP) according to *Tannerella forsythia* presence in atherosclerotic plaques of blood vessels

Parameters	Presence of *Tannerella forsythia* in atherosclerotic plaques		*p**
	yes	no	
Total number of patients, n (%)	48 (53.3)	42 (46.7)	
Degree of periodontal disease, n (%)			
moderate	11 (22.9)	22 (52.4)	0.004
severe	37 (77.1)	20 (47.6)	
Degree of gingival inflammation, n (%)			
mild	0 (0)	3 (7.1)	
moderate	4 (8.3)	12 (28.6)	0.005
severe	44 (91.7)	27 (64.3)	
Bleeding on probing, median (range)	4.2 (2.0-5.0)	3.3 (1.0-4.9)	< 0.001

***χ^2-test or Mann-Whitney *U* test.**

Presence of *Tf* was also analyzed in relation to sociodemographic characteristics of the patients. According to our results, age, gender, level of education and presence of bad habits (smoking and alcohol use) were not statistically significant regarding presence of *Tf* (Table 4).

Table 4

Sociodemographic characteristics of patients with periodontal disease and atherosclerosis in relation to *Tannerella forsythia (Tf)* presence in subgingival or atherosclerotic plaques

Sociodemographic characteristics	Presence of *Tf*		*p*
	yes	no	
Gender, n (%)			
male	49 (72.1)	12 (54.5)	0.127
female	19 (27.9)	10 (45.5)	
Age (years), mean ± SD	59.2 ± 11.8	59.4 ± 15.7	0.937
Education, n (%)			
primary school	10 (14.7)	1 (4.5)	
high school	41 (60.3)	16 (72.7)	0.398
university	17 (25.0)	5 (22.7)	
Smoking, n (%)			
yes	38 (55.9)	14 (63.6)	0.522
no	30 (44.1)	8 (36.4)	
Alcohol, n (%)			
yes	21 (30.9)	4 (18.2)	0.248
no	47 (69.1)	18 (81.8)	
Oral higyene, n (%)			
good	28 (41.2)	14 (63.6)	0.066
poor	40 (58.8)	8 (36.4)	

SD – standard deviation.

Tf demonstrated the following distribution in blood vessels of the patients with atherosclerosis: the highest prevalence was in coronary arteries (41.7%), followed by carotid arteries (35.4%) and aneurisms of *a. abdominalis* (12.5%), *a. mammaria* (8.3%) and *a. femoralis* (2.1%) while in *a. iliaca* *Tf* was not detected at all (Table 5).

Table 5

Distribution of *Tannerella forsythia* in atherosclerotic plaques of different blood vessels

Blood vessel	Patients, n (%)
A. coronaria	20 (41.7)
A. carotis	17 (35.4)
A. abdominalis aneurism	6 (12.5)
A. mammaria	4 (8.3)
A. femoralis	1 (2.1)
A. illiaca	0 (0)
Total	48 (100)

There were no statistically significant differences in values of PI, GI, sulcus bleeding index and PPD among patients with or with no presence of *Tf* in atheromatous plaques of different blood vessels (Table 6).

Table 6
Results of periodonatal examinations in relation to *Tannerella forsythia* presence in atherosclerotic plaques of different blood vessels

Presence of *Tannerella forsythia* in atherosclerotic plaques	Blood vessel	n	Periodontal examination, median (range)			
			PI	GI	SBI	PPD (mm)
Yes	*A. carotis*	17	2.9 (1.9–3.0)	2.7 (2.1–3.0)	4.5 (2.9–4.9)	5.0 (3.0–6.0)
	A. abdominalis aneurism	6	2.8 (1.9–3.0)	2.85 (1.8–3.0)	4.65 (2.8–5.0)	6.0 (3.0–7.0)
	A. femoralis	1	3.0 (3.0–3.0)	2.6 (2.6–2.6)	3.9 (3.9–3.9)	6.0 (6.0–6.0)
	A. coronaria	20	2.85 (1.5–3.0)	2.85 (1.2–3.0)	4.0 (2.0–5.0)	6.0 (3.0–8.0)
	A. mammaria	4	2.2 (1.9–3.0)	2.45 (1.7–2.9)	3.95 (2.8–4.8)	5.0 (4.0–8.0)
	Total	48	$p = 0.587$	$p = 0.336$	$p = 0.214$	$p = 0.655$
No	*A. carotis*	12	1.8 (0.9–2.9)	1.8 (0.9–3.0)	2.9 (1.1–4.6)	3.5 (3.0–6.0)
	A.abdominalis aneurism	4	2.85 (1.9–2.9)	2.3 (1.9–2.8)	4.1 (2.0–4.8)	4. 5 (3.0–6.0)
	A. femoralis	9	2.0 (1.0–2.8)	2.1 (1.5–3.0)	3.1 (2.5–4.9)	5.0 (3.0–5.0)
	A.illiaca	4	2.4 (1.9–2.7)	2.5 (2.2–3.0)	3.95 (3.0–4.9)	4.5 (3.0–6.0)
	A. coronaria	9	2.6 (1.5–3.0)	2.8 (1.7–3.8)	4.0 (1.0–4.3)	5.0 (3.0–6.0)
	A. mammaria	4	2.3 (1.9–2.9)	2.2 (1.5–3.0)	2.95 (1.8–4.1)	4.0 (3.0–6.0)
	Total	42	$p = 0.110$	$p = 0.469$	$p = 0.571$	$p = 0.935$

PI – plaque index; GI – gingival index; SBI – sulcus bleeding index; PPD – periodontal pocket depth.

Discussion

Periodontal disease represents chronic inflammation in tooth supportive tissues (periodontal ligament, connective tissue and alveolar bone), that, if left untreated, leads to periodontal pocket formation and consequent bone loss. It is unclear which pathogens initiate the disease, but several species including anaerobic Gram negative bacteria *Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia* are most strongly associated with the destruction of periodontium, and are routinely found in subgingival plaques in patients with chronic periodontitis [12]. To date, many researches are pointing on correlation between periodontal disease and systemic health. It has been suggested that periodontitis-associated bacteraemias and systemic dissemination of inflammatory mediators produced in the periodontal tissues may cause a systemic inflammation. Therefore, it is consider that periodontal disease can contribute as a risk factor for cardiovascular diseases, endocrine disturbances (e.g. diabetes mellitus), premature birth and low weight on birth, etc. [13, 14]. Atherosclerosis, a progressive disease of medium and large elastic and muscular arteries can lead to ischemic lesions of brain, heart or extremities and can result in thrombosis and infarction of affected vessels [4]. It is considered the primary cause of heart disease and stroke and is the underlying cause of around 50% of all deaths in western societies [15].

Among 90 patients included in this study, 61 of them (67.8%) were males, which make a prevalence of periodontal disease and atherosclerosis higher in men. Patients' mean age was 59.2 years. That is in correlation with common understanding of periodontal disease progress. Even thought, the age itself is not predetermining risk factor for periodontal disease [16] due to lower number of elastic and collagen fibers, and mitotic activity of fibroblasts is usually seen in adults over 40 years. Maybe the reason for the presence of periodontal disease in older persons can be partially explain by association between poor oral hygiene habits and systemic diseases [17–19].

Presence of periopathogen *Tf* in atherosclerotic blood vessels showed a significant correlation in regards to degree of periodontal inflammation. Consequently, prevalence of *Tf* was statistically significantly higher in patients with moderate and severe periodontal disease when compared to patients with average PPD. Presence of *Tf* strongly correlated with level of periodontal inflammation and BOP.

Results from this study are pointing on strong correlation between periopathogens and their presence in atherosclerotic plaques, which is in accordance with many published studies on this topic [20–24]. So far, studies confirmed the presence of *Tf* in 30-61.9% patients with atherosclerotic *a. carotis* [24, 25], and in 86% of patients with *a. abdominalis* aneurism [26]. In our study, among 90 patients *Tf* was detected with highest prevalence in coronary arteries (41.7%) and carotid arteries (35.4%), and *a. abdominalis* aneurisms (12.5%), followed by *a. mammaris* (8.3%) and *a. femoralis* (2.1%), while in *a. iliaca Tf* was not detected at all. Therefore, our results are pointing that most probably the periopathogens after entering the systemic circulation are located in the circulation nearby heart blood vessels. *Tf* presence in far away blood vessels are less, but not unimportant.

Conclusion

The present study suggests strong relationship between periodontal inflammation and atherogenesis, therefore it should be considered as potential risk factor for arherosclerosis. It is, however necessary to control periodontal disease in order to reduce mortality and morbidity associated with atherosclerosis and myocardial infarction.

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Irony, deception and theory of mind in people with intellectual disabilities and dual diagnoses

Ironija, prevara i teorija uma kod osoba sa intelektualnom ometenošću i dualnim dijagnozama

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Abstract

Background/Aim. The ability to comprehend and produce irony and deception is rarely explored in people with intellectual disability (ID) or dual diagnoses (DD). The ability to understand irony and deception appears to be related to many cognitive skills, but some authors point out that the theory of mind is one of the most important factors for this ability. This research was conducted to determine the linguistic aspects of production and comprehension of irony and deception in adults with ID and DD, as well as the relationship of these abilities with theory of mind. **Methods.** The sample consisted of 120 people with ID aged between 20 and 56. Half of the sample comprised people with DD. Four subscales from the Assessment Battery for Communication were used to assess the participants' abilities to produce and comprehend irony and deception. False-belief tasks from "appearance-reality" category were used in theory of mind assessment. The level of intellectual functioning was measured by the Raven's progressive matrices, while the Peabody Picture Vocabulary Test was used to assess speech comprehension ability. **Results.** The results show that participants with DD and ID comprehend and produce false statements better than ironic ones. Participants with ID were more successful in production than in comprehension tasks of both false and ironic statements, while the same was true for participants with DD only for ironic statements. Participants with ID were significantly more successful than participants with DD in irony comprehension tasks. In participants with ID, first-order theory of mind significantly correlated only with the ability to produce irony, and second-order theory of mind significantly correlated with producing irony and deception. There were no significant correlations between theory of mind and producing and comprehending irony and deception in participants with DD. **Conclusion.** Although differences in some aspects of assessed abilities were found between the two groups of participants, the similarities in the profile of these abilities were dominant. Results of variability can be explained by differences in speech comprehension ability more than by differences in nonverbal intellectual functioning or theory of mind acquisition. Future studies should assess the influence of other cognitive factors.

Key words:
intellectual disability; diagnosis, dual (psychiatry); mental processes; deception; theory of mind; comorbidity.

Apstrakt

Uvod/Cilj. Sposobnost razumevanja i produkcije ironije i prevare je retko izučavana u populaciji osoba sa intelektualnom ometenošću (IO) ili dualnim dijagnozama (DD). Sposobnost razumevanja ironije i prevare se dovodi u vezu sa mnogim kognitivnim veštinama, ali neki autori ističu da je upravo teorija uma jedan od najznačajnijih faktora za ovu sposobnost. Ovo istraživanje je sprovedeno radi utvrđivanja lingvističke sposobnosti razumevanja i produkcije ironije i prevare odraslih osoba sa IO i DD, kao i utvrđivanja odnosa između ovih sposobnosti i teorije uma. **Metode.** Uzorak je činilo 120 osoba sa IO starosne dobi između 20 i 56 godina. Polovinu uzorka činile su osobe sa DD. Za procenu ispitanikovih sposobnosti produkcije i razumevanja ironije i prevare korišćene su četiri supskale iz Baterije za procenu komunikacije. Za procenu teorije uma korišćeni su zadaci lažnog verovanja iz kategorije „izgled-realnost". Nivo intelektualnog funkcionisanja proveravan je Ravenovim progresivnim matricama, dok je sposobnost razumevanja govora procenjivana *Peabody Picture Vocabulary* testom. **Rezultati.** Rezultati pokazuju da ispitanici sa IO i DD bolje razumeju i produkuju lažne nego ironične iskaze. Ispitanici sa IO su uspešniji u zadacima produkovanja, nego u zadacima razumevanja, kako lažnih, tako i ironičnih iskaza, dok za ispitanike sa DD ovo važi samo za ironične tvrdnje. U zadacima

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razumevanja ironije, ispitanici sa IO su bili značajno uspešniji od ispitanika sa DD. Kod ispitanika sa IO teorija uma prvog reda ostvarila je značajne korelacije samo sa sposobnošću produkcije ironije, a teorija uma drugog reda sa produkcijom ironije i produkcijom prevare. U grupi ispitanika sa DD nisu ustanovljene značajne korelacije između teorije uma i produkcije i razumevanja ironije i prevare. **Zaključak.** Između dve grupe ispitanika pronađene su razlike u nekim aspektima ispitivanih sposobnosti, ali ipak dominiraju sličnosti u profilu ovih sposobosti. Varijabilnost rezultata više objašnjava razlike u sposobnosti razumevanja govora, nego razlike u neverbalnom intelektualnom funkcionisanju ili usvojenosti teorije uma. Narednim istraživanjima trebalo bi proveriti uticaj drugih kognitivnih faktora.

Ključne reči:
intelektualna ometenost; dijagnoze, dualne (psihijatrija); mentalni procesi; prevara; teorija uma; komorbiditet.

Introduction

The ability to comprehend irony should be considered in terms of the knowledge and context shared by two interlocutors (i.e., one interlocutor understands that the meaning of the other interlocutor's spoken message is in contrast to the background and context of the message) [1]. By uttering ironic contents, the speaker produces a message that is not true and is contrary to the truth, but has no desire to deceive or trick the interlocutor [2].

When the speaker intends to influence the interlocutor's mental state, i.e., to manipulate his/her mental state, we refer to deception. In situations involving deception, the speaker knows that what he/she is saying is a lie but tries to convince the interlocutor that it is true. By uttering a lie, the speaker conveys a message he/she knows is a lie [3–4].

Detecting the literal meaning of a statement represents only the first step in discovering and comprehending the interlocutor's communicative intentions. Bara [5] points out the importance of understanding the interlocutor's mental states in realizing and comprehending a communication act. For communication to be successful, the interlocutor should reconstruct the speaker's mental state, search for the speaker's communicative intention, attribute certain mental states to the speaker with the possibility to change them, form his own communicative intentions and reply to the speaker [5–6].

That is the reason the theory of mind (TOM) ability is singled out as significant cognitive factor for this ability [4]. However, the exact role of the theory of mind in pragmatic aspects of communication has not yet been fully explored, both in typical participants [7], and those with psychiatric disorders [8–9]. Theory of mind (mentalization) represents the ability to understand and attribute different mental states to oneself and others, and as such is associated with communication in which the interlocutors convey messages with an intention, with the possibility to persuade the interlocutor, or deliberately deceive him/her, by sharing mutual attention, mutual plans, and goals of behaviour [10].

According to some authors, first-order theory of mind (the ability to understand personal mental states) correlates with the comprehension of metaphor [11], and second-order theory of mind (a phenomenon that a person has a belief about the belief of another person) correlates with the comprehension of irony [11–13]. On the other hand, some authors have not found significant correlation between theory of mind and irony comprehension [14].

In previous years, the ability to comprehend and produce irony and deception has been studied in children and adults with average intellectual abilities [4, 15–22], but also in persons with traumatic brain injuries [23], autism [24], schizophrenia [14], Parkinson's disease [13], cerebral palsy [25], attention deficit hyperactivity disorder (ADHD) [26].

By analysing the literature, we determined that the abilities to comprehend and produce irony and deception have almost never been studied in people with intellectual disability (ID) or those with dual diagnoses (DD) i.e., people who have ID and a comorbid psychiatric disorder). ID represents a condition which occurs before the age of 18, and which is characterized by significant limitations in intellectual and adaptive functioning. Limitations in adaptive functioning include the deficits in conceptual, social, and practical skills [27]. Insufficiently mastered conceptual and social skills in persons with ID hinder, among other things, the identification and comprehension of relevant social signals, their integration and processing, and thus planning and realization of behaviour in accordance with the existing situation [28]. With regard to that, persons with ID can, to a greater or lesser extent, express difficulties in different aspects of functional communication and pragmatic abilities [29].

ID is often accompanied by comorbid psychiatric disorders [30–32]. The prevalence of these disorders ranges from 14% to 70% [33–36], and such a wide range of the obtained results is attributed to methodological characteristics of different studies [37–38]. It is believed that the presence of psychiatric symptoms has a negative effect on everyday functioning of persons with ID more than that is the case in persons with average intellectual abilities [31].

Studies that aimed to assess general linguistic aspects of production and comprehension in participants with DD indicate that these people express disorganized linguistic production, which is characterized by unclear and poor speech expression, confusion, discomfort and frustration caused by the interlocutor's poor comprehension, and noticeably reduced or absent initiative in conversation [39–41]. The conversation of persons with DD can be described as aimless, disorganized, incoherent, and poor [42–43].

In addition, previous studies have shown theory of mind to be substantially delayed in people with ID. Several authors indicate that people with ID do not exhibit a theory of mind deficit relative to typically developing people of the same mental age [44–45], while the others reveal significant impairments, especially in people with specific aetiologies [46–47].

There are also many studies examining alterations of theory of mind in patients with schizophrenia and average intellectual abilities [48–51], but no one investigated the ability of theory of mind in people with DD.

The aim of this research is to investigate the linguistic abilities to produce and comprehend irony and deception in adults with ID with regard to the level of ID and the presence or absence of DD. Additionally, the relationship between theory of mind ability and the ability to produce and comprehend irony and deception was tested.

Methods

Participants

The sample consisted of 120 participants of both genders. The complete sample was divided into ID and DD subsamples, with 60 participants in each subsample. The exclusion criteria for both groups were severe visual and hearing impairment, bilingualism, autism spectrum disorder and brain injury.

The groups did not differ significantly in terms of age ($t[118] = 1.42$, $p = 0.158$).

Data on gender, age, speech comprehension ability, and intellectual functioning are presented in Table 1.

The participants with ID were those with below average intellectual and adaptive functioning of unknown aetiology. No comorbid psychiatric disorders were detected in their clinical presentations, and thus these participants did not use medications.

In participants with DD, comorbid psychiatric disorders were diagnosed along with below average intellectual and adaptive functioning, and they were all classified as schizophrenia spectrum disorders according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) classification [52]. Because of the existing psychiatric disorder, the participants with DD used antipsychotics, and their medical charts included information on occasional hospitalization in psychiatric institutions, while their below average intellectual functioning had no known cause.

All of the participants were diagnosed in childhood, and repeated diagnosis and obligatory psychiatric assessments were conducted upon the participants' admission to a social care institution. Data on the dual diagnoses were taken from the participants' personal records. Raven's progressive matrices were used to determine the level of intellectual functioning. Independent samples *t*-test was used to compare scores on Raven's progressive matrices in participants with ID and DD, and the obtained results indicated that there were no statistically significant differences ($t[118] = 1.20$, $p = 0.232$).

Speech comprehension ability was assessed using the Peabody Picture Vocabulary Test. Both groups of participants were compared using independent samples *t*-tests, and the results showed that speech comprehension ability was more developed in the participants with ID than in the participants with DD ($t[118] = 2.13$, $p = 0.035$).

Bearing in mind the importance of language abilities for understanding irony [53] and deception [54], as well as for theory of mind [55], speech comprehension ability was used as the covariate in this research. Apart from speech comprehension ability, the score on Raven's progressive matrices was also used as the covariate.

Measures

The abilities to comprehend and produce irony and deception were assessed using four subscales from The Assessment Battery for Communication (ABaCo) [6], a clinical instrument for evaluating pragmatic abilities. The ABaCo was translated in full from Italian into Serbian using the "double-blind translation" method. The video tasks were synchronized by male and female synchronizers. The instrument has five scales: Linguistic scale (e.g. the examiner asks the subject "Tell me that you are cold"), Extralinguistic scale (e.g. the examiner asks the subject "Order me to be quite", the subject has to produce gestural acts), Paralinguistic scale (e.g., saying "I like it very much" while one's voice and attitude reveal that one doesn't like it at all), Context scale (the actor asks "Where are you going precisely?" and the partner replies "I'm going out"; the subject has to detect and explain the adequacy/inadequacy of the partner's reply), and Conversational scale (which assesses participants' ability to get involved in conversation, answer questions, respect the given topic, introduce new topics and speak when it is their turn). Within each scale, except the Conversational scale, the tasks were grouped into two subcategories – for the assessment of comprehension abilities and for the assessment of production abilities. There were 172 items in total, where 100 items were presented as video clips and 72 items were direct items in which the examiner asked questions and the participant was his interlocutor. In video tasks, the examiner showed a video clip and then asked questions related to communicative interaction presented in it. Video clips were 20 to 25 seconds long, and the number of words uttered in them ranged between five and nine. Each correct answer was marked with 1, and incorrect with 0. Maximum number of points in one task differed with regard to the scale it belonged to and the type of the task itself.

Table 1

The participants' characteristics

Subsamples	n	Gender		Age (years)				Peabody Picture Vocabulary Test				Raven's progressive matrices			
		F	M	min	max	mean	SD	min	max	mean	SD	min	max	mean	SD
ID	60	30	30	20	55	32.95	8.333	10	176	95.22	44.394	6	35	13.73	4.974
DD	60	30	30	20	56	30.70	8.494	9	159	78.12	43.379	4	24	12.73	4.104

ID – intellectual disability; DD – dual diagnoses; n – number of participants; F – female; M – male; SD – standard deviation.

According to the authors [6] of the scale, the whole battery has high internal consistency which ranges from α = 0.63 to α = 0.91; the authors [6] of the scale also point out that the agreement among the evaluators was high and that it ranged from 0.76 to 0.96.

Only the following subscales from the entire ABaCo battery were used for the purpose of this research: The Subscale of Linguistic Comprehension of Irony (hereinafter Comprehension of Irony), The Subscale of Linguistic Comprehension of Deception (hereinafter Comprehension of Deception), The Subscale of Linguistic Production of Irony (hereinafter Production of Irony), The Subscale of Linguistic Production of Deception (hereinafter Production of Deception). Although irony and deception have both linguistic and non-linguistic aspects, the given subscales assessed only linguistic aspects.

Each of the four subscales consists of four video tasks, all of which are graded on three levels. A participant can obtain a maximum of 12 points for each subscale. The mentioned levels within the production tasks include the following: 1. whether the participant expressed (formulated) a message, 2. whether the message was clear, understandable and acceptable, and 3. whether the message was sent with a specific purpose. On the other hand, the following items are evaluated in tasks which assess comprehension: 1) whether the participant understood what the actor in the video said, 2) whether the participant understood the truthfulness of that statement (is it a lie or the truth), and 3) whether the participant understood the purpose of that message (what was the person's intention when he said that).

For the tasks from Comprehension of Irony, after watching a video depicting a two-way communication interaction in which one actor utters an ironic statement (e.g. A girl in the shop tries on a dress that is obviously too tight. She asks her boyfriend whether a dress fits and he replies "It's a bit wide"), the participants are directed to answer the following questions: "What did the actor want to say? Did he really say that? Why did he answer in that way?"

Tasks from Comprehension of Deception include video scenes in which one actor gives a false reply to deceive the interlocutor (e.g. A boy and a girl sit at a table in a reading room. The girl gets up and leaves the room. He accidentally pours coffee over her notes. The girl comes back and asks: "Who has spilled coffee on my notes?" and the boy answers: "I really do not have a clue"). On the basis of what they see and hear, the participants are directed to answer the following questions: "What did the actor want to say? Did he tell the truth? Why did he say that?"

Tasks from Production of Irony include a video that presents a communicative interaction in which one actor says something, and the participants are expected to formulate an ironic answer for the second actor that completes the conversation (e.g. A girl is studying a radio that is on the table. A young man enters the room, leans on the table and observes an unplugged cable. The girl says desperately: "I don't know why the radio does not work"). The following aspects are assessed in this task: whether the participant formulated the message, whether the message is clear and understandable, and whether the participant said it to make a joke, amuse somebody, or achieve the effect of irony.

In Production of Deception, the participants are expected to complete a chain of communication by formulating and producing an answer to deceive the interlocutor on the basis of the video presented (e.g. A young man enters a room, spots a bottle of juice, drinks it and throws the empty bottle in the bin. Straight after a girls enters the room and asks: "What has happened with my juice?"). The participant's answers to these tasks are graded on three levels: whether the participant formulated the message, whether the message is clear and understandable, and whether the participant lied to deceive somebody, i.e., so that the participant was not revealed.

False-belief tasks, the ones from the category of "appearance-reality" or "deceiving object" tasks, were used to assess the theory of mind ability [56, 57]. The original task assesses the participants' ability to understand that objects can resemble each other and that appearances can vary from reality. Prior to setting the task, the examiner asks each participant about the names of their two best friends. Real friends' names are used in asking questions. A participant is presented with an object that he/she is asked to identify immediately; then, upon manipulating the object, it becomes clear that it is something else that only resembles the initially identified item. The participants are required to answer the following questions: a) "What does this object look like?", b) "What is the object?", c) "What do you expect your friend [the name of the participant's first friend] would think if he saw this object?" [57]. A successful answer to all three questions in three attempts indicates that the participant has adopted first-order theory of mind. For the purpose of this research, the applied tasks were modified and supplemented with questions which enable the assessment of second-order theory of mind ability. The examiner put additional question in order to assess "beliefs about beliefs": d) "What do you expect your friend [the name of the participant's first friend] to think about what someone else [the name of the participant's second friend] thinks this object represents?". For the purpose of this research, the participants were presented with three objects: a candle that looks like an apple, a box that looks like a book, and a bank that looks like a ladybird. Each correct answer was awarded one point, and incorrect zero. A successful answer to the fourth question in all three attempts indicates that second-order theory of mind has been adopted.

The Peabody Picture Vocabulary Test [58] was used to assess the ability to comprehend speech, as a control variable. The items were grouped in 19 categories each consisting of 12 words. The total number of words was 228. The original version of the instrument was translated into Serbian, and then the Serbian version was translated back into English. After minor changes made by comparing two versions of the test (the original and the translation), a final version was created. The participants were expected to show one out of four given pictures, which corresponded to the uttered word. The Peabody Picture Vocabulary Test has a high internal consistency ranging from 0.89 to 0.97 [58]. Electronic version of this test was used for the purpose of this research. The partici-

pants were presented with pictures on a computer screen. The testing was stopped once a participant gave eight incorrect answers in one set. In accordance with the recommendations of the test authors and scoring instructions, raw score was obtained by subtracting the total number of incorrect answers from the total number of given items.

Raven's progressive matrices [59] were used to determine the level of intellectual functioning. This instrument consists of non-verbal tasks designed to measure general intelligence factor. The tasks within this test are organized as patterns but always with one segment missing. Participants are expected to recognize the pattern rule and accordingly choose the missing one from several offered. The applied version of the matrices consisted of 60 tasks organized in five series. The tasks were arranged according to difficulty, and the series were organized according to topics: completing patterns, determining analogies between pairs of figures, progressive changing of patterns, permuting figures and breaking the figures into parts. Reliability coefficient determined by even-odd method was high and was 0.96, while test-retest reliability was somewhat lower (0.88) [59]. The test was assigned individually for the purpose of this research. It was shown and explained to the participant that there was one segment missing from the top of the page in each task, and that possible answers were given at the bottom of the page. It was also explained to the participant that each of the given answers was in a shape which could fit in the place of the missing segment, but that there was only one correct answer. Before starting the assessment, it was explained to the participant that he/she was expected to point to the answer he/she believed was the correct missing segment. After the introductory explanation of the task and a trial item, the examiner started the assessment starting from the first set and the first task. The examiner wrote down the answers which the participant pointed. All participants solved this test in less than 30 minutes. Raw score was calculated by adding up all the correct answers.

Ethical notes

All of the participants voluntarily participated in the research. The informed consent to participate in the research was obtained for each of the participants or by their parents or guardians. The participants were informed about the nature and content of the applied instruments and about the possibility of withdrawing from the procedure at any time. Additionally, the participants and their guardians were aware that the obtained results would be used solely for scientific purposes and that the confidentiality of any information obtained would be respected. The research was approved by the Ethics Committee of the Faculty of Special Education and Rehabilitation, University of Belgrade, Serbia.

Procedure

The assessment was conducted after the sample was formed and written consent was obtained from the participants and their guardians. The participants with ID and DD were interviewed in their social care institutions. After providing introductory explanations and familiarizing the participants with the nature of tasks, the examiner assessed the participants individually in a space without any distractions. Video tasks from the applied scales were presented on a laptop, after which each participant was asked questions about the contents of the video. The video clips were 20 to 25 seconds long.

Data analyses

Descriptive data analysis included calculating the mean value, standard deviation (SD), and standard error of measurement (SE). Two separate mixed three-factor analysis of covariance (ANCOVAs) were used to examine the differences between groups with regard to different factors. The Spearman's correlation was used to assess the relationship between the abilities to comprehend and produce irony and deception, intellectual functioning and theory of mind. The Pearson's correlation was used to determine the relation between the subscales for assessing the abilities to comprehend and produce irony and deception.

Results

Table 2 shows the participants' scores on subscales that assess comprehension and production of irony and deception with regard to the presence of DD.

For the purpose of examining the differences in achievements on comprehending and producing irony and deception tasks, two separate three-factor ANCOVAs were performed, with diagnosis (ID, DD) as between subject factor and irony/deception and comprehension/production as repeated factors (within subject). Speech comprehension ability presented as the Peabody test score was used as the covariate in the first analysis, while intelligence presented through score on Raven's progressive matrices was the covariate in the second analysis.

Table 2
Participants' scores on scales assessing the comprehension and production of irony and deception with regard to the presence of dual diagnoses (DD)

Parameters	Mean		SD		SE	
	ID	DD	ID	DD	ID	DD
Comprehension of irony	5.37	4.20	1.93	2.08	0.25	0.27
Comprehension of deception	6.97	7.65	4.06	3.55	0.52	0.46
Production of irony	6.50	5.58	2.59	2.95	0.33	0.38
Production of deception	8.82	7.30	3.43	3.84	0.44	0.49

ID – intellectual disability; SD – standard deviation; SE – standard error of measurement.

Table 3

Three-factor ANCOVA with Peabody test score as the covariate

Parameters	F	*p*	Partial η^2
Comprehension/production	0.177	0.674	0.002
Comprehension/production * Peabody test score	5.076	**0.026**	0.042
Comprehension/production * ID/DD	1.578	0.212	0.013
Irony/deception	16.981	**0.000**	0.127
Irony/deception * Peabody test score	0.041	0.840	0.000
Irony/deception * ID/DD	1.778	0.185	0.015
Comprehension/production * irony/deception	0.475	0.492	0.004
Comprehension/production * irony/deception * Peabody test score	0.043	0.836	0.000
Comprehension/production * irony/deception * ID/DD	6.856	**0.010**	0.055
Peabody test score	58.959	**0.000**	0.335
ID/DD	0.722	0.397	0.006

η^2 – eta squared; ANCOVA – intellectual disability; DD – dual diagnoses.

Table 4

Three-factor analysis of covariance (ANCOVA) with score on Raven's progressive matrices as the covariate

Parameters	F	*p*	Partial η^2
Comprehension/production	0.417	0.520	0.004
Comprehension/production * score on Raven's progressive matrices	3.562	0.062	0.030
Comprehension/production * ID/DD	2.232	0.138	0.019
Irony/deception	12.181	**0.001**	0.094
Irony/deception * score on Raven's progressive matrices	0.169	0.682	0.001
Irony/deception * ID/DD	1.604	0.208	0.014
Comprehension/production * irony/deception	0.795	0.374	0.007
Comprehension/production * irony/deception * score on Raven's progressive matrices	0.311	0.578	0.003
Comprehension/production * irony/deception * ID/DD	6.940	**0.010**	0.056
Score on Raven's progressive matrices	10.657	**0.001**	0.083
ID/DD	2.728	0.101	0.023

η^2 – eta squared; ID – intellectual disability; DD – dual diagnoses.

In the analysis in which speech comprehension ability was used as the covariate, only main effects of the covariate (speech comprehension) and irony/deception factor appeared as significant (Table 3). Apart from that, the analysis revealed significant interactions of speech comprehension ability with comprehension/production, as well as the interaction of all three factors, comprehension/production, irony/deception and ID/DD. The interaction showed that effects of all three factors depended on each other; for instance, effects of irony/deception differed in comprehension and production, and also in ID and DD participants.

In the analysis in which intellectual functioning was used as the covariate, only main effects of the covariate (intellectual functioning) and irony/deception factor appeared as significant (Table 4). Apart from that, the analysis revealed significant interactions of all three factors, comprehension/production, irony/deception and ID/DD. The interaction showed that effects of all three factors depended on each other; for instance, effects of comprehension/production were different for irony and deception, and also in ID and DD participants.

Participants with ID were better in producing irony than in comprehending it in both analyses (comprehending speech covariate $p = 0.003$; intellectual functioning covariate $p = 0.002$), as well as in producing deception than in comprehending it (comprehending speech covariate $p = 0.010$; intellectual functioning covariate $p = 0.007$). DD group was better in producing irony than in comprehending it ($p = 0.000$ in both analyses), while there were no statistical differences in deception tasks; ID group had higher scores compared to DD group for comprehension of irony (comprehending speech covariate $p = 0.016$; intellectual functioning covariate $p = 0.004$) and for production of deception only in the second analysis in which the covariate was intellectual functioning ($p = 0.044$).

With regard to achievements in first-order theory of mind assessment tasks, we could conclude on the basis of mean values that participants with ID (M = 0.31, SD = 0.469) had somewhat higher achievements than participants with DD (M = 0.26, SD = 445), but not statistically significant ($t[118] = 0.598$, $p = 0.234$). A similar relation was present in second-order theory of mind tasks in participants with ID (M = 0.15, SD = 0.360) and DD (M = 0.13, SD = 0.345) ($t[117] = 0.223$, $p = 0.657$). Nonparametric techniques of the Spearman's rank correlation were used to assess the relations between first- and second-order theory of mind. Statistically significant correlations were obtained both in persons with ID (r = 0.617, $p = 0.000$) and in persons with DD (r = 0.649, $p = 0.000$).

The Pearson's correlation was used to determine the relation between individual subscales for assessing the abilities to comprehend and produce irony and deception.

Table 5

Correlations between comprehension and production of irony and deception[a]

Parameters		Comprehension of irony	Comprehension of deception	Production of irony	Production of deception
Comprehension of irony	r	α = 0.672	0.120	**0.359****	**0.302***
Comprehension of deception	r	0.163	α = 0.891	-0.021	0.130
Production of irony	r	**0.507****	0.125	α = 0.800	**0.576****
Production of deception	r	**0.406****	0.059	**0.598****	α = 0.885

[a] Above diagonal – correlations for intellectual disability; below diagonal – correlations for dual diagnoses; on diagonal – Cronbach's alpha reliability coefficients for linguistic production and comprehending irony and deception subscales; *$p < 0.05$; **$p < 0.01$.

Table 6

Correlations among comprehension and production of irony and deception, theory of mind intellectual functioning, and speech comprehension

Parameters		Raven's score		TOM I		TOM II		Speech comprehension	
		ID	DD	ID	DD	ID	DD	ID	DD
Comprehension of irony	r_s	0.137	**0.306***	0.223	0.097	0.146	-0.022	**0.433****	**0.459****
Comprehension of deception	r_s	-0.006	0.069	-0.052	0.127	0.145	0.040	0.203	0.235
Production of irony	r_s	0.208	**0.426****	**0.341****	0.114	**0.263****	0.055	**0.521****	**0.532****
Production of deception	r_s	0.163	**0.319***	0.222	0.079	**0.312***	0.051	**0.527****	**0.305***

$^{**}p < 0.01$; $^{*}p < 0.05$; ID – intellectual disability; DD – dual diagnoses; TOM I – first-order theory of mind; TOM II – second-order theory of mind.

The obtained results showed irony comprehension correlated positively with irony production and deception production, in both groups (ID and DD), but in DD the correlations were a bit higher. Also, irony production correlated positively with deception production in both groups quite similarly. Cronbach's alpha coefficients showed satisfactory reliability for almost all scales except for irony comprehension which was a bit lower (Table 5).

The relationship between first- and second-order theory of mind ability and the production and comprehension of irony and deception was assessed using nonparametric techniques of the Spearman's rank correlation, since preliminary analyses determined that there was no normal distribution in theory of mind results. By means of the same technique, we tested the relations among speech comprehension ability, success in comprehending and producing irony and deception, and the level of intellectual functioning presented as the score on Raven's progressive matrices (Table 6).

There were no significant correlations between intellectual functioning and comprehension and production of irony and deception in the group of participants with ID, while in the group with DD significant correlations were determined between intellectual functioning and comprehending irony, producing irony and producing deception. In participants with ID, first-order theory of mind had significant correlations only with producing irony, while second-order theory of mind had significant correlations with producing irony and producing deception. In participants with DD, there were no significant correlations between theory of mind and producing and comprehending irony and deception. With regard to speech comprehension ability, statistically significant correlations were determined in both groups of participants (ID and DD) with all subscales except with comprehending deception subscale.

Discussion

The obtained results showed that participants with ID were significantly more successful in comprehending irony tasks compared to participants with DD. Apart from comprehending irony, participants with ID were also more successful in producing deception. All participants were more successful in deception tasks compared to irony tasks. Production was easier for all participants, except for participants with DD in deception tasks.

Regarding differences between the participants with DD and the participants with ID, after using speech comprehension and intelligence as covariates, the ability to comprehend irony was singled out in both analyses indicating the existence of significant differences at an advantage of persons with ID. Colle et al. [60] note that participants with psychotic disorders and average intellectual abilities show more prominent difficulties with solving irony tasks. Gavilán and García-Albea [61] also indicate that in participants with symptoms of schizophrenia, the ability to comprehend figurative aspects of language (e.g. metaphor, irony, proverbs) is more strongly influenced by theory of mind ability than by intelligence. Although according to these authors, theory of mind deficit has a negative influence on semantic-pragmatic processing and on comprehending figurative meaning, bearing in mind the absence of statistically significant correlations (which will be interpreted with caution due to reduced variability of the variables which were correlated) between theory of mind and irony obtained in this research, we are closer to the opinion of the authors who argue that the theory of mind could not be considered a crucial factor in irony comprehension [4, 62].

The following notions also indicate that the relation between theory of mind and comprehending irony in persons

with mental disorders depends on several different factors. In line with the above mentioned, some studies have found that, in persons with schizophrenia who do not have acute symptoms, comprehension of irony is not significantly related to the theory of mind [14, 63], which is in accordance with the results of this research. Some authors believe that the absence of relation between the theory of mind and comprehension of irony can be explained by the fact that persons with schizophrenia may have deficits in implementation and execution, i.e. that they understand mental states of others, but they fail to apply this knowledge due to limitations related to processing (e.g. general cognitive deficit) [14].

Some authors believe that cognitive flexibility, the integration of different contextual elements, and the ability to reject literal interpretations and make connections between a statement and a totally opposite meaning are necessary to comprehend irony [64]. All of the mentioned factors (theory of mind and other cognitive factors) are in accordance with the ideas stated in the Theory of Cognitive Pragmatics, which indicate that irony is a non-standard form of communication in which it is not enough for the listener to follow the usual chain of communication; instead, he or she must flexibly observe both contextual segments and the speaker's mental state. In this regard, the participants with DD in our research had somewhat more noticeable difficulties detecting discrepancies in meaning and/or uttered messages whose meanings were in opposition to the contextual background and the speaker's intention, which may point to the fact that the characteristics attributed to these participants – such as cognitive disorientation, poor analytic abilities, and insufficient motivation, can contribute to such results. This assumption is in accordance with the results of the research conducted by Colle et al. [60] which indicate that severity of schizophrenic symptoms have a negative correlation with overall pragmatic abilities of persons with schizophrenia.

Apart from differences in the ability to comprehend irony, the results of the mixed three-factor ANCOVA analysis, in which the covariate was intellectual functioning, indicate another difference between participants with ID and DD in producing deception tasks, again at an advantage of persons with ID.

On the other hand, the results show that all participants were more successful in deception tasks compared to irony tasks regardless of the presence of ID and DD. The fact that all participants were more successful in deception tasks is not surprising, and is supported by the literature which states that tasks containing irony are more demanding and more complex than tasks containing deception, both for participants with ID [65], and for typically developing individuals [4]. Also, the results of this research indicate that all participants with ID were more successful in production tasks than in comprehending ironic and false statements. Persons with DD had higher scores in producing irony than in comprehending it. Bearing in mind that the obtained results indicate that speech comprehension ability had significant interactions with the comprehension/production factor ($p = 0.026$, Partial $\eta^2 = 0.042$), the obtained differences between comprehending and producing irony and deception can also be observed in the context of the relation between this variable and receptive speech. We can conclude that better understanding of speech leads to more pronounced differences between comprehension and production.

The results of our research indicate that TOM did not correlate with comprehending and producing irony and deception in participants with DD at all, while in participants with ID, first-order TOM correlated with producing irony, and second-order TOM correlated with producing irony and producing deception. It is possible that the absence of correlations was caused by the limited variability (range restriction) in both TOM and comprehending/producing irony/deception (although the restriction appears to be larger for TOM). This finding which points to the relation between TOM and production is not uncommon in studies on other clinical populations, and thus Bosco et al. [66] indicate that in participants with traumatic brain injury TOM can account for the production of irony and deception to some extent, but not their comprehension. Executive functions were singled out as significant for speech comprehension, but not for production ability.

Achievements of participants with ID and DD in irony and deception tasks with regard to their comprehension and production can be explained by speech comprehension ability (Partial $\eta^2 = 0.335$) to a greater extent than by intellectual functioning (Partial $\eta^2 = 0.083$). Supporters of linguistic theories of non-literal comprehension development also point out the significant influence of general linguistic comprehension and scope of vocabulary for comprehending non-literal language in persons with language difficulties[67,68]. Language skills have also proved to be crucial in comprehending ironic statements in studies on typically developing children [69]. Norbury [68] emphasizes that it cannot be argued that TOM fails to play any role in persons with language difficulties in comprehending non-literal language, not because of the theory of mind itself, but because of its close relation to language skills which are considered dominant in non-literal language.

The ability of producing and comprehending both irony and deception in persons with ID cannot solely be explained by the presence of mental disorders, since it appeared as significant only in interaction with other factors. Presence of mental disorders showed significant effect only on irony comprehension and deception production, which were higher in the group without mental disorders. Furthermore, the results of our research also point to the complexity of this relation, indicating that skills of producing and comprehending irony are significantly positively correlated with each other, and correlations were also determined with producing deception. Only comprehending deception did not correlate with other skills in any group. Partial absence of correlations between comprehending deception and irony can also be observed as a part of differences between these skills and their partially different neurological basis. For example, in a neuroimaging study, Bosco et al. [70] found that the left middle temporal gyrus activated in comprehending ironic statements, while it did not activate in comprehending lies.

Limitations

One of the limitations of this research is the use of only one instrument to assess the production and comprehension

of irony and deception, which did not allow a comparison of the results among instruments measuring the same group of abilities. A second limitation could be the sample structure of this research. Thus, future studies should extend the scope and structure of the assessed groups with regard to age and different intellectual disabilities aetiologies. Also, one of the limitations of this study may be the absence of assessment of certain aspects of executive functions (e.g. planning skills, reasoning skills, cognitive flexibility, etc.), which can potentially be related to non-standard linguistic abilities. Additionally, further studies should include the assessment of other cognitive and adaptive factors that influence the differences in non-standard achievements between participants with intellectual disabilities and those with dual diagnoses.

Conclusion

Although differences were found in some aspects of assessed abilities between the two groups (participants with intellectual disability were better than participants with dual diagnoses in comprehension of irony and production of deception), similarities in the profile of these abilities were dominant in all participants (both groups were better in comprehending and producing deception than irony). Examining the relations indicated that results variability can be explained by differences in speech comprehension ability more than by differences in non-verbal intellectual functioning or differences in theory of mind acquisition.

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Association of PAX3 and TMTC2 genes polymorphism with the face morphology changes after excision of skin tumors

Povezanost polimorfizma PAX3 i TMTC2 gena sa promenama morfologije lica nakon ekscizije tumora kože

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Abstract

Background/Aim. The group of genes, known as PAX (paired box), has a great role in organogenesis, as well as in maintaining the normal function of certain cells after the birth. In addition to these genes, the impact on the organogenesis, at the cellular level, has a transmembrane tetratricopeptid group of genes (TMTC). The term polymorphism in the human genome implies variations in the hereditary basis that occur in human populations, the presence of two or more different alleles of one genome in the population. The aim of the work was to determine whether there is an association of PAX3 and TMTC2 genes polymorphism with changes of the face morphology after skin tumor excision and direct suture closure. **Methods.** The study included 130 patients of both sexes, older than 50 years, with the medical indication for the elliptical surgical excision of the skin tumor. DNA was isolated from 5 mL of peripheral blood. Gene polymorphisms were analyzed with pre-designed single nucleotide polymorphisms (SNP) assays, by allelic discrimination method on REAL-TIME apparatus. The patients were subjected to a laser scanning preoperatively, and 7 and 90 days postoperatively, in order to obtain x, y and z coordinates of 5 cephalometric points on the face, which determined the shape of the medial cheek region. The shape of the medial cheek region, as well as the coordinates of 5 cepahlometric points, were compared among genotypes of both genes preoperatively, as well as 7 days and 90 days postoperatively. **Results.** A statistically significant difference in the shape of the medial cheek region between wild-type and mutant of PAX3 gene was found preoperatively, while the statistically significant difference in the shape of the medial cheek region was not found between wild-type and heterozygote, nor between wild-type and heterozygote and mutant of PAX3 gene, nor among genotypes of TMTC2 gene. Seven days and 90 days postoperatively, there were no statistically significant differences in the shape of the examined region among genotypes of both genes. **Conclusion.** Polymorphisms of PAX3 and TMTC2 genes are not associated with the change in the face morphology after the skin tumor excision and direct suture closure of the defect.

Key words:
polymorphism, genetic; face; skin neoplasms; surgical procedures, operative.

Apstrakt

Uvod/Cilj. Grupa gena, poznata pod nazivom PAX (eng. *paired box*), ima velikog udela u organogenezi, kao i u održavanju normalne funkcije izvesnih ćelija nakon rođenja. Pored ovih gena, uticaj na organogenezu, na ćelijskom nivou, ima i transmembransko-tetratrikopeptidna grupa gena (TMTC). Pod pojmom polimorfizam u genomu čoveka podrazumevaju se varijacije u naslednoj osnovi koje se javljaju u humanim populacijama, prisutnost dva ili više različitih alela jednog gena u populaciji. Cilj rada bio je da se utvrdi da li postoji povezanost polimorfizma PAX3 i TMTC2 gena sa promenom morfologije lica nakon ekscizije tumora kože i postekscizione direktne suture. **Metode.** Istraživanjem je bilo obuhvaćeno 130 ispitanika, oba pola, starijih od 50 godina, kod kojih je postavljena medicinska indikacija za hiruršku elipsastu ekscisiju tumora kože lica. DNK je izolovana iz 5mL periferne krvi. Polimorfizmi gena analizirani su predizajniranim *single nucleotide polymorphisms* (SNP) esejima, metodom alelske diskriminacije na REAL-TIME aparatu. Ispitanici su skenirani laser skenerom preoperativno, kao i sedam dana i 90 dana postoperativno, kako bi se za svakog ispitanika dobile x, y i z koordinate pet kefalometrijskih tačaka na licu, koje su određivale oblik medijalne obrazne regije. Upo-

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rađivan je oblik medijalne obrazne regije, kao i koordinate pet kefalometrijskih tačaka između genotipova oba gena, preoperativno, kao i 7 i 90 dana postoperativno. **Rezultati.** Preoperativno je nađena statistički visoko značajna razlika u obliku medijalne obrazne regije između *wild type* i mutanata PAX3 gena, dok statistički značajna razlika u obliku ispitivane regije nije nađena između *wild type* i heterozigota, kao ni između *wild type* u odnosu na heterozigote i mutante PAX3 gena, kao ni između genotipova TMTC2 gena. Sedam i 90 dana postoperativno, nije nađena statistički značajna razlika u obliku ispitivane regije između genotipova, kod oba gena. **Zaključak.** Polimorfizmi PAX3 i TMTC2 gena nisu povezani sa promenom morfologije lica nakon ekscizije tumora kože lica i zatvaranja defekta direktnom suturom.

Ključne reči:
geni, polimorfizam; lice; koža, neoplazme; hirurgija, operativne procedure.

Introduction

In recent years, efforts have been intensified to determine an influence of polymorphism of genes on morphological characteristics of the face.

It has been demonstrated that a group of genes, known as PAX (Paired box), has a great role in organogenesis, as well as in maintaining the normal function of certain cells after the birth. There are four groups of PAX genes [1]. In the first group are PAX 1 and 9, in the second group are PAX 2, 5 and 8, in the third group are PAX 3 and 7, while in the fourth group are PAX 4 and 6. During embryonic development, PAX 3 gene is active in cells of the neural crest. These cells migrate from the spinal cord in certain regions in the embryo [2]. The protein encoded by PAX 3 gene influences the activity of other genes, inducing cells to form neural crest limb muscles, bones of the face and scalp, certain neural structures and melanocytes that determine the color of hair, eyes, and skin. Melanocytes are also found in some regions of the brain and the inner ear. Therefore, PAX 3 gene, associated with the development of the ear, eye and face, is highly expressed in melanoma, and also contributes to the survival of tumor cells (alveolar rhabdomyosarcoma, which is more common in adolescents). It is located on the second chromosome (2q36.1). Mutations in the gene lead to the Waardenburg syndrome. The disease is characterized by varying degrees of deafness, minor defects in structures that originate from the neural crest and anomalies in pigmentation [3].

In addition to these genes, a transmembrane tetratricopeptid group of genes (TMTC 1, 2, 3, 4) has the impact on the organogenesis, of which TMTC 2 gene encodes protein 2, which is a transmembrane building element of the cell membrane, and endoplasmic reticulum. TMTC 2 gene is located on chromosome 12 (12q21.31) [4]. At the molecular level, it has a role in binding of one molecule to one or more specific sites of other molecules [5]. The specific role of TMTC 2 gene has not yet been established, although it is known that it has a role in cellular calcium homeostasis [6].

Deoxyribonucleic acid (DNA) polymorphisms are now widely studied as markers of possible genetic susceptibility for certain diseases. The Genome-Wide Association Studies (GWAS) explain the genetic basis of complex diseases by comparing the frequency of different genetic variants in the population in relation to healthy-population. One of the most common types of genetic polymorphisms is the polymorphism of the single nucleotide polymorphism sequence (SNP), replacing one of the four nucleotides in DNA molecule. Substitutions may occur in the coding (exon) or non-coding (intron) portion of the gene, or in the promoter region. SNPs are commonly used in genetic studies of the association. Previous research has shown that SNPs can be associated with the development of various types of disease, response to pathogens, drugs and other agents. Besides, in some studies, an association between SNP of PAX3 (rs7559271, G/A) and TMTC2 (rs10862567, T/A) and differences of face morphology were found [1], but there were no studies about the association between PAX3 and TMTC2 SNP and postoperatively differences in face morphology, after skin tumor excision.

In accordance with the reconstructive ladder, in plastic and reconstructive surgery after facial skin tumor excision, we primarily use the direct closure, as this is the simplest method of covering defects [7].

However, in addition to general medical and surgical principles, it is necessary to take into account the aesthetics of the face, and the consequential symmetry after excision. If the symmetry is violated, direct suture does not apply, and we use skin graft or flap [8].

The aim of the study was to determine the association of polymorphisms of PAX3 (rs7559271, G/A) and TMTC2 (rs10862567, T/A) genes with changes of face morphology after skin tumor excision and direct suture closure of the defect.

Methods

The study included 130 patients of both sexes, older than 50 years, with the medical indication for the surgical elliptical excision of facial region skin tumors.

Before the surgical elliptical excision, 5 mL of peripheral blood of all the patients was taken by venipuncture. All the patients signed the consent to participate in the research, by the decision of the Ethics Committee of the Military Medical Academy in Belgrade. Peripheral blood with anticoagulant was kept in a freezer at -20°C. DNA from peripheral blood was isolated by commercial kit PureLink®Genomic DNA Kit (Invitrogen, Thermo Fisher, USA), according to manufacturer instructions.

Polymorphism of genes was determined with predesigned SNP (single nucleotide polymorphism) assays (TaqMan® Pre-designed SNP Genotyping Assay, Applied Biosystems, for PAX3 rs7559271, and TMTC2 rs10862567), by allelic discrimination method on REAL-TIME apparatus (ABI Prism 7500, USA).

Immediately before the surgery, in all patients, the elliptical excision of skin tumors lines around the margin of clinically unaffected skin, 2 mm width, was marked and the elliptical excision, parallel to the lines of minimum tension, was done, after which patients were scanned preoperatively with laser scanner (Laserscanner, the Institute for Robotics and Process Control, University of Braunschweig, Germany, 2009). The patients were also scanned postoperatively, 7 and 90 days after the surgery [9].

Five cephalometric points (nasion, endocanthal central point, pronazale, lower palpebral point, endocanthion), and their x, y and z coordinates, were determined from scans of the patients' face, using extraction of coordinates by C++ software, and using the characteristics of cephalometric points: nasion is the most anterior point of the junction of the nasal and frontal bones in the midsagittal plane, endocanthal central point is in the middle between bilateral most deep points of endocanthus, pronasale is the most prominent point on tip of nose, lower palpebral point is the lowest point of lower eyelid, endocanthion is the most deep point of endocanthus. For five cephalometric points we got 15 coordinates (x1-5, y1-5, z1-5). Those coordinates determined the shape of the polygonal line, as a border of the space of operated region, in the region of the medial cheek of the face. Changes of the shape of the operated region were assessed by using Procrustes analysis. As the first, superimposition of landmark points was done by translation, rotation and scaling, after what we used Procrustes distance (Pd), given by Procrustes coordinates (x, y, z), as a squared root of sum of squared distances between corresponding landmarks of two shapes, which is a measure of shape difference between two groups of shapes. We compared Pd, as a measure of shape differences, among all the genotypes, and among all three scanning times (preoperatively, 7 and 90 days postoperatively), using ANOVA and *Post Hoc* Scheffe test. Besides, as we wanted to know which coordinate has influence on changing the shape of the operated region, we compared x, y and z coordinates of five cephalometric points among the groups of patients with different genotypes of PAX3 and TMTC2 genes, using MANOVA. Determination of Pd distances was done in the software program MorphoJ, version 1.06d, 2014, while ANOVA, *Post Hoc* Scheffe test, and MANOVA, were done in SPSS 23, IBM, 2015 [10].

Results

Distribution of genotypes of PAX3 and TMTC2 gene was presented in Table 1. The most presented genotype was wild-type, in both genes.

The values of Pd means between the coordinates of each patient and average coordinates in all of three genotypes of PAX3 gene [wild-type (G/G), heterozygote (G/A), and mutant (A/A)], preoperatively, and 7 and 90 days after surgery, was presented in Figure 1. We found the highest value of Procrustes distances in all of three genotypes seven days postoperatively, while 90 days postoperatively values of Pd were lower than preoperatively. The preoperative median was lower than mean in all of the genotypes, while the median was higher than mean 7 and 90 days postoperatively, with the exception of mutants 90 days postoperatively.

Table 1
Distribution of genotypes of PAX3 and TMTC2 genes in the examined groups of patients

Genotypes	PAX3	TMTC2
	n (%)	n (%)
Wild type	72 (55.4)	103 (79.2)
Heterozygote	34 (26.1)	17 (13.1)
Mutant	24 (18.5)	10 (7.7)
Total	130 (100)	130 (100)

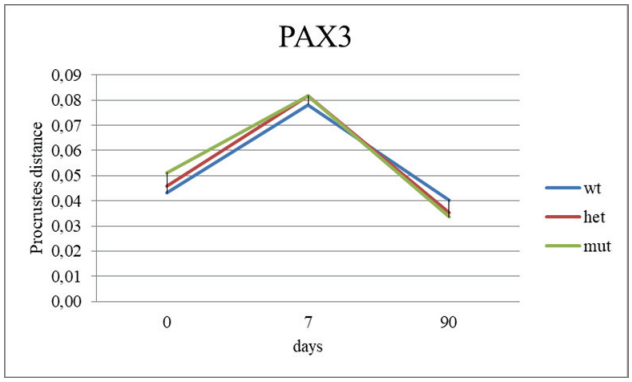

Fig. 1 – Procrustes distances in patients with different PAX3 genotypes [preoperatively (0), 7 and 90 days postoperatively].
***wt – wild type; het – heterozygote; mut – mutant.**

The statistical significance of Pd differences among the patients with different PAX3 genotypes in all of three scanning times was analyzed using ANOVA and *Post Hoc* Scheffe test and was presented in Table 2. We found the statistically highly significant difference only between wild-type and mutant preoperatively, while 7 and 90 days postoperatively there was no statistically significant difference among the genotypes.

Table 2
Significance of differences of Procrustes distances in patients with different PAX3 genotypes [preoperatively (0), 7 and 90 days postoperatively]

Genotypes	Days		
	0	7	90
	p-value		
wt vs. het	0.419	0.847	0.248
wt vs. het + mut	0.738	0.924	0.597
wt vs. mut	**0.005**	0.868	0.128

wt – wild type; het – heterozygote; mut – mutant.
***p*-values < 0.01 are bolded.**

Results of difference testing among the coordinates of PAX3 genotypes in all three scanning times (0, 7, 90 days) by MANOVA were presented in Table 3.

Table 3
Significance of differences between x, y and z coordinates in patients with different PAX3 genotypes [preoperatively (0), 7 and 90 days postoperatively]

Coordinates	Genotypes								
	wt vs. het			wt vs. het + mut			wt vs. mut		
	days								
	0	7	90	0	7	90	0	7	90
	p-value								
x1	0.292	0.292	0.292	0.934	0.901	0.874	0.677	0.677	0.677
x2	0.477	0.399	0.227	0.981	0.837	0.701	0.537	0.856	0.856
x3	0.207	0.207	0.237	0.802	0.861	0.834	0.644	0.644	0.642
x4	0.292	0.292	0.292	0.921	0.912	0.894	0.677	0.677	0.677
x5	0.292	0.292	0.292	0.934	0.911	0.901	0.677	0.677	0.677
y1	0.292	0.292	0.292	0.901	0.909	0.902	0.677	0.677	0.677
y2	0.292	0.992	1.000	**0.005**	0.961	0.574	**0.007**	0.885	0.449
y3	0.301	0.298	0.301	*0.015*	0.318	*0.019*	**0.000**	*0.016*	**0.000**
y4	0.292	1.000	0.992	**0.002**	0.981	0.521	**0.007**	0.978	0.574
y5	0.292	0.292	0.292	0.913	0.921	0.901	0.677	0.677	0.677
z1	0.590	0.281	0.281	0.958	0.825	0.827	0.706	0.723	0.723
z2	0.301	0.169	0.301	*0.016*	0.134	*0.015*	**0.000**	**0.001**	**0.000**
z3	0.572	0.508	0.508	0.928	0.922	0.924	0.476	0.861	0.861
z4	0.301	0.169	0.301	*0.017*	0.134	*0.017*	**0.000**	**0.001**	**0.000**
z5	0.292	0.292	0.292	0.901	0.945	0.847	0.677	0.677	0.677

wt – wild type; het – heterozygote; mut – mutant; x15, y1-5, z1-5 – coordinates of 5 cephalometric points.
***p*-values < 0.01 are bolded.**

We found the statistically highly significant difference between wild-type and mutant in y2-4, z2 and z4 preoperatively, in z2 and z4 7 days postoperatively, and in y3, z2 and z4 90 days postoperatively, as well as in y2 and y4 between wild-type vs. heterozygote and mutant. The statistically significant difference was found between wild-type and mutant 7 days postoperatively in y3, as well as between wild type vs. heterozygote and mutant preoperatively and 90 days postoperatively, in y3, z2 and z4.

The value of Pd means between the coordinates of each patient and average coordinates, in all of three genotypes of TMTC2 gene [wild type (T/T), heterozygote (T/A), and mutant (A/A)], preoperatively, and 7 and 90 days after the surgery, are presented in Figure 2. We found the highest value of Pd 7 days postoperatively in all of three genotypes, while 90 days postoperatively the value of Pd were lower than preoperatively. Preoperatively, the median was lower than mean in all of the genotypes, while the median was higher than mean 7 and 90 days postoperatively.

The statistical significance of Pd differences between TMTC2 genotypes in all of three scanning times was analyzed using ANOVA and *Post Hoc* Scheffe test. There were no statistically significant differences among the genotypes of TMTC2 gene in all three scanning times.

Using MANOVA, we also tested the statistically significant differences among the coordinates of TMTC2 genotypes in all three scanning times (0, 7, 90 days). There were no statistically significant differences among the genotypes of TMTC2 gene in all three scanning times, for all the coordinates.

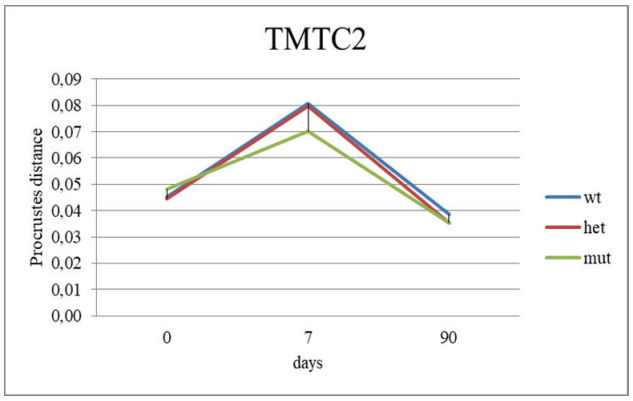

Fig. 2 – Procrustes distances in patients with different TMTC2 genotypes [preoperatively (0), 7 and 90 days postoperatively].
wt – wild type; het – heterozygote; mut – mutant.

Discussion

Previous studies have shown that polymorphisms of PAX3 and TMTC2 genes have a role in determination of the face morphology [11]. The genetic-phenotypic relationship of PAX3 gene is characterized by gene expression in the craniofacial syndrome, as well as in the alveolar rhabdomyosarcoma 2, and in Waardenburg syndrome (types 1 and 3) [12].

In previous studies [1, 4, 12], it was found that PAX3 gene has a role in the endocanthal region growth. This finding provokes the difference in landmark coordinates in this region. As the shape differences are dependent on x, y and z

coordinates of landmarks, it is expected that different genotypes could be associated with differences in the shape of that region [1, 13–15]. On the other hand, there were no studies about associations between PAX3 SNP and x, y and z coordinates of cephalometric points postoperatively.

In our study, we found that there was the statistically highly significant difference in the shape of the examined region between patients with PAX3 wild-type and mutants, preoperatively, while there was no statistically significant difference in the shape between wild-type and heterozygotes as well as between wild-type vs. heterozygotes and mutants. This result showed that PAX3 alleles were expressed differently in facial morphology of the examined region, which was correlated with the expression of the PAX3 gene in Waardenburg syndrome [16–18].

PAX3 gene product is a DNA-binding protein that is expressed during early neurogenesis [19]. Transfection experiments have shown that PAX3 and SOX10 have a direct binding effect for the proximal region of the MITF promoter, which contains sites for both factors [20, 21]. The mutated SOX10 or PAX3 proteins cannot transact this promoter, directly indicating that the two genes directly affect the regulation of MITF expression [22, 23]. Hybridization experiments in dominant mouse megacolon have confirmed that SOX10 dysfunction reduces MITF expression, as well as the development and survival of melanocytes. Authors have suggested that the interaction among three genes that have been altered in Waardenburg syndrome can explain the auditory and pigment symptoms of the disease [24, 25]. MITF and PAX3 gene mutations, encoding transcription factors, are responsible for Waardenburg syndrome 2A [26].

Also, in previous studies, it was found that PAX3 and TMTC2 SNP were associated with cephalometric points' distances in the endocanthal region [1].

Analyzing x, y, and z coordinates of the cephalometric points, we found that there was statistically significant difference between wild-type and mutants, preoperatively in y2-4, z2 and z4, on the basis of which could be concluded that the PAX3 gene has a role in defining the morphology of the medial canthal region, and in the nasion-endocanthion angle. Postoperatively, a statistically significant difference in y2 and y4 was not found. Accordingly, it could be concluded that other factors affected the postoperative change of z2 and z4 and not PAX3 gene polymorphism. This finding could be explained by the influence of surgical intervention on the change in the morphology of the medial canthus due to the concavity of the medial cantus.

The significant differences among shapes of the examined region in relation to genotypes of PAX3 gene were not found postoperatively. As there was no significant difference between wild-type and mutants, postoperatively, we could conclude that there was a role of PAX3 gene to the facial morphometric characteristics, but only preoperatively. As there was no significant correlation between preoperative and postoperative results in general, we could assume that there was no association between PAX3 gene polymorphism and postoperative facial morphometric characteristics.

Also, we did not find any difference in the shape of the examined region among TMTC2 genotypes preoperatively, neither postoperatively [27, 28]. Besides, like in PAX3 gene, there was no correlation between preoperative and postoperative results, so we could suppose that there was no association between TMTC2 gene and facial morphology in the medial cheek region [29].

Postoperative results are based on preoperative morphology, but also are dependent on the postsurgical healing process. Accordingly, we can assume that other factors could affect the changes of three-dimensional coordinates of tested cephalometric points.

Conclusion

Polymorphisms of PAX3 and TMTC2 genes are not associated with changes in the face morphology after the skin tumor excision and direct suture closure of the defect. Other factors might have a role in postoperative changes of three-dimensional coordinates of cephalometric points.

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Pre-trabeculectomy intravitreal injections of bevacizumab for treating neovascular glaucoma in diabetic patients

Intravitrealna injekcija bevacizumaba pre trabekulektomije za lečenje neovaskularnog glaukoma kod bolesnika sa dijabetesom

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Abstract

Background/Aim. Neovascular glaucoma (NVG) is a secondary glaucoma caused by occlusion of the trabecular network of newly formed blood vessels. The aim of this study was to evaluate the efficacy and safety of intravitreal injections of bevacizumab before trabeculectomy with mitomycin C (MMC) for the treatment of NVG. **Methods.** A prospective and open-label study was conducted from May 2013 to December 2014 on consecutive NVG patients who underwent intravitreal injections of bevacizumab and a primary trabeculectomy with MMC. All patients were followed-up at least for 12 months. Success was defined as an intraocular pressure (IOP) of ≤ 21 mm Hg with or without topical ocular hypotensive medication. **Results.** Fourteen eyes of 12 diabetic patients fulfilled the respective demands of the inclusion and exclusion criteria. The mean (± standard deviation) follow-up period was 15.0 (± 2.0) months (range, 12 to 19 months). After one year of follow-up, 11 (78.6%) eyes had an IOP ≤ 21 mmHg. The mean IOP was significantly reduced from 42.4 (± 9.7) mmHg preoperatively to 18.4 (± 2.9) mmHg postoperatively ($p < 0.0001$). Regarding surgical complications, 6 months of trabeculectomy, hyphaema was observed in 3 (21.4%) eyes, macular edema in one (7.1%) eye and recurrence of neovascularization requiring intravitreal bevacizumab injection in 2 (14.2%) eyes. **Conclusion.** Preoperative intravitreal bevacizumab may be effective as adjunctive treatment for trabeculectomy with mitomycin-C for neovascular glaucoma patients.

Key words:
diabetes mellitus; glaucoma, neovascular; bevacizumab; mitomycin; trabeculectomy; treatment outcome.

Apstrakt

Uvod/Cilj. Neovasularni glaukom (NVG) je sekundarni glaukom uzokovan okluzijom mreže novoformiranih krvnih sudova. Cilj rada bio je procena efikasnosti i sigurnosti intravitrealne injekcije bevacizumaba pre trabekulektomije sa mitomicinom C (MMC) u lečenju NVG. **Metode.** Prospektivna i *open-label* studija sprovedena je od maja 2013. do decembra 2014. na uzorku bolesnika sa NVG, kojima je data intravitrealna injekcija bevacizumaba i koji su bili podvrgnuti primarnoj trabekulektomiji sa MMC. Svi bolesnici su bili praćeni najmanje 12 meseci. Uspeh je bio definisan kao intraokularni pritisak (IOP) od ≤ 21 mm Hg, uz ili bez topikalne okularne hipotenzivne terapije. **Rezultati.** Četrnaest očiju od 12 bolesnika sa dijabetesom je ispunjavalo odgovarajuće kriterijume za uključenje u studiju. Prosečni (± standardna devijacija) period praćenja bio je 15,0 (± 2,0) meseci (opseg, 12 do 19 meseci). Posle jedne godine praćenja kod 11 (78,6%) očiju IOP je bio ≤ 21 mmHg. Prosečna vrednost IOP je bila značajno smanjena sa 42,4 (± 9,7) mmHg preoperativno na 18,4 (± 2,9) mmHg postoperativno ($p < 0,0001$). Što se tiče hirurških komplikacija, šest meseci nakon trabekulektomije, hifema je bila uočena kod 3 (21,4%) oka, makularni edem na 1 (7,1%) oku, a recidiv neovaskularizacije sa potrebom davanja intravitrealne injekcije bevacizumaba kod 2 (14,2%) oka. **Zaključak.** Preoperativni intravitrealni bevacizumab može biti delotvoran kao pomoćna metoda lečenja trabekulektomijom sa mitomicinom-C kod bolesnika sa neovaskularnim glaukomom.

Ključne reči:
dijabetes melitus; glaukom, neovaskularni; bevacizumab; mitomicini; trabekulektomija; lečenje, ishod.

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Introduction

Neovascular glaucoma (NVG) is a secondary glaucoma caused by occlusion of the trabecular network of newly formed blood vessels. When new blood vessels appear within the anterior chamber angle, aqueous outflow can be compromised with extension of these new vessels across the scleral spur and subsequent obstruction of the trabecular meshwork. The new blood vessels are usually accompanied by a fibrous membrane, and contraction of this membrane results in formation of peripheral anterior synechiae and progressive angle closure. Neovascularization is caused by proliferative diabetic retinopathy (PDR), central retinal vein occlusion (CRVO), and ocular ischemic syndrome (OIS) [1].

Surgical procedures such as trabeculectomy with antimetabolite agents, are often used to manage elevated intraocular pressure in NVG patients. Intraoperative mitomycin-C (MMC) during trabeculectomy (TMC) may be considered as the gold standard for glaucoma surgical treatment [2].

Anti-vascular endothelial growth factor (anti-VEGF) represents an alternative treatment for neovascular glaucoma [3, 4].

Intravitreal bevacizumab (IVB) injection has been shown to reduce ocular neovascularization and vascular permeability in patients with ischemic retinal diseases and age-related macular degeneration, firstly reported in 2006 [5, 6]. Additionally, IVB caused regression in the anterior chamber angle neovascularization, providing better intraocular pressure (IOP) control in NVG patients in open angle stage [7, 8].

The aim of this study was to evaluate the efficacy and safety of intravitreal injections of IVB, administered preoperatively, as adjunctive therapy for trabeculectomy with MMC in neovascular glaucoma patients.

Methods

A prospective and open-label study was conducted from May 2013 to December 2014 on consecutive patients with NVG who underwent a TMC with preoperative intravitreal application of bevacizumab.

Recruitment was ongoing for a period of 6 months with an aim to collect a sufficient number of patients.

The off-label use of bevacizumab (Avastin; Roche Pharmaceuticals, Basel, Switzerland) was also approved by the Institutional Review Board (IRB) of the University Eye Clinic, Clinical Centre of Serbia, Belgrade. Written informed consent about the glaucoma treatment was obtained from all patients after thorough discussion of the potential benefits and risks of IVB injection.

Neovascular glaucoma was defined as the presence of iris and/or anterior chamber angle neovascularization with extensive fibrous synechiae (at least 2/3 of the angle area) in the angle and an IOP equal or higher than 21 mmHg with antiglaucoma medication, either topical or systemic. Eyes with no light perception were excluded from the study.

Procedures

Neovascular glaucoma patients who were included in the study, before arriving at our glaucoma department on the surgical treatment, had several retinal photocoagulation (standard spot size 200 μm, 300-400 spots per treatment, average 3 sessions and average 2100 spots) sessions covering average 2/3 of retina, but not complete panretinal photocoagulation before neovascular glaucoma forming.

Intravitreal injection of 50 mL (1.25 mg; ec. 0.5 mg/0.05 mL) of bevacizumab through 30 G needle were inserted through the pars plana, under topical anesthesia with 4% lidocaine. Monitoring of regression of iris neovascularization lasted for two weeks. A fornix-based conjunctival flap technique for trabeculectomy with 0.2 mg/mL MMC was performed within 2 weeks – 1 month after IVB.

Trabeculectomy was performed by the same experience surgeon (IM). The trabeculectomy procedure included creation of a fornix-based conjunctival flap and a 3 x 4 mm, half-thickness scleral flap. Small pieces of surgical sponge soaked in 0.2 mg/mL MMC were then inserted under the conjunctival flap for up to 2 minutes. Trabeculectomy was done with a Kelly Descemet's Membrane Punch (Inami, Tokyo, Japan). After that, a peripheral iridectomy was done. The scleral flaps were closed with 10–0 nylon sutures, and the conjunctival flap was closed with an 8.0 absorbable suture.

The visual acuity, IOP, anti-glaucoma medications, and the appearance of the iris neovascularization (NVI) by ophthalmoscopy were estimated at baseline and post-IVB and before eculectomy (pre-trab) and after trabeculectomy (post-trab). The intraoperative and postoperative complications were also recorded.

Postoperatively, patients were examined at the day 1, the day 7, every month till month 3, and every 3 months thereafter.

IOP was measured by Goldmann applanation tonometry. The mean of two IOP measurements immediately before trabeculectomy were adopted as the preoperative IOP.

Surgical success was defined as IOP of ≤ 21 mm Hg with or without topical ocular hypotensive medication. On the other hand, surgical failure was defined as insufficient IOP reduction (IOP ≥ 22 mmHg, $< 20\%$ IOP reduction, use of a systemic carbonic anhydrase inhibitor, or further glaucoma surgeries), devastating complications (loss of light perception, *phthisis bulbi*, and *endophthalmitis*), or significant hypotony (IOP equal or lower than 5 mmHg continuing six months and until the last follow-up visit or hypotony that required intervention).

Statistical analysis

Statistical analysis was performed using SPSS software (IBM SPSS Statistics 20, IBM Corp., New York) and Stata software (version 13.1; StataCorp, TX). Descriptive statistics [mean ± standard deviation (SD)] and 95% confidence intervals (95% CIs) were used for demographic and clinical characteristics.

Data were tested for normal distribution using the Kolmogorov-Smirnov test. For comparisons between baseline and post-IVB, and for pre-trab and post-trab, mixed-effects models using clustered robust standard errors by Stata software were used to account for the correlation of both eyes in

the same patient. For all analyses, a p-value of < 0.05 was considered statistically significant.

Results

Fourteen eyes of 12 diabetic patients were included in the study. Their main clinical and demographic characteristics are shown in Table 1.

Table 1
Baseline demographic and clinical characteristics of diabetic patients with neovascular glaucoma (14 eyes of 12 patients)

Characteristics	Values
Age (years)	
mean (± SD)	57.3 (12.0)
range	28–69
Sex, n (%)	
male	9 (75.0)
female	3 (25.0)
Pre-trabeculectomy IOP, mm Hg	
mean (± SD)	42.4 (9.7)
range	30–69
Pre-trabeculectomy VA	
mean (± SD)	0.35 (0.30)
range	0.01–0.5
Antiglaucoma medications, n (%)	
beta-blockers	14 (100)
prostaglandin analogues	14 (100)
CAI	14 (100)
Systemic medications, N (%)	
HBP	12 (100)
diabetes	12 (100)

SD – standard deviation; n - number of eyes; N – number of patients; IOP – intraocular pressure; VA – visual acuity; CAI – carbonic anhydrase inhibitors; HBP – high blood pressure

Two weeks post-injection, all cases showed complete regression of the iris an angle new vessels. Recurrence of iris neovascularization was observed in 2 (14.2%) eyes at month 6 where reinjection was done; a second recurrence of neovascularization was not observed.

The mean (± SD) preoperative IOP was 42.4 (± 9.7) mmHg. TMC significantly reduced IOP at all time point measurements ($p < 0.0001$) (Figure 1).

After one year of follow-up, 11 (78.6%) eyes had IOP ≤ 21 mmHg and were considered as success.

The mean (± SD) number of antiglaucoma medications at baseline was significantly higher, 3.0 (± 0.0) than that reported at the month 12 after the treatment, 1.1 (± 1.5) ($p = 0.0003$).

Visual acuity did not show any significant change between pre-trab measurements [0.35 (± 0.3)] and after one year of follow-up [0.40 (± 0.3)] ($p = 0.150$).

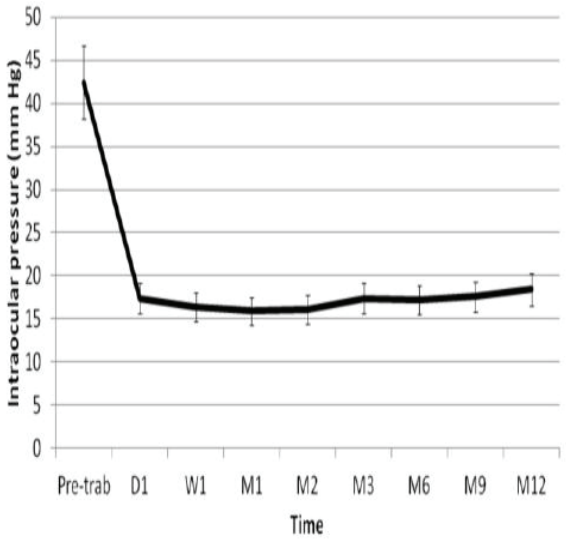

Fig. 1 – Evolution of intraocular pressure (IOP) over the lenght of follow-up. The mean IOP, at all point measured, was significantly lower than the pre-trabeculectomy IOP ($p < 0.0001$).
Pre-trab – Pre-trabeculectomy; D – day; W – week; M – month.

As regards complications, hyphaema was seen in 3 (21.43%) eyes at the early postoperative period; at month 6, 1 (7.1%) patient had macular edema and 2 (14.2%) eyes showed recurrence of neovascularization requiring IVB injection.

Discussion

The results of our study suggested that intravitreal injections of bevacizumab, administered preoperatively, as adjunctive therapy for trabeculectomy with MMC in neovascular glaucoma patients is effective not only for reducing IOP but also for preventing bleeding.

Additionally, our study also found a complete regression of the iris neovascularization in all the eyes 2 weeks after IVB injection. These results are in agreement with Iliev et al. [3] and Marey and Ellakwa [9] who reported complete regression of neovascularization at the end of follow-up period in 100% of cases. Conversely, Oshima et al. [7] observed complete regression in only 29% of their cases.

The rate of reinjection was lower than that reported by Oshima et al. [7] (29% at the month 2 after the tratment) and Marey and Ellakwa [9] (20% at the month 4 and 70% at the month 8 after the treatment).

The success rate of trabeculectomy with MMC in NVG is quite low [10]. In our study, 11 (78.6%) eyes had IOP ≤ 21 mmHg after 12 months of follow-up. These results are in line with those reported by Marey and Ellakwa [9] who reported a success rate of 77.8%. However, it is slightly lower than that observed by Elmekawey and Khafagy [11], who found a success rate (IOP between 10 and 21 mm Hg with or without medication) of 90%, but using ranibizumab as adjunctive therapy for the TMC.

Regarding visual acuity, our study did not find any significant improvement after the treatment. This is in contradiction with Marey and Ellakwa [9] who observed a significant improvement of the visual acuity ($p = 0.0001$).

This study has some limitations that should be mentioned: an open-label design, a single centre study, a limited number of patients. Nevertheless, the fact that statistical analyses were conducted in a masked fashion could reduce the potential for bias.

Conclusion

Intravitreal bevacizumab, administered preoperatively as adjunctive therapy for trabeculectomy with mitomycin-C in neovascular glaucoma patients, successfully produced regression of iris neovascularization and also increased the success rate of the trabeculectomy with mitomycin-C. Further studies are needed to elucidate the long term effect of trabeculectomy with adjunctive bevacizumab injections in neovascular glaucoma patients.

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The effects of fish-based and milk-based diets on liver tissue antioxidant enzymes and lipid peroxidation in female Wistar rats: A pilot study

Efekti hrane obogaćene ribljim brašnom i mlekom u prahu na antioksidativne enzime i lipidnu peroksidaciju u jetri ženki pacova Wistar soja: pilot studija

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Abstract

Background/Aim. Recently, there has been an increased interest in novel dietary antioxidants, including omega-3 fatty acids and bioactive proteins present in milk. The aim of this study was to examine potential antioxidant effects of four-weeks long fish-based and milk-based diets in female Wistar rats. **Methods.** Four-months old rats were divided into three groups receiving either: control diet, diet enriched with fish meal, or diet enriched with milk. The activities of antioxidant enzymes: glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), and concentration of thiobarbituric acid reactive substances (TBARS) were determined in liver homogenates obtained at the end of the treatment period. **Results.** Statistically significant higher activities of GPx (3.52 ± 0.73 U/mg) and CAT (147.25 ± 15.93 U/mg) were detected in rats fed with fish-based meal in comparison with both the control (GPx: 1.93 ± 0.11 U/mg; CAT: 99.37 ± 10.03 U/mg) and the group fed with milk-based diet (GPx: 1.72 ± 0.52 U/mg; CAT: 104.18 ± 37.49 U/mg). Despite somewhat lower concentration of TBARS in the milk-treated group (0.88 ± 0.23 nmoL/mg), no significant differences were detected in comparison with other groups (the control group: 1.00 ± 0.08 nmoL/mg; the fish-based diet group: 1.13 ± 0.15 nmoL/mg). **Conclusion.** Diet enriched with fish could improve one's oxidative status by enhancing activities of antioxidant enzymes in the liver tissue. On the contrary, we failed to obtain results suggesting that milk could serve as a source of dietary antioxidants.

Key words:
antioxidants; catalase; enzymes; fatty acids, omega-3; fishes; food; lipid peroxidation; milk; rats; superoxide dismutase.

Apstrakt

Uvod/Cilj. U poslednje vreme povećano je interesovanje za istraživanja novih antioksidanasa u ishrani**,** uključujući omega-3 masne kiseline i bioaktivne proteine prisutne u mleku. Cilj ove studije bio je ispitivanje potencijalnih antioksidativnih efekata hrane obogaćene ribljim brašnom i hrane obogaćene mlekom u prahu kod ženki Wistar pacova, u trajanju od četiri nedelje. **Metode.** Pacovi, starosti četiri meseca, podeljeni su u tri grupe koje su bile hranjene standardnom hranom (kontrolna grupa), hranom obogaćenom ribljim brašnom i hranom obogaćenom mlekom u prahu. U homogenatima jetre, posle četiri nedelje, određene su aktivnosti antioksidativnih enzima: glutation peroksidaze (GPx), superoksid dismutaze (SOD) i katalaze (CAT), kao i koncentracija reaktivnih supstanci tiobarbiturne kiseline (TBARS). **Rezultati.** Statistički značajno veće aktivnosti GPx (3,52 ± 0,73 U/mg) i CAT (147,25 ± 15,93 U/mg) nađene su kod pacova koji su dobijali hranu obogaćenu ribljim brašnom u odnosu na kontrolu (GPx: 1,93 ± 0,11 U/mg; CAT: 99,37 ± 10,03 U/mg) i grupu koja je hranjena hranom obogaćenom mlekom u prahu (GPx: 1,72 ± 0,52 U/mg; CAT: 104,18 ± 37,49 U/mg). Uprkos nešto nižoj koncentraciji TBARS u grupi koja je primala hranu obogaćenu mlekom u prahu (0,88 ± 0,23 nmoL/mg), nisu utvrđene statistički značajne razlike u poređenju sa drugim grupama (kontrola: 1,00 ± 0,08 nmoL/mg; grupa na ishrani obogaćenoj ribljim brašnom: 1,13 ± 0,15 nmoL/mg). **Zaključak.** Ishrana bogata ribom mogla bi delovati povoljno na oksidativni status preko poboljšanja aktivnosti antioksidativnih enzima jetre. Sa druge strane, rezultati ne pokazuju da bi mleko moglo biti dobar izvor dijetarnih antioksidanasa.

Ključne reči:
antioksidansi; katalaza; enzimi; masne kiseline, omega-3; ribe; hrana; lipidi, peroksidacija; mleko; pacovi; peroksid dismutaza.

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Introduction

Use of dietary antioxidants is highly recommended in order to maintain overall health and prevent metabolic disorders, cardiovascular diseases, cancer and other pathological conditions. Beneficial effects of plant foods rich in antioxidant vitamins and phytochemicals are well established and confirmed in numerous epidemiological and human and/or animal studies [1, 2]. On the contrary, more investigations are needed on novel dietary antioxidants such as omega-3 fatty acids (FA), bioactive proteins and peptides. High intake of fish and fish oil has been associated with lower incidence of cardiovascular, neurological diseases and cancer, well known oxidative stress-related states [3, 4]. However, data on antioxidant effects of omega-3 FA are still contradictory and need to be further investigated. Some reports suggest that intake of these highly unsaturated FA contributes to the increase in lipid peroxidation and oxidative damage [5, 6]. Other studies indicate potential role of omega-3 FA in stimulation of endogenous antioxidant defence and homeostasis of free radicals. Antioxidant effects of fish oil have been reported in animal models of asthma, diabetes, and nephrotoxicity and in normal rats as well [5–8]. Still, firm conclusions can not be drawn, since so far studies have varied in design and duration ranging from 15 days to 13 weeks [7–10]. The most recent study, testing the combined effects of fish oil and α-lipoic acid on liver fatty acid oxidation and parameters of oxidative stress, lasted for 21 days [11].

Among other novel functional foods, dairy products and milk-based supplements are indicated as potential antioxidants with beneficial impact on health and healthy ageing [12, 13]. Milk and dairy products have a unique protein and FA composition that could beneficially affect one's oxidative status. Potential antioxidant activity of milk proteins is related to their ability to scavenge radicals, chelate metals, and enhance the endogenous antioxidant defence. In addition, studies have revealed potential impact of milk proteins on oxidative stress-related states, such as insulin resistance and cardiomyopathy [14, 15]. In comparison with the fish and omega-3 FA, data on interventions with milk are far scarcer. Available intervention studies on animals are quite different in the tested organ models as well as in the study design and duration ranging from 14 days to eight weeks [14–17]. One of the latest studies on the influence of milk-based diets on, among others, liver enzymatic antioxidant defence, reported an experimental period of 30 days [17].

The aim of our study was to investigate potential antioxidant effects of fish-based and milk-based diets in female Wistar rats. Since lipid peroxidation is commonly used indicator of oxidative status, we measured concentration of thiobarbituric acid (TBA) reactive substances (TBARS). In addition, we determined the activities of antioxidant enzymes: glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT). The mentioned parameters were measured in rats' livers at the end of the 4-weeks long treatment period. This treatment period was chosen based on the available research and it was considered to be long enough for obtaining significant changes in measured parameters.

Methods

Animals and treatments

The experiments were carried out on four-months old female Wistar rats, housed under controlled conditions (room temperature 23°C–25°C, 12 h light-dark cycle, food and drinking liquid *ad libitum*) and provided by the vivarium of the Institute for Biological Research (University of Belgrade, Serbia). The experimental protocols, as well as the maintenance of animals were in accordance with the Official Institutional Guide for Experimental Work on Animals, adjusted to the European Communities Council Directive (86/609) and the Guide for Care and Use of Laboratory Animals, NIH publication No. 85–23. The approval was obtained from the Ethics Committee for the Use of Laboratory Animals of the Institute for Biological Research (University of Belgrade, Serbia), under the decision No 02-56/12.

The animals were randomly assigned into one of the three treatment groups, and were well matched in terms of body weight (250–300 g). The control group (n = 6) was fed with the standard laboratory chow, while the fish-based group (n = 5) had added fishmeal (based on anchovy) and the milk-based group (n = 8) had dried-milk powder added. The formulation of rat chow was made by the Department of Animal Nutrition and Botany, Faculty of Veterinary Medicine, University of Belgrade, Serbia. A detailed analytical characterisation of food (rat chow), its chemical composition, respectively is given in previously published article [18].

Preparation of tissue homogenate

At the end of the treatment period, the rats were sacrificed by decapitation, livers were dissected and immediately frozen on dry ice. Further on, the livers were stored at -80ºC until the following procedures were applied: livers were weighed, and homogenates were prepared by homogenization of 1 g of the tissue in 10 mL of buffer containing: 50 mM Tris pH 7.4, 150 mM sodium chloride (NaCl), 2 mM ethylenediaminetetraacetic acid (EDTA) and 1% Igepal® (Sigma Aldrich®, Darmstadt, Germany). Homogenization was performed using a homogenizer Ultra Turex followed by the centrifugation of the tissue at 37 °C for 90 min 500 rpm at 4ºC. The supernatants were further used for the analyses of the antioxidant enzymes' activities and TBARS concentration. All the results were expressed per mg of proteins.

Determination of TBARS level

The concentration of TBARS, as by-product of lipid peroxidation, was measured with the use of TBARS Assay Kit (Cayman chemical, Ann Arbor, Michigan, USA) based on the reaction with TBA in acidic pH at 90–100°C [19]. After boiling the mixtures for 1 h, they were cooled, centrifuged and the absorbance of resulting pink products measured by plate reader at 540 nm. Malondialdehyde (MDA), 500 μM, was used to prepare standard curve for the calculation of TBARS levels (MDA equivalents) in liver samples.

Determination of SOD

The SOD activity was determined by Ransod kit (Randox, Crumlin, UK) based on the superoxide radical anion production in a xanthine–xanthine oxidase system and its further reaction with 2-(4-iodophenyl)-3-(4-nitrophenol)-phenyltetrazolium chloride, resulting in the formation of red formazan dye. Samples were diluted 150- to 200-fold and the decrease in the absorbance was measured at 505 nm and 37°C for 3 min. The units of SOD activity were calculated from the absorbance changes per minute with the use of the standard curve made with purified enzyme. One unit of SOD was defined as the amount of enzyme resulting in the 50% inhibition of red formazan dye formation.

Determination of GPx

The GPx activity was measured by the Ransel kit (Randox, Crumlin, UK) based on the oxidation of reduced glutathione in the presence of cumene hydroperoxide. The formed glutathione-disulphide was further reduced by glutathione reductase in the presence of NADPH coenzyme. The samples were 100-fold diluted and the activity was determined by monitoring the decrease in absorbance due to the disappearance of the NADPH at 340 nm and 37 °C. One unit of GPx was defined as amount of NADPH (expressed in nmol) oxidized per min, and calculated based on the NADPH molar absorption coefficient.

Determination of CAT

The CAT activity was determined by the slightly modified method described by Aebi [20], based on the degradation of hydrogen peroxide – H_2O_2 (Sigma Aldrich®, Darmstadt, Germany), reaction that can be measured directly by the decrease in absorbance at 240 nm. Liver homogenates were diluted 100- to 150-fold, added to 1 M phosphate buffer (pH 7.0) and reaction was initiated by adding 10 mM H_2O_2 and the decrease in absorbance recorded for 3 min at 25ºC. One unit of CAT activity was defined as the amount of enzyme decomposing 1 μmoL of H_2O_2 per min.

Determination of protein content

Protein content in tissue homogenates was determined by the use of bicinchoninic acid (BCA) Protein Assay Macro Kit (SERVA Electrophoresis, Heidelberg, Germany), according to the given instructions. The method combined Biuret reaction, and colorimetric detection of the cuprous cation (Cu^{1+}) with a BCA containing reagent. The absorbance of the purple-coloured product, formed by chelating two molecules of BCA with one Cu^{1+} ion, was measured at 562 nm. Bovine serum albumin was used to prepare a standard curve, and the liver homogenates were diluted 50-fold prior the analyses.

Statistical analysis

All variables were tested for normality using the Shapiro-Wilk's test. For normally distributed variables, comparisons between the groups were performed by one-way analysis of variance (ANOVA) with Tukey *post hoc* test. Data are shown as mean values ± standard deviation. Due to the absence of normal distribution, SOD values were compared by Kruskal-Wallis nonparametric test and data presented as medians with interquartile ranges (25th and 75th percentiles). Correlations between measured parameters were evaluated by Pearson's coefficients of correlation. Analyses were performed with SPSS software (Chicago, IL, USA).

Results

Effects on TBARS

Despite the somewhat lower concentration of TBARS in the milk-treated group (0.88 ± 0.23 nmoL/mg), no significant differences were detected in comparison with the other groups (the control group: 1.00 ± 0.08 nmoL/mg; the fish-based diet group: 1.13 ± 0.15 nmoL/mg) (Figure 1).

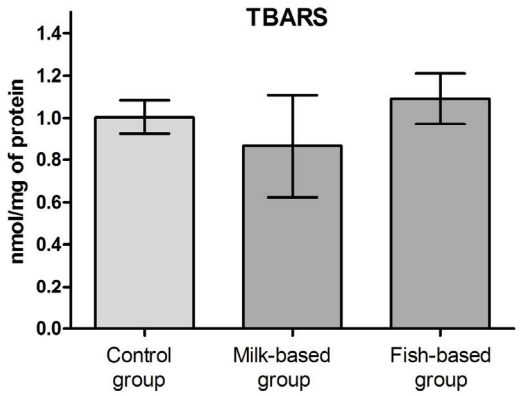

Fig. 1 – Levels of lipid peroxidation – thiobarbituric acid reactive substances (TBARS) at the end of four-weeks long consumption period.
Data are shown as mean values ± standard deviation; Comparisons were performed by one-way analysis of variance (ANOVA) with Tukey *post hoc* test.

Effects on antioxidant enzymes

Statistically significantly higher activities of GPx (3.52 ± 0.73 U/mg) and CAT (147.25 ± 15.93 U/mg) were detected in rats fed with fish-based meal, in comparison with both the control (GPx: 1.93 ± 0.11 U/mg, $p < 0.001$; CAT: 99.37 ± 10.03 U/mg, $p < 0.05$) and the milk-fed rats (GPx: 1.72 ± 0.52 U/mg, $p < 0.001$; CAT: 104.18 ± 37.49 U/mg, $p < 0.05$), as presented in Figure 2. There were no significant differences in the activities of SOD among the groups.

Correlation between measured parameters

In the group fed with the milk-based diet, TBARS levels positively correlated with the activities of the measured enzymes (Table 1), although the correlation was not significant for SOD. Furthermore, a significant correlation was found between GPx

and CAT activities ($p < 0.05$, r = 0.795), while SOD positively correlated with both GPx and CAT, but not significantly. In the control group, we observed significant correlation ($p < 0.05$) between the GPx and CAT activity as well.

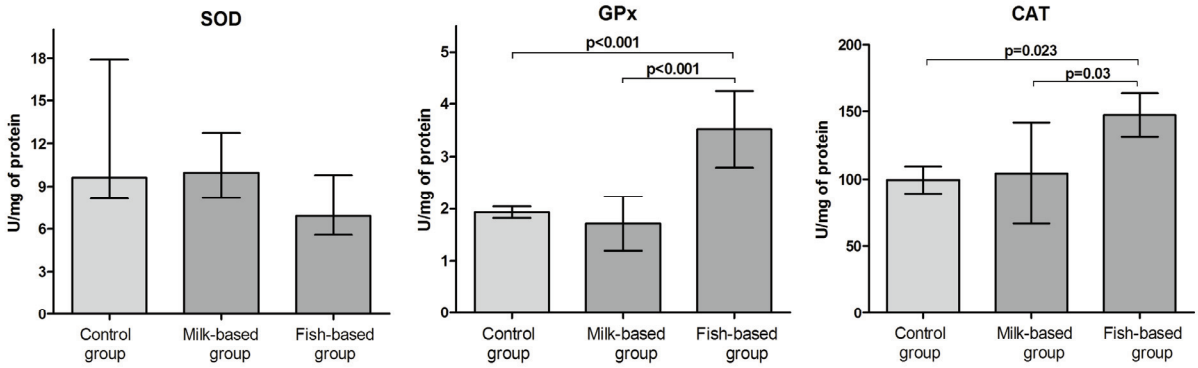

Fig. 2 – Activities of antioxidant enzymes at the end of four-weeks long consumption period.
SOD- superoxide dismutase, GPx- glutathione peroxidase, CAT- catalase; For GPx and CAT, data are shown as mean values ± standard deviation and comparisons were performed by one-way analysis of variance (ANOVA) with Tukey post hoc test; SOD values were compared by Kruskal-Wallis nonparametric test and data are presented as medians with interquartile ranges (25th and 75th percentiles).

Table 1
Correlations between activities of antioxidant enzymes and level of lipid peroxidation

Parameters	r	*p*
Milk-based group		
TBARS/GPx	0.811	0.014
TBARS/CAT	0.980	< 0.001
TBARS/SOD	0.681	0.062
GPx/CAT	0.795	0.018
GPx/SOD	0.668	0.070
CAT/SOD	0.670	0.069
Control group		
GPx/CAT	0.816	0.047

r – Pearson's coefficient of correlation;
TBARS – thiobarbituric acid reactive substances, index of lipid peroxidation; GPx – glutathione peroxidase;
CAT – catalase; SOD – superoxide dismutase.

Discussion

In the present study, we found significantly higher activities of GPx and CAT in female Wistar rats treated with fish-based meal for four weeks in comparison with those in both the control and milk-fed animals. In comparison with the control group, the milk-based diet showed no effects, but we observed some significant correlations in this group.

Our results contribute to previous suggestions that omega-3 FA could improve enzymatic antioxidant defense [21]. A study that investigated effects of 30 days long fish oil supplementation in male Wistar rats revealed significantly higher erythrocytes' CAT activity in treated animals [7]. Similarly, supplementation with fish oil enhanced activities of both GPx and SOD in asthmatic rats [10] and raised CAT activity in animal model of nephrotoxicity [9]. Furthermore, treatment with omega-3 FA caused an increase in glutathione concentration and GPx activity in streptozotocin-induced diabetic rats [8]. Although the use of fish and fish-based meals as dietary antioxidants is yet to be established, their ability to scavenge radicals, down-regulate activity of NADPH activity [22], and increase microRNA expression of antioxidant enzymes [23], have been reported. Besides evaluating activities of antioxidant enzymes, we measured concentration of TBARS, as by-product of lipid peroxidation [24] and found no significant impact of tested diets. Similarly to our results, dietary supplementation with fish oil in duration of 13 weeks caused no significant changes in liver lipid peroxidation in female rats, comparing with the supplementation with soybean and linseed oil. Still, research to date is quite ambiguous particularly on the impact of fish-based meals on lipid peroxidation. Since double bonds are the main targets of free radical attack, long-chain polyunsaturated FA, with numerous double bonds, represent particularly prone FA to the lipid peroxidation. Thus, it could be expected that higher intake of fish and fish based meals could lead to the increase in the lipid peroxidation, as confirmed previously [5, 6]. On the contrary, reducing effect of omega-3 FA and fish oil on increased TBARS concentration was reported in diabetic rats, rats with uranyl nitrate-induced nephrotoxicity [8, 9], as well as in healthy rats [7]. In our previous work, we have investigated the impact of fish and milk-based diets on liver phospholipids' FA composition in rats of both genders. We observed sex-specific changes in FA profile. In case of fish-based diet, there was more pronounced increase in the concentration of omega-3 FA (both total and individual FA) in female comparing with the male rats [18]. Considering this and the fact that polyunsaturated FA could be used as indirect measure of lipid peroxidation (as the most prone FA to lipid peroxida-

tion), we assumed that the changes in lipid peroxidation and other parameters of oxidative stress would be more pronounced in female rats [25]. This is why we have included female rats in here presented research.

Although we observed to some extent lower concentration of TBARS in rats fed with milk-based diet in comparison with the control group and the group fed with fish-based diet, these differences were not significant. In addition, we found no significant impact of milk-based diet on activities of antioxidant enzymes in rats' liver. Still, we recorded significant positive correlations between TBARS concentration and both GPX and CAT activities. In other words, lower lipid peroxidation was followed by lower activities of antioxidant enzymes. Indeed, we found significantly lower activities of GPx and CAT in the group fed with milk-based diet in comparison with the fish-based diet. Comparing the levels of TBARS in these two groups, lipid peroxidation was to some extent higher in the group fed with fish-based diet. The higher lipid peroxidation could serve as a trigger for the upregulation of antioxidant defence in the body. The lack of effect on antioxidant enzymes in the group fed with milk-based diet could be derived from the lower concentration of TBARS in this group. In accordance with previous findings, significant correlations were found between the activities of GPx and CAT in both the milk-fed and the control group.

Besides being highly nutritious and tasty, milk and dairy products could serve as food supplements with promising health effects. This is because these products are rich in FA, proteins, amino acids, such as cysteine and glutamic acid, and vitamins, such as A and E [13]. Novel findings suggest that milk proteins and their break down products could serve as potent dietary antioxidants, based on their radical scavenging and metal chelating activity [26, 27]. Antioxidant activity of milk and milk products still needs to be evaluated *in vivo* in both animals and humans. One of the few animal studies investigated the impact of 2-week feedings with breads enriched with milk powder. The authors revealed higher CAT activity in groups fed with breads containing, among other compounds, milk powder in comparison with the group fed with plainbread [16]. In addition, whey protein-enriched diet significantly increased total antioxidant capacity, SOD activity and glutathione concentration, while it decreased lipid peroxidation in insulin resistant rats [14]. Whey protein also ameliorated effects of iron load on oxidative damage in male rats, demonstrated as an increase in total antioxidant defence and a decrease in lipid peroxidation [15].

In spite of the promising results, our study has some limitations. Firstly, the duration of the treatment (four weeks) was relatively short. A longer supplementation and possibly an assessment of other parameters, in other tissues as well, would provide broader conclusions and would help identifying mechanisms underlying observed antioxidant activity of fish-based diet. Secondly, final conclusions are limited due to the low number of animals especially in the group fed with fish-based meals. Still, we believe that this study provides valuable and novel findings, bearing in mind scarce and ambiguous literature data, especially on milk as potential functional food.

Conclusion

Based on the results of the current study and on the research available to date, we conclude that the intake of fish and fish-based meals could improve one's oxidative status by enhancing activities of antioxidant enzymes. On the contrary, we obtained no effects in favour of statements that milk could serve as a good source of novel dietary antioxidants. Overall, possible applications and physiological relevance of our findings should be further tested in long-term animal and human intervention studies.

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Author disclosure statement

The authors declare that they do not have any actual or potential financial interests or any conflict of interests in the findings from this manuscript.

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CASE REPORTS

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Pediatric mandibular fracture therapy – A case report

Lečenje preloma donje vilice kod dece

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Abstract

Introduction. Frequency of pediatric mandibular fractures is relatively uncommon. Apart from rare exceptions, there is minimal invasive access in the treatment of those injuries in order to avoid the future developmental disorders. **Case report**. During the game with a colt, a 6-year-old boy was kicked by hoof in the chin. The child did not lose consciousness and did not experience nausea or vomiting. According to clinical examination and radiological analysis, diagnosis was assigned as dislocated mandibular fracture in the parasymphysis part of the jaw and luxation injury of teeth 31 and 72. The surgical treatment under general anesthesia encompassed reduction and bimanual manipulation of bone fragments up to the optimal restoration of the dental occlusion, along with osteosynthesis with titanium miniplates. Luxated deciduous tooth 72 at the fracture line was extracted and luxated permanent tooth 31 was fixed to tooth 41 with wire. The patient was given antibiotic therapy. Additional immobilization of the luxated tooth 31 and mandibular fracture was performed after surgery by composite resin splint. During five-month follow-up period there were no signs of pathological movements in the fracture line, no luxation of tooth 31 and no restriction in mouth opening. **Conclusion.** Osteosynthesis with miniplates is adequate and very efficient treatment method in dislocated mandibular fracture that is recommended in children with both deciduous and mixed dentition. It is necessary to remove miniplates after fracture consolidation.

Key words:
child, preschool; fracture fixation, internal; mandible; mandibular fractures; oral surgical procedures; treatment outcome.

Apstrakt

Uvod. Rasprostranjenost preloma donje vilice je relativno mala kod dece. Da bi se izbegli naknadni poremećaji u razvoju vilice, sem retkih izuzetaka, u lečenju se primenjuje minimalno invazivni pristup. **Prikaz bolesnika.** U toku igre sa ždrebetom, šestogodišnji dečak je dobio udarac kopitom u bradu. Nije gubio svest, niti je imao gađenje i povraćanje. Na osnovu kliničkog pregleda i rendgen analize postavljena je dijagnoza dislokovanog preloma donje vilice u parasimfiznoj regiji i luksacija zuba 31 i 72. U opštoj anesteziji je izvršena hirurška repozicija, uz bimanuelnu manipulaciju koštanih fragmenata do uspostavljanja optimalne dentalne okluzije, a zatim je izvršena osteosinteza donje vilice sa titanijumskim mini pločicama. Luksirani mlečni donji levi lateralni sekutić (72), koji se nalazio u liniji preloma, je ekstrahovan, a luksirani donji levi centralni sekutić (31) fiksiran čeličnom žicom za donji desni centralni sekutić (41). Ordinirana je antibiotska terapija. Dodatna imobilizacija luksiranog zuba (31) i frakture donje vilice izvršena je postoperativno, pomoću kompozitnog splinta. Posle petomesečnog opservacionog perioda nije bilo znakova patološkog pomeranja u predelu frakturne linije, luksacije zuba 31, kao ni ograničenog otvaranja usta. **Zaključak.** Osteosinteza mini pločicama pokazala se kao adekvatna i vrlo efikasna metoda lečenja i preporučuje se u slučajevima dislokovanog preloma donje vilice kod dece sa mlečnom i mešovitom denticijom. Neophodno je ukloniti mini pločice posle konsolidacije preloma.

Ključne reči:
deca, predškolska; prelomi, fiksacija, unutrašnja; mandibula; mandibula, prelomi; hirugija, maksilofacijalna, procedure; lečenje, ishod.

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Introduction

The mandible fractures frequency is low in children, and occurs in 5% of all maxillofacial traumas [1]. The most of pediatric mandible fractures are not dislocated because of the bone elasticity and existing tooth buds that holds firmly the fragments together "like glue" [1]. Frequency of the mandible injury is more commonly present in boys than girls by a ratio of 2 : 1 [2]. Treatment of pediatric mandible fractures is different in relation to that in adults, concerning the age of a child, the level of tooth development along with the teeth that start to grow-ups, or others still unerupted [3]. Fracture treatment is basically more difficult concerning deciduous teeth as their roots size is not enough strong to support fixation of mandible fragments with maxillary-mandibular fixation (MMF) [4]. Younger patients also have better potential for restitution and remodeling comparing to the sclerotic type of remodeling seen in adults [2]. The principal conditions for successful bone healing are: early specific treatment, morphological reduction of bone fragments, immobilization and prevention of the infection. In the case of displaced bone fragments the use of closed reduction and immobilization are carried out a priori to avoid future functional disorders [2, 3]. Most fractures in children without dislocation of the fragments have been treated conservatively by dental splints, occlusal splint with circum-mandible wires, or absorbable plates and screws, all of them being well eligible and quite effective [1].

In this paper we described a case of rare pediatric parasymphysis mandible fracture with large dislocation of fragments that was successfully treated by a rigid internal fixation with titanium miniplate system.

Case report

During the game with a colt, a 6-year-old male child was hit by hoof in the chin. After the injury, the patient came to the General Hospital where he got the first aid, and his soft-tissue wounds were thoroughly debrided. There was no history of bleeding from nose, ears or the head injury. The father reported no syncope, vomiting or drowsiness in the child. The patient was sent to the Dentistry Clinic of Vojvodina, Novi Sad, Serbia, where the further injury management was organized after pediatric dentist examination by joint work of surgeon, pediatric dentist and orthodontist. Extraoral examination revealed an one inch lacerated wound on the chin with gaping borders but homeostasis had already been achieved. The child had swelling and bruising in the submental region and mouth floor. There were also facial asymmetry, restricted mouth opening, deviation of the mandible to the affected side, incorrect speech and pain in the left part of the chin during the examination. An intraoral examination, revealed laceration presented in lower labial vestibule, luxation injury of the central mandible incisors (31, 41) and lateral deciduous incisor (72). Fractured segments mobility, step defect and tenderness were observed in the left parasymphysis region. Radiological examination showed left parasymphysis fracture between left mandible, central displaced permanent incisior (31) and lateral deciduous incisior (72), with fracture line runs downward and backward (Figure 1). There was a large (7 mm) dislocation of fragments which it is not very common type of mandibular fracture at this young age. Usually, this injury is associated with unilateral or bilateral condylar fracture but not in this case.

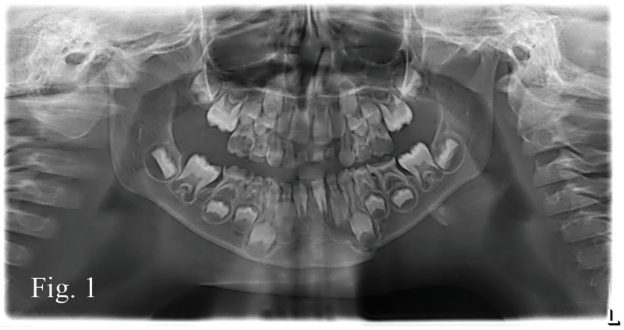

Fig. 1 – Preoperative orthopantomograph view of left parasymphyseal fracture.

Management

Informed written consent was obtained prior to the treatment beginning. Surgery was performed under general anesthesia. We used a rigid internal fixation to reduce the mandible parasymphysis fracture with two 2.0 mm thickness 8-hole with gap titanium mini plates with 4 screws (1.7 mm x 5.0 mm) and 3 screws (1.2 mm x 3.0 mm). The chosen treatment in this case was an open reduction of fracture through oral lower sublabial incision. During intraoperative treatment, manual reduction of mandible fragments was performed to obtain proper dental occlusion until miniplate was placed in, to fix the fracture. As luxated lower left deciduous lateral incisor (72) was situated at the fracture line, it was extracted, and the teeth 31, 41 were fixed with wire (Figure 2). Patient's soft tissue wounds were debrided and sutured. Postoperative ortopantomograph showed reduction and left parasymphysis fracture fixed with titanium miniplate with restoration of occlusion (Figure 3). Composite resin splint (Hager&Werken) was placed after surgery (Figure 4), from the left deciduous molar to the right one (75-85) for stabilizing luxated teeth (31, 41). Postoperative antibiotic treatment was prescribed for two weeks period with antitetanus protection checked. There was recommendation for soft diet, antibacterial mouth rinse use, physical inactivity and postoperative control on a weekly basis. There were no complications observed during the healing period in 5-month follow-up with quite effective restoring of complete chewing function (Figure 5).

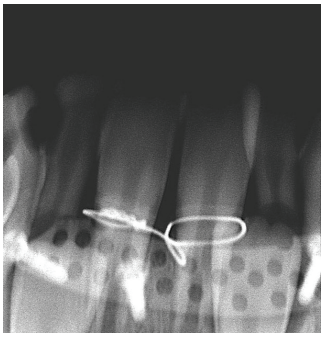

Fig. 2 – Postoperative retroalveolar radiograph view of fixed teeth (31, 41) with wire.

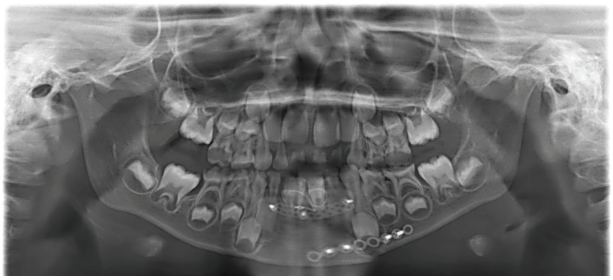

Fig. 3 – The reduction and rigid internal fixation of left parasymphysis fracture with a titanium mini plate.

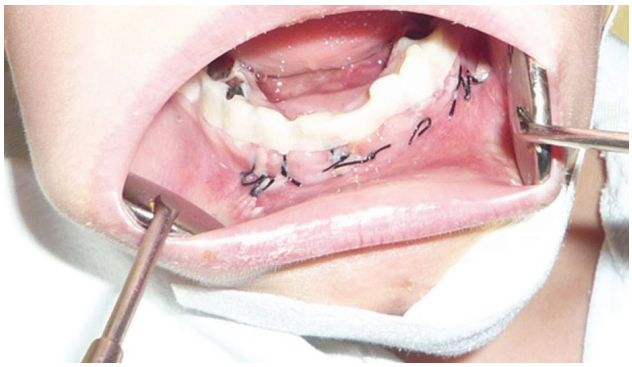

Fig. 4 – Composite resin splint (Hager&Werken) from the left deciduous molar to the right one (75-85).

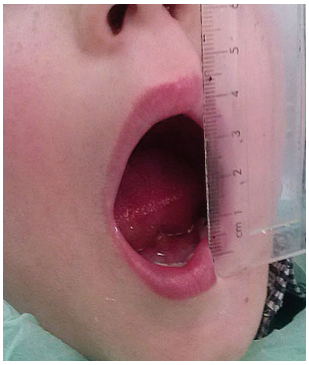

Fig. 5 – Postoperative photograph showing increased mouth opening at the fifth month of follow-up.

Discussion

Very young age, a rare type of injury, the animal as a rare etiological factor for mandible trauma, different and contradictory opinions concerning therapy management, no consent concerning the matter of the appropriate time for plates removal in our case, incertitude therapy outcome especially in small children with deciduous dentition, the lack of cooperation among surgeon, pediatric dentist and orthodontist, as well as no unique methods presented for treatment in such young age, gave us the reason to show this case report. It also can be very helpful, especially to pediatric dentists because they are in the position to give the first aid to the young patient before oral surgeons, and also dentists are the ones who will further follow-up injured children.

Analysis of the literature indicates the lower prevalence of pediatric mandible fractures comparing to adult population. The highest frequency of mandible fractures occurred in younger patients, 6-12 years old (0.6%-1.2%) [5]. Kicks from animals as the etiological factor for mandible fracture was relatively rare, with 3.3% up to 6.0% of all maxillofacial fractures [2]. Various management protocols of mandible fracture treatment are discussed in the literature [1]. Nevertheless, some techniques may be better than others, but no one technique can be used in all situations. The treatment of fractured pediatric mandible differs from that of adults, because of anatomic variation, rapidity of healing, degree of cooperation and the potential for interference with the mandible growth [6]. Children have great ability for healing with few possible complications, aided by well blood supplied tissues with greater osteogenic potential than adults. Anatomic reduction in children should be accomplished earlier and immobilization time should be shorter ie. 2-3 weeks as compared to 4-6 weeks in adults [7, 8]. Although there is no clear consensus about optimal method for fixation of mandible parasymphysis fractures, the most effective and the less invasive method is the best one. Using conservative therapy in the majority of cases of “greenstick” or minimally displaced fractures in children with a short period of MMF is satisfiying. Additionally, there are many treatments of those fractures, and some of them are: acrylic splints, circumferential wiring, the skeletal fixation through the skin, compressive and noncompressive plates, isolated screws, absorbable plates and screws, open reduction, modified orthodontic brackets, etc. [9]. The applied treatment of displaced fractures mostly varied from MMF to cap splints and either regular or absorbable miniplates insert. A lot of serious mandible fractures demands the wide range of therapeutic approaches from open to close reduction and rigid or non rigid fixation along with or without MMF. In our case, the reason for the pediatric mandible trauma was an accident at home. For treatment of these injuries, Davison et al. [10] said that the risks of facial growth disturbance in open reduction and internal fixation (ORIF) has not been supported. In contrast to that opinion, unappropriate treatment in unrecognized mandible fractures leads to high incidence of orthognathic surgery. Technique utilized to repair the fractured mandible parasymphysis, in our case, was the conventional approach of ORIF with titanium miniplates and screws. Intraoral approach through an oral mucosal incision, allow direct control of appropriate occlusion during the incorporation of the titanium miniplates which stabilize the fracture site. The use of miniplates changed the treatment of mandible fractures in the past twenty years, with varying degrees of success [11]. Koshy et al. [12] reported that ORIF is not commonly performed until late mixed dentition, but may be indicated in the early mixed dentition in severely dislocated fractures. In our case the treatment is complicated by the presence of teeth (31, 41) instability and a lot of the teeth that did not grow yet. The potential damage to tooth roots and follicles can be minimized with careful technique, which places monocortical screws in the lower mandible edge. In the majority of cases of minimally displaced fragments of the pediatric mandible fracture, using conservative methods with MMF during a short period is generally satisfying. If surgical treatment is indicated, occlusal acrylic splints, interdental wiring, and monocortical plates and screws are all eventual

option [2]. The use of titanium mini plates systems in relation to absorbable plates permits a stable rigid or semirigid fixation that may eliminate the necessity for MMF [2]. The monocortical bone plates are smaller in size and easily adaptable for application to any type of fractured site. Zimmerman et al. [6] consider that ORIF insures stable three dimensional reconstructions, encourages the primary bone healing, reduces the treatment time and eliminates the need for MMF. On the other hand, closed treatment of the parasymphysis fractures usually demands extended periods of MMF from 3 to 5 weeks. This can become an extremely important factor when it comes to the treatment of pediatric patients, since the level of cooperation is greatly reduced. A patient has to stay on the liquid diet, hospitalized for a longer period of time, with difficulties in regular oral hygiene, and speech is also affected [2]. Application of fabricated acrylic splints is more reliable than ORIF or MMF techniques with regard to the cost/ effectiveness ratio, ease of use and removal, reduced operation time, maximum stability during healing period, minimal trauma for surrounded anatomic structures etc. [1], but it was not suitable in our case. Based on the literature data we can clearly recommend "minimally invasive" internal fixation by means of the monocortical plate and screws, as reported Cole et al. [7]. We believe that choice of ORIF should always be recommended to treat children younger than 6 years of age. With this system of fixing, we obtained the same success reported by the other authors [8]. This method provides better stability of fractured fragments, primary bone healing, the low infection rate and possibility to avoid MMF [11]. However, this system has an important disadvantage, because plate and screws removal is recommended in order to minimize the risk of interference with normal growth of the mandible. There is a great possibility, as Bos et al. [13] reported, that metal implants may cause stress shielding with local osteoporosis after later removal. Certainly, ORIF could have a negative effect on skeletal growth and unerupted teeth because there is a need of plate removal after complete healing [2, 3, 8, 12]. Hogg and Horswell [14] has not seen any growth disturbances caused by miniplate osteosynthesis when they remove it after a period of 6 months. The use of absorbable plates is less likely to disturb facial skeletal growth but is still associated with risk of damaging the teeth that have not yet erupted even when using monocortical plates and screws [2].

Conclusion

ORIF treatment is suggested in large dislocated pediatric mandible fractures and must be carefully done, because of rapid growing up and developmental phenomenon that continues in children. Plates system should be removed as soon as healing period is over. Minimized invasive therapy should always be the choice, especially in children younger than 6 years of age. Joint work of surgeons, pediatric dentists and orthodontists is needed during the whole recovery period.

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CASE REPORT
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Miliary tuberculosis presenting with acute respiratory distress syndrome in a patient with Down syndrome

Milijarna tuberkuloza sa akutnim respiracijskim distres sindromom kod bolesnice sa Daunovim sindromom

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Abstract

Introduction. Miliary tuberculosis (TB) is a rare and potentially fatal form of disseminated TB. It is caused by a widespread haematogenous dissemination of *Mycobacterium tuberculosis* from an active caseous focus to different organs. Sometimes it can have an acute presentation with a rapid-onset clinical deterioration and death. Miliary TB complicated with an acute respiratory distress syndrome (ARDS) requiring mechanical ventilation (MV) is rare, even in countries with a high incidence of TB. **Case report.** A 35-year-old woman with Down syndrome (DS) was admitted to the Clinic for Pulmonology, Clinical Centre Kragujevac, due to an evaluation of cough and weight loss during last 2 months. Laboratory findings revealed anaemia, leukocytosis, elevated C-reactive protein (CRP) and hypoalbuminemia. A chest x-ray showed bilateral reticulonodular shadows, predominantly in the mid and lower right lung lobes. A purified protein derivative (PPD) skin test and induced sputum smear for acid-fast bacilli (AFB) were both negative. On the fifth day following admission, her health condition suddenly declined, and after developing a moderate ARDS, she was put on the mechanical ventilation. Due to a high clinical suspicion of miliary TB and the fact that her life was compromised, an empirical anti-tuberculosis therapy was initiated. Despite all therapeutic and supportive measures, the patient expired 3 days later. The diagnosis of miliary TB was established *post-mortem*. **Conclusion.** Miliary TB should be kept in mind in patients with DS due to immunosuppression associated with deficient cell-mediated immunity. The development of ARDS as a complication of miliary TB is difficult to identify due to a low causal association. High clinical suspicion and a chest radiograph with a typical appearance of miliary pattern justify the initiation of empirical anti-tuberculosis treatment in such patients, as an attempt to change poor prognosis.

Key words:
down syndrome; tuberculosis, miliary; respiratory distress syndrome, adult.

Apstrakt

Uvod. Milijarna tuberkuloza (TB) je retka, potencijalno fatalna forma diseminovane TB. Nastaje masivnom hematogenom diseminacijom *Mycobacterium tuberculosis* iz aktivnog kazeoznog fokusa u različite organe. Ponekad može da ima akutnu prezentaciju sa brzim kliničkim pogoršanjem i smrću. Milijarna TB komplikovana akutnim respiratornim distres sindromom (ARDS) koji zahteva mehaničku ventilaciju (MV) je retka čak i u zemljama sa visokom incidencijom TB. **Prikaz bolesnice.** Trideset petogodišnja žena sa Daunovim sindromom (DS) je bila primljena u Kliniku za pulmologiju zbog ispitivanja kašlja i gubitka telesne mase tokom poslednja dva meseca. Laboratorijski nalazi su ukazali na anemiju, leukocitozu, povećanu vrednost C reaktivnog proteina (CRP) i hipoalbuminemiju. Radiografijom grudnog koša su ustanovljene bilateralne retikulonodularne senke, dominantno u srednjem i donjem plućnom polju, desno. Tuberkulinski kožni test (*Purified protein derivative* – PPD test) i razmaz sputuma na acidorezistentne bacile (ARB) su bili negativni. Petog dana od prijema, stanja bolesnice se naglo pogoršalo, razvio se umereni ARDS i bolesnica je stavljena na mehaničku ventilaciju. Zbog visoke kliničke sumnje na milijarnu TB i životno ugrožavajućeg stanja bolesnice uvedena je empirijska terapija antituberkuloticima. Uprkos preduzetih terapijskih i potpornih mera, nakon tri dana došlo je do letalnog ishoda. Dijagnoza milijarne TB je postavljena obdukcionim nalazom. **Zaključak.** Milijarnu TB treba imati na umu kod bolesnika sa DS zbog imunosupresije povezane sa deficitom ćelijski posredovanog imuniteta. Razvoj ARDS, kao komplikacije milijarne TB, težak je za prepoznavanje zbog njihove slabe uzročne povezanosti. Visok stepen kliničke

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sumnje i tipični milijarni uzorak na radiografiji grudnog koša opravdavaju započinjanje empirijske terapije antituberkuloticina kod ovih bolesnika, u pokušaju da se promeni loša prognoza.

Ključne reči:
daunov sindrom; tuberkuloza, milijarna; respiratorni distres sindrom kod odraslih.

Introduction

Tuberculosis (TB) is an infectious disease caused by the aerobic bacterium *Mycobacterium tuberculosis*. Despite the available effective therapy, TB is still a global emergency because of its high morbidity and mortality rates (10.4 million new cases and 1.3 million died in 2016) [1]. It is estimated that almost 1/3 of the world population has a latent TB infection (LTBI) and of these, 10% are at risk of developing an active form of the disease during their lifetime [2]. The lungs are usually the first site of TB infection, and they are involved in more than 90% of cases. In case of extrapulmonary TB (EPTB) the most commonly affected sites are lymph nodes, pleura, genitourinary and osteoarticular systems. However, any organ can be affected [3].

Miliary TB is a rare form caused by a widespread hematogenous dissemination of *M. tuberculosis* from an active caseous focus (usually lung) to different organs. The definite diagnosis of miliary TB is established by: 1) microbiological (positive acid-fast bacilli smear and/or culture), 2) radiological [evidence of miliary nodules on the chest radiograph or on a high resolution computed tomography (HRCT)], and 3) histopathological findings. A characteristic histopathological finding in miliary TB is caseous granuloma measuring approximately 2 mm in diameter, in two or more noncontiguous organs [4]. However, even today, miliary TB remains a formidable diagnostic and therapeutic challenge. The diagnostic dilemma is even greater if the disease is presented with complications.

Down syndrome (DS) is the most common cause of intellectual disability of mostly mild or moderate range. Only rare, individual cases of individuals with mosaic DS have severe mental retardation, with IQ ranging 10–30 [5]. Improved living conditions and lifestyle have resulted in an increase of life expectancy of, not only, general population, but also of individuals with DS whose life expectancy is 60 years at present [6].

DS is the most common human chromosomopathy (1 : 600–700 live births) caused by the presence of all or part of a third copy of chromosome 21 [7]. Autoimmune diseases such as primary hypothyroidism [8], coeliac disease [9] and diabetes mellitus [10] as well as haematological malignant diseases, acute lymphoblastic leukaemia and myeloproliferative diseases [11] are more frequent in patients with DS than in people with normal karyotype.

Similarly, there is an increased susceptibility of these patients for recurrent infections, mostly lower respiratory tract infections, characterized by increased severity and a prolonged course of the disease as well as the need for extra or augmented treatment compared to the general population [12]. Anatomical and immunological problems associated with DS are thought to be the reason for this [13].

TB has been occasionally reported in patients with DS. Patients with chromosomal abnormalities such as DS may be at a higher risk of unusual forms of TB, mostly extrapulmonary with rare and serious complications [5, 14–16].

Case report

A 35-year-old female with DS was hospitalized in the Clinic for Pulmonology, Clinical Centre Kragujevac due to cough with scanty expectoration for 2 months. She had on and off night sweats and chills. Body temperature was not measured. Over this time period, she lost 5 kilograms. A few days back, due to frequent diarrhea, she reported more pronounced weakness and fatigue.

The patient had no congenital anomalies of the heart and gastrointestinal tract. Apart from moderate hearing impairment, according to data provided from the father, during childhood the patient did not have any serious respiratory infections requiring hospitalization. She was a non-smoker, and does not take any alcohol. The obtained data on maternal death caused by TB, about 1 year ago, were not supported by medical documentation.

At the examination, the patient had temperature 36.8 °C, pale color of the skin and mucous membranes, pulse rate 120/min, blood pressure 120/75 mmHg and a respiratory rate of 20/min with oxygen saturation of 95% on room air. Auscultatory finding on the lungs registered decreased breathing sound over basal part of the right lung with inspiratory crackles. Cardiovascular system examination revealed tachycardia without any murmurs. Physical examination of the abdomen was normal. There was no lymphadenopathy.

Results of laboratory analyses showed positive biohumoral inflammatory syndrome [erythrocyte sedimentation rate 66 mm/first hour, white blood cell (WBC): 19.5 x 10⁹/L with 91.7% of neutrophils, C-reactive protein (CRP): 121.7 mg/L, normochromic normocytic anaemia with hemoglobin (Hgb) of 88 g/L, hypoalbuminemia of 18 g/L] and there were no significant variations in liver function tests, compounds of nitrogen metabolism, and electrolyte levels. Chest radiograph showed bilateral reticulonodular shadows, predominantly in the middle and lower field of the right lung (Figure 1A). HRCT of the lung was not done because of technical malfunction of the appliance. Antibiotic treatment was initiated (cefrtiaxon and azithromycin combination) under the suspicion of bilateral bronchopneumonia with albumin substitution, probiotic and rehydration.

Microbiological analysis of the induced sputum, including anaerobes, and fungi, did not isolate any potential pathogenes. Tuberculin skin test was negative, and 3 sputum specimens were negative for acid-fast bacillus (AFB). Serological analysis for *Mycoplasma pneumoniae* infection, enzyme-linked immunosorbent assay (ELISA) for human immunode-

ficiency virus (HIV) antibodies, anti hepatitis C virus (HCV) antibody and hepatitis B surface antigen (HBsAg) were negative. Immunology parameters did not show any significant disturbances. Coproculture did not isolate any pathogen (bacteria or parasites), including *Clostridium difficile*. The patient was afebrile, hemodynamically stable, active, although diarrhea persisted.

On the fifth day of hospitalization, in the evening, sudden appearance of high fever of 40°C was noted, with a sharp deterioration in the general condition of the patient with progressive dyspnea, tachycardia (138/min) and mental confusion with decrease of oxygen saturation to 70% on room air. Auscultatory finding in the lungs registered mass of inspiratory crackles in the lower and middle areas of both lungs. Arterial blood gas analysis on room air [fraction inspired oxygen (FiO_2) 21%] showed severe hypoxemia, partial arterial oxygen pressure (PaO_2) – 4.8 kPa, [partial pressure of arterial carbon dioxide ($PaCO_2$) – 4.0 kPa; pH-7.46 and bicarbonate-25.3 mmol/L]. On oxygen by face mask, the calculated PaO2/FiO2 ratio was 151 mmHg.

Chest X-ray showed a distinct radiographic progression with confluent nodular infiltrates bilaterally, diffuse reduction of parenchymal transparency, without changes in the size and configuration of the cardiac silhouette (Figure 1B). The patient was transferred to the intensive care unit (ICU), intubated electively and put on mechanical ventilation (MV).

On the basis of clinical, PaO2/FiO2 and radiological findings, working diagnosis of ARDS was set. Due to the high index of suspicion of milliary TB (the fulminant course of the disease, despite antibiotic therapy, medical history data about maternal death of TB and DS as a primary disease) after intubation the patients was started on antituberculosis medications: rifampicin (RIF), isoniazid (INH), pyrazinamide (PYZ) and ethambutol (EMB). Despite therapy and MV, the patient died on the 8th day of admission. Because of unexplained etiology of fulminant course of the disease, clinical autopsy and post mortem lung biopsy were performed.

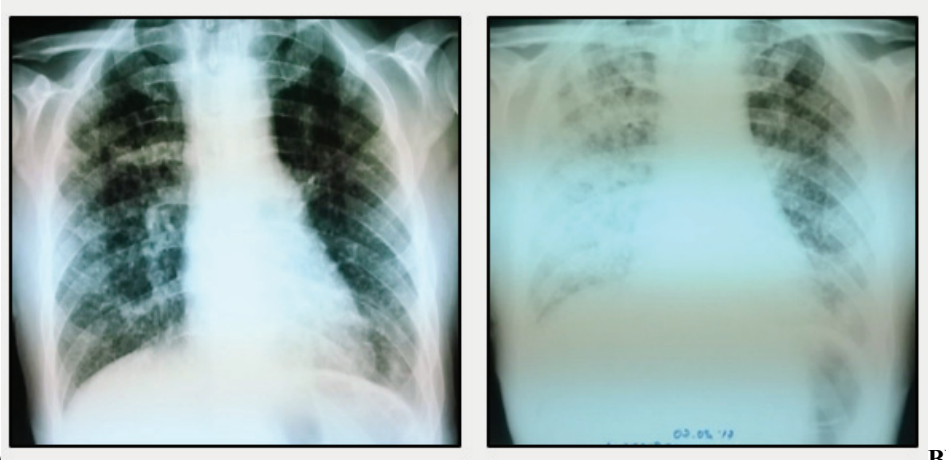
Autopsy showed multiple whitish nodules, individual and plums, present in the lungs (Figure 2A). Miliary seeding of lymph nodes were detected in the small intestine mesentery (Figure 2B). On the cross section of the kidney, there were cystic formation filled with greyish-white, mucoid material (Figure 2C). Histopathological findings of the lung tissue showed numerous granulomas composed of epithelioid multinuclear cells, gigantic cells of Langhans type and lymphocytes, with caseous necrosis within the center (Figure 3). Histopathological findings of the small intestine mesentery and kidney biopsy were identical. This confirmed the diagnosis of miliary TB.

Discussion

Miliary TB is a potentially fatal form of disseminated TB. If not diagnosed and treated early, death is imminent. It can be a manifestation of a primary progressive infection or the result of a latent TB focus reactivation. Large amounts of bacillemia in miliary TB mostly involve organs with high blood supply including lungs, liver, spleen, lymph nodes, meninges, bone marrow and adrenals [17, 18].

In various clinical studies, among immunocompetent adults, miliary TB accounts for less than 2% of all TB cases. It has been reported that immunocompromised states such as advanced age, malnutrition, terminal stage of renal disease, organ transplantation, poorly controlled diabetes, immunosuppressive and cytotoxic therapy (including biologic agents antitumour necrosis factor – anti-TNF), malignant diseases, corticosteroids, smoking, alcoholism, as well as infection such as human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) as the most important among them, are associated with a significantly increased susceptibility to TB [17, 18].

There is a very small number of studies dealing with immunological parameters in adults with DS, especially in patients with both DS and tuberculosis. In view of the fact that the aetiology of immunodeficiency is still not clear, the immune system in DS remains the subject of numerous studies. Possible abnormalities of the immune system include: mild to moderately reduced T and B lymphocyte subpopulation with a marked decrease of naive lymphocytes, impaired mitogen-induced T cell proliferation, defects of neutrophil chemotaxis role and reduced specific antibody responses to immunizations. These abnormalities contribute to an increased susceptibility to infections and inflammatory processes [13].



A) **B)**

Fig. 1 – A) Chest x-ray on admission showing bilateral reticulo-nodulare shadows, predominantly right; B) Chest x-ray at the 6th day of hospitalization showing bilateral infiltrates (an acute respiratory distress syndrome – ARDS).

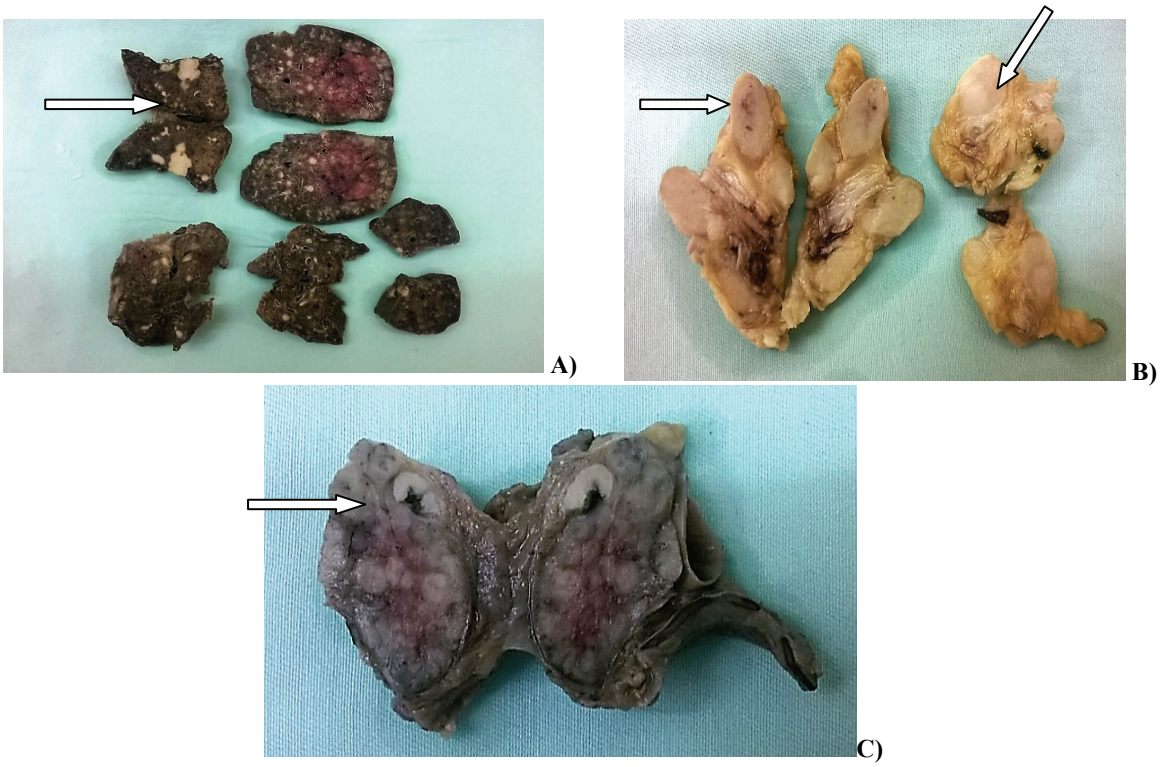


Fig. 2 – A) Miliary seeding of the lungs; B) Miliary seeding of the mesentery and lymph nodes; C) Cut section of the kidney with white cystic lesions and mucoid material.

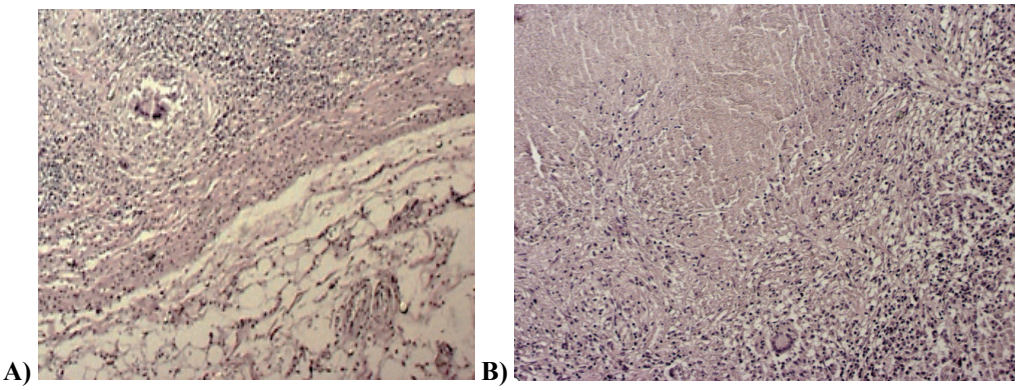


Fig. 3 – A) Histopathological examination of the lung showing features of tuberculosis with granulomas, caseous necrosis, and multinucleated, gigant cells of Langhan's type;
B) Mesenteric lymph nodes showing tuberculous lymphadenitis.

While there is ample evidence of dysfunction or dysregulation of almost every arm of the immune system in DS, the most significant one is the impairment of both the number and activity of T lymphocytes (CD4+ and CD8 suppressor) and natural killer (NK) cells. Thus, DS is associated with T cells dysfunction [19, 20]. This immune dysfunction contributes to recurrent infections and poor microbial clearance [19, 21]. Patients with DS are at a 12-fold increased risk of infectious diseases, especially pneumonia because of their impaired cellular immunity [5]. Although it is justifiable to expect a higher incidence of TB in DS, no data till date has commented about any change in prevalence of tuberculosis in such patients [5]. Therefore this paradoxical finding calls for more research and study.

In the case of our patient, a high risk of future disease was due to a positive family history, namely as a result of the direct contact with her mother who was diagnosed with active TB, resulting in an progressive infection. Approximately 5% of infected individuals get sick within 12–24 months of being infected [2]. As no other members of the family were ill, immunological dysfunction of our patient was a likely factor that contributed to the development of the disease. Although the patient was HIV negative and presented with normal immunoglobulin levels, unknown phagocytic dysfunction in DS could have increased her susceptibility to the disease. Immunocompromised status of the patient, contributed to disseminated TB infection in the lung, intestinal lymph nodes and kidney, which was confirmed by the autopsy report.

Despite a high level of clinical suspicion and a characteristic radiological finding, the diagnosis of miliary TB in our patient was difficult to reach, due to the absence of a microbiological findings and the inability to perform HRCT as a more sensitive method than chest radiography.

An additional problem in reaching the diagnosis of miliary TB in individuals with DS is that in order to get adequate material for bacteriological analyses and depending on the level of their intellectual disability, diagnostic procedures have to be adjusted. In one documented case, an extremely poor general condition of a DS patient made it impossible for a sputum sample to be collected, so *M. tuberculosis* was isolated from oral mucosa brushing instead [16]. She was found to have negative AFB in induced sputum smear. Even though AFB smear was negative, TB could not be ruled out, since the positive AFB [4] was reported only in 20%–40% of patients with miliary TB. On the other hand, there is a case report of a positive AFB sputum result of a boy with DS and miliary TB [22]. The finding was associated with a very high bacterial load in immunocompromised state in DS, which increases the chance for isolation of AFB in sputum.

On the fifth day of hospitalization, our patient developed ARDS, and given that her life was compromised, an empirical antituberculosis treatment was initiated. However, due to advanced stage of a TB infection, she expired on the 8th day of admission.

ARDS is a syndrome of acute respiratory failure, clinically presented as appearance of acute bilateral pulmonary infiltrates on chest radiograph and severe hypoxemia, refractory to oxygen therapy. According to a new definition, ARDS is classified into 3 groups by the degree of hypoxemia, defined as PaO2/FiO2 ratio: mild, moderate and severe [23].

Miliary TB is a rare cause of ARDS, with estimated comorbidity rates of 1%–2% [24]. A possible pathophysiology for the development of ARDS in patients with miliary TB may be a strong pro-inflammatory response to *Mycobacterium tuberculosis* infection – leading to inflammatory cells accumulation in alveolar spaces, following a release of granular enzymes and oxidants, and resulting in damage to the alveolar-capillary membrane. Damage to the alveolar-capillary membrane allows an increase in cellular permeability, which aggravates oxygen dysfunction and consequently causes ARDS [25].

Aside from immunocompromised status, other independent predictors of ARDS development in patients with miliary TB include: diabetes mellitus as general condition, alanine aminotransferase-ALT(> 70–100 U/L), aspartate aminotransferase-AST (> 94 U/L), D-dimer (>1,6 mg/L), Hgb (< 90 g/L) and albumin (< 25 g/L) [26]. In our case study, a patient had decreased haemoglobin and hypoalbuminemia, which could present additional risk factors for the development of ARDS due to miliary TB. Hypoalbuminemia accelerates fluid exudation, promotes alveolar oedema and contributes to ventilation-perfusion ratio mismatch. Hypoalbuminemia and weight loss in our patient suggested malnutrition, which was clinically presented as well. It is well known that malnutrition affects cell-mediated immunological processes and is a risk factor of developing TB [26]. Anaemia caused by chronic infections, including TB, is the result of the effect of cytokines mediating the inflammatory response. Considering that the severity of anaemia is mainly determined by Hgb level, it is assumed that the decrease of Hgb is a result of inflammation itself, which, in turn, can explain the relationship between low haemoglobin levels and ARDS [26].

Compared with miliary TB alone or other causes of ARDS, miliary TB with ARDS portends a higher mortality of 33%–90% [24]. In the study Deng et al. [26] of 471 patients with miliary TB, 85 had the diagnostic criteria for ARDS, with a mortality rate of 47.1%. In the study Lee et al. [27] of 67 patients with adult respiratory distress syndrome caused by milliary TB, mortality rate was also very high 61.2%. The main reasons for such a high mortality rate should be attributed to the fact that diagnosing and subsequent treatment of miliary TB is delayed and often missed, as well as to a low causal association of miliary TB and ARDS [26]. The duration of symptoms before clinical worsening of miliary TB to ARDS is usually gradual, ranging from 5 to 90 days. However, sometimes the onset of symptoms may be unpredictably rapid. In many reported cases the diagnosis was established *post-mortem*, as was the case of our patient [28].

Conclusion

Miliary TB should be kept in mind in patients with DS due to immunosuppression associated with deficient cell-mediated immunity. The development of ARDS as a complication of miliary TB is difficult to identify due to a low causal association. High clinical suspicion and a chest radiograph with a typical appearance of miliary pattern justify the initiation of empirical anti-tuberculosis treatment in such patients, as an attempt to change poor prognosis.

Careful monitoring of patients is crucial for early detection of ARDScomplication, and laboratory findings, such as anaemia, hypoalbuminemia and high levels of alanine aminotransferase can be of help in identifying such patients. Treatment of miliary TB in advanced disease stages has a poor outcome, due to the development of ARDS and a mortality rate can be predicted according to a relation: PaO_2/FiO_2, in line with the updated "Berlin definition".

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CASE REPORT

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Infective endocarditis of partial atrioventricular septal defect – A case report

Infektivni endokarditis parcijalnog atrioventrikularnog septalnog defekta

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Abstract

Introduction. Partial atrioventricular septal defect (AVSD) is a form of congenital heart disease (CHD) rarely detected in adults. Infective endocarditis represents a severe complication that carries a substantial risk. **Case report.** We here reported a case of a 43-year-old female with previously diagnosed adult CHD (partial AVSD and bicuspid aortic valve) presented to the hospital with fever and malaise 14 days prior to admission. On the lung computed tomography scan inflammatory consolidations were found and dual antibiotic therapy (ceftazidime and clarithromycin) was administered without significant regression of pulmonary inflammatory consolidations. The antibiotic treatment was continued with amoxicillin/clavulanic acid combined with levofloxacin and metronidazole. Transthoracic and transesophageal echocardiography revealed a large vegetation (dimension, 3.6 x 1.8 cm) attached to the septal leaflet of the tricuspid valve floating between right atrium and right ventricle through tricuspid valve with high embolic potential. Endocarditis team reached a decision for immediate surgical intervention. The operative findings revealed the partial AVSD, common atrioventricular valve with cleft of the anterior mitral leaflet in the A2 segment and detached and cleft septal leaflet of the tricuspid valve. Vegetation (size 4 x 3 cm) was attached to the septal side of the tricuspid annulus, basal segment of the anterior mitral leaflet and edge of the atrial septal defect freely floating between right atrium, right ventricle and left atrium. Excision of the vegetation and AVSD plastics were done, as well as the reconstruction of the mitral and tricuspid annuli and leaflets. The treatment was continued with antibiotics and completed in 18 days with full recovery. **Conclusion.** Early and precious diagnosis and optimal management that combines both conventional and surgical approaches are crucial for reducing the risk of complications and mortality in patients with infective endocarditis in grown-up congenital heart disease.

Key words:
endocarditis, bacterial; atrioventricular septal defect; tricuspid valve; diagnosis; cardiovascular surgical procedures; anti-bacterial agents; treatment outcome.

Apstrakt

Uvod. Parcijalni atrioventrikularni septalni defekt (AVSD) je oblik kongenitalne bolesti srca koja se retko dijagnostikuje u odraslom dobu. Infektivni endokarditis predstavlja tešku komplikaciju koja je potencijalna opasnost po život bolesnika. **Prikaz bolesnika.** Kod bolesnice, stare 43 godine, sa prethodno dijagnostikovanom urođenom srčanom manom [parcijalni atrioventrikularni septalni defekt (AVSD) i bikuspidna aortna valvula] u adultnom dobu, 14 dana pred prijem u regionalnu zdravstvenu ustanovu javile su se tegobe u vidu febrilnosti i slabosti. Na kompjuterizovanoj tomografiji grudnog koša uočene su inflamatorne konsolidacije u plućnom parenhimu, te je započeto lečenje dvojnom antibiotskom terapijom (ceftazidim i klaritromicin). Obzirom na nedovoljnu regresiju promena u plućima ordinirana je trojna antibiotska terapija: amoksicilin/klavulanska kiselina, levofloksacin i metronidazol. Urađena je transtorakalna i transezofagealna ehokardiografija kojom je otkrivana vegetacija (veličina 3,6 x 1,8 cm) vezana za septalni deo atrioventrikularnog (AV) anulusa u području trikuspidne valvule koja je slobodno flotirala u šupljini desne pretkomore sa povremenim delimičnim prolaskom kroz trikuspidni otvor i koja je imala veliki embolijski potencijal. Tim za endokarditis indikovao je hitno operativno lečenje. Intraoperativno, ustanovljen je parcijalni AVSD koji je angažovao septalni deo trikuspidnog anulusa, bazu A2 prednjeg mitralnog kuspisa i ceo donji deo interatrijalnog septuma. Vegetacija (veličina 4 x 3 cm) je bila vezana za baze septalnog listića, baze prednjeg mitralnog listića i ruba defekta na nivou atrijalnog septuma i slobodno se kretala između desne pretkomore, desne komore i leve pretkomore. Urađena je ekscizija vegetacije, plastika AVSD, kao i rekonstrukcija mitralnog i trikuspidnog anulusa i suture kuspisa. Lečenje je nastavljeno antibiot-

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skom terapijom i završeno za 18 dana uz potpuni oporavak. **Zaključak.** Rana i precizna dijagnostika i optimalno lečenje kombinacijom konvencionalnog i hirurškog lečenja presudno je za smanjenje rizika od komplikacija i smrtnosti kod odraslih bolesnika sa urođenom srčanom manom i infektivnim endokarditisom.

Ključne reči:
endokarditis, bakterijski; srce, atrioventrikularni septalni defekt; zalistak, trikuspidni; dijagnoza; hirurgija, kardiovaskularna, procedure; antibiotici; lečenje, ishod.

Introduction

Infective endocarditis (IE) in adults with congenital heart disease (CHD) has been increasing along with the increasing number of patients with CHD who were reaching adulthood [1–3] as grown-up congenital heart disease (unrepaired, repaired, palliated) [4]. An underlying CHD is found in 11% to 13% of all IE [5]. Despite latest advances in diagnosis and treatment (conventional or surgical management), mortality and the rate of complications are high [6] and late course is unpredictable [5].

The term atrioventricular septal defect (AVSD) covers a spectrum of heart anomalies with a common atrioventricular (AV) junction. With an incidence of 4–5.3 per 10,000 live births, AVSD comprises 7% of all CHD. Controversies exist on nomenclature and subdivision of the varying morphology of AVSDs, and several different descriptions are currently used. There are three types of AVSD: 1) Complete AVSD includes an ostium primum atrial septal defect and inlet ventricular septal defect (VSD). Clefts in the mitral and tricuspid valve leaflets result in one common, large AV valve connecting the atrial and ventricular chambers; 2) Partial AVSD includes an ostium primum AVSD with separate mitral and tricuspid valve orifices and clefts in the mitral and/or tricuspid valve leaflets. Partial AV canal occurs in 1-2% of all congenital heart defects; 3) The term intermediate AVSD (also called transitional) is variably defined and is an infrequent form of AVSD. Two-orifice AV valve with a single valve annulus is usually present and often restrictive VSD just below AV valves [7].

Case report

A 43-year-old woman was admitted to a secondary health care hospital with a history of fever up to 39°C. Two weeks prior to admission the patient complained of malaise, poor appetite, cough and pain at the right side of chest. Antibiotic therapy with ciprofloxacin was started 10 days prior to admission, but she remained febrile. The patient was diagnosed grown-up CHD in the age of 38. Transthoracic echocardiography (TTE) and transesophageal echocardiography (TOE) showed AVSD as a combination of ostium primum AVSD (diameter of atrial septal defect was 14 mm), with left to right shunt (LR shunt), cleft of the anterior mitral valve leaflet (AML) with mild mitral regurgitation as well as cleft of the tricuspid septal leaflet. Also, the bicuspide aortic valve was present without stenosis and regurgitaion. The right chamber was normal in size with thickened wall (0.7 cm) and preserved systolic function. The left ventricle was normal in size and function. Contrast echocardiography with agitated saline contrast showed negative contrast effect. Diagnostic cardiac catheterization demonstrated a moderately significant left-to-right shunt Qp:Qs of 1.2 and confirmed the diagnosis of partial AVSD, without significantly elevated pulmonary artery pressure (PA 36/16/24 mmHg). The patient refused surgical correction that was proposed. She had history of arterial hypertension and type 2 diabetes.

Physical examination during admission to the regional hospital revealed systolic heart murmur audible on mitral area. Laboratory tests revealed leukocytosis (white blood cells count 12.0 × 10^9/L) and raised inflammatory markers (C-reactive protein 41 mg/L). Chest X-ray and thoracic computed tomography (CT) scan showed inflammatory consolidations in left middle and lower parts and right lower parts of the lung with accompanied mediastinal lymphadenomegaly. The patient was treated with dual antibiotic therapy (ceftazidime and clarithromycin) for 14 days without significant regression of pulmonary inflammatory consolidations. Repeated blood cultures remained sterile. TTE reveled tricuspid valve endocarditis. The antibiotic treatment was continued with amoxicillin/clavulanic acid combined with levofloxacin and metronidazol for 8 days. Then, the patient was referred to a tertiary health center, for TOE and further diagnostic testing and treatment. The antibiotic treatment was continued with amoxicillin/clavulanic acid and levofloxacin. TOE showed large vegetation (3.6 x 1.8 cm) attached to the septal leaflet of the tricuspid valve floating between the right atrium and right ventricule through the tricuspid valve with mild tricuspid regurgitation (Figure 1).

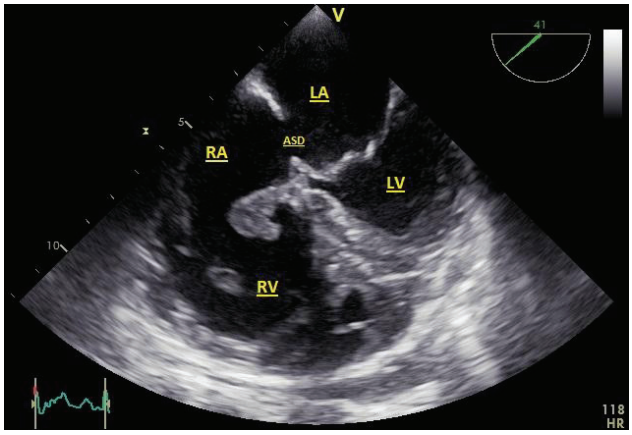

Fig. 1 – Transesophageal echocardiography of four chambers shows atrial septal defect (ASD), type primum. Mitral and tricuspid valve orifices are in the same level. Vegetation is connected to the septal leaflet of the tricuspid valve floating between right atrium (RA) and right ventricle (RV) through the tricuspid valve. LA – left atrium; LV – left ventricle.

The embolic potential of vegetation was high. Thoracic CT scan with pulmonary angiography revealed bilateral segmental and subsegmental septic pulmonary emboli. Endocarditis team reached a decision for immediate surgical intervention. The operative findings revealed the AVSD type primum (dimension 4 x 4 cm), cleft of the anterior mitral leaflet in the A2 segment and cleft septal leaflet of the tricuspid valve. There was direct communication between the left ventricle and the right atrium through AML and ostium primum. Vegetation size was 4 x 3 cm and it was attached to the septal leaflet of the tricuspid valve, basal segment of the AML and edge of the primum AVSD freely floating between the right atrium, right ventricle and left atrium. Excision of vegetation, AVSD plastics with Xeno Sure Biologic patch, as well as the reconstruction of the anterior mitral and septal tricuspid cusps with mitral and tricuspid annuloplasty were done (Figure 2). The intervention was performed without any complications. TTE was repeated following the procedure, and there was no remaining AVSD, mitral and tricuspid regurgitation and no signs of infective endocarditis. After the surgery, antibiotic therapy was continued with amoxicillin/clavulanic acid in combination with levofloxacin and metronidazole for 14 days. The patient was discharged from the hospital in good general condition with no signs of inflammation with recommendations for oral therapy with moxicillin/lavulic acid and ciprofloxacin for 10 days more. The treatment was completed in 18 days with full recovery. The patient was doing well at 3 months follow-up.

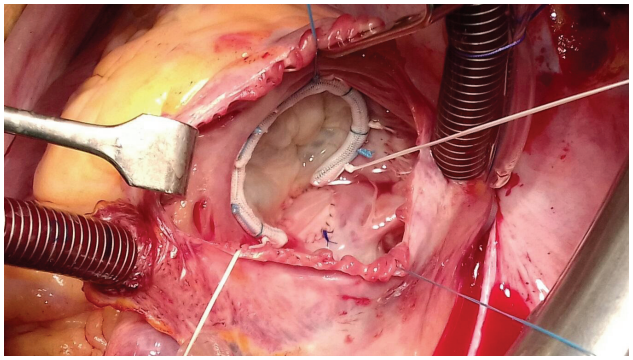

Fig. 2 – Intraoperative images: atrial septal defect (ASD) plastics and reconstruction of the mitral and tricuspid annuli and leaflets.

Discussion

We present herein a rare case of CHD in adulthood followed by IE. To our knowledge no such case of IE is yet described in literature. In large cohort study on 84,308 adult patients admitted to hospitals with CHD Rodriguez et al. [8] found that only 0.4% had AVSD. Partial AVSD is often discovered in early childhood, but sometimes it may go undetected with no symptoms up to adulthood as in the case of our patient whose symptoms presented for the first time at the age of 38. Somerville [9] has reported that patients with partial AVSDs face symptoms with increasing frequency after the third decade of life. Gatzoulis et al. [10] have reported that three quarters of patients with grown-up congenital heart disease who underwent primary repair of partial AVSD are symptomatic at mean age 37.7 years like our patient.

AVSD is typically present in the neonatal period being an important cause of cardiac morbidity and mortality in this age group [11]. Mortality is higher in children with a complex AVSD and in those with 2 or more major noncardiac malformations [12]. Children with partial and transitional AVSD are mostly asymptomatic so recommendation for surgical repair is might be delayed to preschool or older ages [13–16]. Surgery for partial AVSDs with large left-to-right atrial shunts in adults can be performed with low mortality and morbidity [10]. On the other hand, in operated or medically treated patients, the long-standing course is unpredictable. The risk of developing IE in adult CHD is more than 10 times increased than in normal population [17]. Infective endocarditis might be late complication that carries substantial mortality risk. Recently, Tutarel et al. [18] studied IE in adults with CHD and found that during follow-up of 6.7 years, 19.4% died. High mortality rate highlights the need for consciousness of IE and adult CHD. The majority of studies about IE in adult CHD are retrospective and limited in number so valid conclusions cannot always be made. There is an evident need for future long-term follow-up studies on this disease. Awareness and understanding of the disease and its complications are essential in order to determine an early treatment and prevent complications.

Conclusion

This is extremely rare case of partial AVSD, uncommonly seen in adulthood, complicated with infective endocarditis. Early diagnosis and optimal management that combines both conventional and surgical approaches are crucial for reducing the high embolic risk, risk of complications and mortality. For obtaining the best results, complicated forms of infective endocarditis with underlying congenital heart disease should be evaluated and treated at early phase in tertiary surgical centers by an experienced endocarditis team.

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LETTER TO THE EDITOR/
RESEARCH LETTER

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Effects of social interactions on psychoactive substance use by medical students

Efekti socijalne interakcije na zloupotrebu psihoaktivnih supstanci među studentima medicine

To the Editor:

Psychoactive substance (PS) use is a wide-spread problem causing economic losses, ecologic damage, population and moral decline which makes it a major threat to social stability and development as well as national health and well-being. Russia is currently facing a serious and dramatic problem associated with PS use by young people which is characterized by high prevalence showing a tendency to increase, a significant number of people at risk, and a growing rate of PS use by females that has never been seen before [1].

Alcohol consumption by medical students remains a pressing issue affecting health, promoting risky sexual behavior, and impeding academic progress [2–8]. Higher education institutions often become environments that facilitate regular alcohol use and form a certain mode of behavior that leads to alcohol addiction [9]. Popular mentality and youth culture (including student culture) often form a strong social and psychological stereotype which either makes PS use an acceptable popular norm of behavior to be followed, or equates PS use to a normal aspect of everyday life, tradition or obligatory ritual [10].

Compared to other educational institutions, medical schools are known for heavy study loads, increased stress levels, and specific psychological characteristics of students. Several researchers note three critical periods in higher education – during the first, third and fifth year [11]. These aspects along with other predisposing factors may lead to heavy drinking in some students potentially triggering addiction [12, 13].

Some researchers [14–17] divide risk factors for PS use by young people into external (geopolitical, demographic, as well as social environment factors) and internal (personal and behavioral) ones.

The role of social interactions of young people in developing a habit of PS use merits closer inspection due to the fact that environment (both family and academic) may affect biological and individual factors in different ways [14, 18–22].

We analyzed the results of 880 students who were asked to fill in a special questionnaire related to PS use.

The prevalence of smoking in students was 28.4% ± 1.5%. Smoking was more common in males (39.0% ± 2.9%) than females (23.4% ± 1.7%) ($p < 0.01$).

Alcohol use within the last 6 months was reported by 66.6% ± 1.6% of respondents. About half of them (51.2%) consumed alcohol sporadically, less than a half (42.7%) several times a month, 5.8% several times a week and 0.3% reported daily use.

Wine and hard liquor were the most popular beverage types (24.6% and 20.9%, respectively) followed by champagne (17.8%), beer (16.0%), cocktails made of hard liquor (14.9%) and others.

Presented results indicate that 6.0% ± 0.8% of students used drugs. Drug use over the period of 6 months was more prevalent in males (10.6% ± 1.8%) than in females (3.8% ± 0.8%) ($p < 0.01$). Marijuana and hashish were the most common drugs among medical students who used drugs with 78% reporting using these substances. Other PS types were much less common with tranquilizers, amphetamines, mushrooms and ecstasy used by 8.3%, 5.1%, 5.1% and 3.5% of respondents, respectively.

Among social interaction aspects influencing PS use by students, family environment is the first to draw attention. Living apart from parents was associated with a significantly higher prevalence of smoking and alcohol consumption in contrast to living with them ($p < 0.01$). Apart from that, students living in dormitories reported higher rates of smoking and drinking than those living in a flat or a house (Table 1).

Table 1
Smoking and alcohol consumption within the last 6 months in students living in different conditions

Living conditions	Smoking	Drinking
Flat	26.5 ± 1.7	65.8 ± 1.9
Room in communal apartments	28.2 ± 7.2	64.1 ± 7.7
House	22.8 ± 5.6	52.6 ± 6.6
Dormitory	39.2 ± 4.1*	76.2 ± 3.6**

Note: Results are given as mean ± standard deviation in percentage of respondents.
*** – $p < 0.05$; ** – $p < 0.01$.**

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The results indicate that PS use can be reliably predicted by PS use in high school. However, there are certain changes in PS use patterns that are caused by changes in living conditions. Young people who continued living with their parents had the lowest level of alcohol and marijuana use compared to those who had other living conditions. The biggest changes were observed in respondents who left home. The authors suggest that higher PS use is associated with lack of parents' control [23].

Married people consumed alcohol more frequently than single ones ($p < 0.05$). This factor may be associated with financial aspects and certain individual risk factors. The results indicate that respondents who have to impose limits on some aspects of their lives due to financial problems (having to eat away from home, refrain from purchasing desired devices or appliances or skip on entertainment with friends) consumed alcohol 17% more frequently than their peers who faced no financial issues ($p < 0.01$).

Students who had family members abusing PSs were more frequent smokers ($p < 0.05$), drinkers ($p < 0.01$) and drug users ($p < 0.01$). Therefore, living in a family with no history of PS abuse may be considered a protective factor.

Difficulty in socialization and interaction with fellow students contributed to more frequent alcohol consumption. Among students who had healthy relationships with their group mates, the prevalence of alcohol consumption was 65.5%, and among students who failed to have rapport with their peers – 77.9% ($p < 0.05$). Students who showed no interest in socializing with group mates consumed alcohol 20% more frequently and used drugs 4 times more often than those who established good communication with peers ($p < 0.01$). Communication problems with peers including fellow students were previously studied by other researchers [24].

If student's self-esteem becomes too low, it may result in withdraw from the social group, stop attending classes and ignore social norms altogether as these factors are the reasons behind negative attitudes and critical feelings towards oneself. In that case, he/she starts to search a new social group which approves their deviant behavior. In cases such as this, PS use may become a means of improving self-esteem [25].

Therefore, it can be concluded that only a multifaceted approach to PS use incorporating social aspects (educational and family in particular) will effectively decrease the influence of negative factors on medical students' health and lifestyle.

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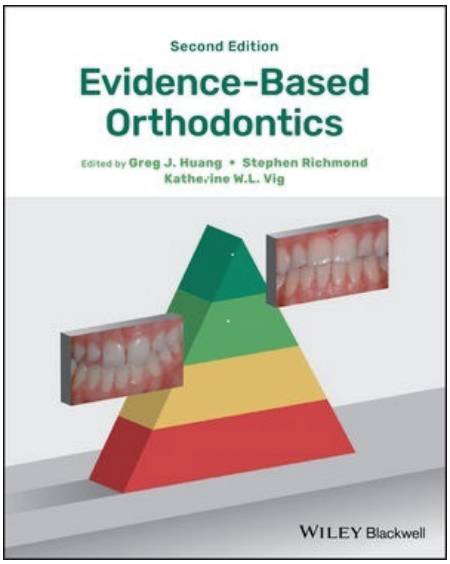
BOOK REVIEW/PRIKAZ KNJIGE

Evidence-Based Orthodontics, 2nd edition



Naslov knjige/Title: Evidence-Based Orthodontics, 2nd edition
Urednici/ Editors: Greg J. Huang, Stephen Richmond, Katherine W. L. Vig
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Knjiga „Evidence-Based Orthodontics“, čiji su urednici Greg J. Huang, Stephen Richmond, Katherine W. L. Vig, u izdanju John Wiley & Sons, Inc, objavljena je 2018. godine u drugom, proširenom izdanju. U odnosu na prvo izdanje iz 2011. godine, čiji je izdavač bila kompanija Blackwell Publishing Ltd, drugo, dopunjeno izdanje donosi značajno veći fond istraživanja principa i praksi ortodoncije zasnovane na dokazima, praktične savete za njihovo uključenje u svakodnevni rad stručnjaka-ortodonata, brojne kliničke teme i sistematske preglede kliničkih studija iz ove oblasti. Knjiga je napisana na 288 strana, organizovana u okviru šest osnovnih poglavlja i pokriva, ujedno, i teorijske i kliničke aspekte ortodoncije zasnovane na dokazima.

Ortodoncija zasnovana na dokazima podrazumeva stručno mišljenje bazirano na visokokvalitetnim metaanalizama i sistematskim pregledima randomizovanih kontrolisanih kliničkih ispitivanja (*Randomized Clinical Trials* – RCT) sa malim rizikom od pristrasnosti. Za ovu disiplinu od velike važnosti je razumevanje i identifikacija karakteristika kvaliteta RCT-a i njihov sistematski pregled.

Ortodoncija zasnovana na dokazima obezbeđuje alate za korišćenje odgovarajuće literature za utvrđivanje alternativnih strategija upravljanja pacijentima. Izraz „medicina zasnovana na dokazima“ prvi put se pojavio u medicinskoj literaturi 1991. godine. U stvari, medicina zasnovana na dokazima i ortodoncija zasnovana na dokazima uključuju informisanu i efikasnu upotrebu svih vrsta dokaza, ali posebno dokaza iz medicinske literature, u nezi pacijenata. Ortodoncija zasnovana na dokazima se u poslednjih 20 godina uveliko razvila, uvažavajući princip da kliničko odlučivanje treba da bude podržano snažnim naučnim dokazima, kada za to postoje uslovi.

Početna poglavlja ove knjige bave se naučnom osnovom ortodoncije zasnovane na dokazima. Prvo poglavlje predstavlja pristup zasnovan na dokazima koji je rezultirao brzim promenama u sistemu zdravstvene zaštite i obrazovanju studenata upisanih na visokoškolske ustanove iz medicinskog naučnog polja, ali i šire javnosti. Jedna od metoda postizanja pristupa zasnovanog na dokazima u stomatologiji odnosi se na randomizovane kliničke studije, odnosno njihove sistematske preglede. U ortodonciji, kliničko iskustvo sugeriše da je neka stanja najbolje lečiti rano, iz bioloških, socijalnih ili praktičnih razloga, dok lečenje drugih treba odložiti.

Drugo poglavlje daje kratak pregled razvoja kliničkih istraživanja, uobičajenih dizajna ovakvih istraživanja, njihove upotrebe, snage i ograničenja, kao i diskusiju o najboljim praksama koje se široko primenjuju u istraživanjima s

akcentom na proučavanje strategija za širenje novih dokaza i za njegovu primenu u rutinskoj kliničkoj praksi.

Treće poglavlje, pod nazivom „Elektronsko traženje informacija o kliničkim ispitivanjima“, ukazuje na značaj elektronskih baza naučne literature kao što su MEDLINE i EMBASE, Cochrane Library uključujući prikaz kreiranja strategije pretrage tih baze i daje podatke o dodatnim izvorima informacija o kliničkim ispitivanjima.

Četvrto poglavlje, „Razumevanje randomizovanih kliničkih ispitivanja i sistematskih pregleda“, pruža informacije koje olakšavaju tumačenje tih ispitivanja i sistematskih pregleda kliničkih studija i ukazuje na značaj CONSORT smernica za izveštavanje koje podržava većina ortodontskih časopisa.

Peto poglavlje govori o trenutnom statusu ortodoncije zasnovane na dokazima, bavi se dizajnom i ocenom kliničkih ispitivanja i razmatra buduće istraživačke pravce i izazove u toj oblasti.

Šesto poglavlje predstavlja faktore koji utiču na oblik lica i daje kratak pregled genetskih faktora i faktora okruženja koji utiču na oblik lica, odnosno koji mogu imati uticaj na ishod bilo koje intervencije. U ovom poglavlju istaknut je značaj utvrđivanja biološke osnove normalne varijacije lica i uticaja faktora okruženja na oblik lica kao što su trauma, operacija, infekcije i opekotine, dok drugi uticaji predstavljaju kombinaciju interakcija okoline i genetike.

Nakon šestog poglavlja predstavljeno je 56 sažetaka izabranih sistematskih pregleda kliničkih studija u ortodonciji, i na kraju, dat je prikaz korišćene literature, indeks pojmova i sistematskih pregleda u ortodonciji.

Knjiga „Evidence-Based Orthodontics“, kroz implementaciju naučnih dokaza u svakodnevnu kliničku praksu, što je preduslov moderne ortodontske prakse, daje smernice za uspešno sprovođenje ortodontskog lečenja sa ciljem obezbeđenja maksimalne efikasnosti i bezbednosti po pacijenta.

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

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

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

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

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

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

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

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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.

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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.

Detaljno uputstvo može se dobiti u redakciji ili na sajtu: www.vma.mod.gov.rs/vsp