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Number one goal of the World Health Organization (WHO) is the universal health coverage. Key to achieving it is ensuring that everyone can obtain the care they need and when they need it.

Although the progress has been made in all regions of the world, millions of people still have no access to all health care services. This is why, this year, for the World Health Day, on 7 April, WHO is focusing on universal health coverage – everyone, everywhere.

Univerzalna zdravstvena zaštita je cilj broj 1 Svetske zdravstvene organizacije (SZO). Ključ za postizanje tog cilja je obezbediti da svako može dobiti potrebnu negu kada mu je potrebna.

Iako je napredak učinjen u svim delovima sveta, milioni ljudi još uvek nemaju odgovarajući pristup zdravstvenoj zaštiti. Zbog toga je SZO, na ovogodišnji Svetski dan zdravlja, 7. april, u fokus svojih aktivnosti stavila, upravo, postizanje tog cilja - obezbeđenje univerzalne zdravstvene zaštite za svakoga, na svakom mestu.

ORIGINAL ARTICLES

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Criterion validity of metabolic and anthropometric predictors in diabetic foot syndrome

Kriterijumska validnost metaboličkih i antropometrijskih prediktora u sindromu dijabetesnog stopala

Dragana Bubanja*, Zorica Jovanović†, Mira Vuković‡

Clinical Center Kragujevac, *Center for Endocrinology, Diabetes and Metabolic Diseases, Kragujevac, Serbia; University of Kragujevac, Faculty of Medicine, †Institute for Pathophysiology, Kragujevac, Serbia; General Hospital Valjevo, ‡Education Center, Valjevo, Serbia

Abstract

Background/Aim. The diabetic foot syndrome (DFS) appears in 15% of diabetes mellitus (DM) patients and is the most common cause of hospitalization, prolonged hospital stay and lower extremity amputation. This study assesses the discriminant validity of the indicators of glycemic control, lipoprotein status and the body mass index (BMI) in diagnosing DFS in the DM patients. **Methods.** A comparative observational study was conducted with the study group composed of patients diagnosed with DM and DFS and a control group, composed of healthy volunteers. Metabolic predictors measured in the study were: fasting glycaemia (FG), postprandial glycaemia (PPG), glycated hemoglobin (HbA1c), total cholesterol, total triglyceride, low density lipoprotein (LDLc) and high density lipoprotein (HDLc). The BMI was measured as an anthropometric variable. The validity criterion of both metabolic and anthropometric variables was estimated by the Receiver Operating Characteristic (ROC) procedure. **Results.** A total of 70 patients with DM and 60 healthy volunteers were observed. Using the ROC procedure, five significant predictors of DFS were proved. The validity criterion for HbA1c, FG, PPG, LDLc and the BMI were in the following order: 6.3%, 6.3 mmol/L, 7.1 mmol/L, 4.39 mmol/L and 25 kg/m^2, respectively. Significantly larger surfaces were found under the curve for all glycometabolic variables, compared to the surface under the curve for LDLc, as well as relative to the surface under the curve for BMI. **Conclusion.** Preventing DFS in patients with DM has to include intensification of diet measures along with the treatment of the increased value of fasting glycaemia, postprandial glycaemia and LDLc, even when they lower compared to the current recommended values for the patients with DM. Lowering body fat in the patients with DM has to be approached in the period of their pre-obesity.

Key words:
diabetes mellitus; diabetic foot; syndrome; glycated hemoglobin; body mass index; prognosis.

Apstrakt

Uvod/Cilj. Sindrom dijabetesnog stopala (SDS) javlja se kod skoro 15% pacijenata sa dijabetes melitusom (DM) i najčešći je uzrok njihove hospitalizacije, prolongiranog bolničkog lečenja i amputacija donjih ekstremiteta. U studiji je procenjena diskriminaciona validnost pokazatelja glikoregulacije, lipoproteinskog statusa i indeksa telesne mase (ITM) u detekciji SDS kod pacijenata sa DM. **Metode.** U uporednoj, opservacionoj studiji, ispitivanu grupu sačinjavali su pacijenti sa DM i SDS, a kontrolnu zdravi dobrovoljci. Metabolički prediktori izmereni u studiji bili su: glikemija našte (GN), post-prandijalna glikemija (PPG), glikozilirani hemoglobin (HbA1c), ukupni holesterol, ukupni trigliceridi, lipoproteini male gustine (LDLc) i lipoproteni visoke gustine (HDLc). ITM je izmeren kao antropometrijska varijabla. Kriterijumska validnost metaboličkih i antropometrijskih varijabli procenjena je procedurom prijemno operativnih karakteristika. **Rezultati.** Ukupno je opservirano 70 pacijenata sa DM i 60 zdravih dobrovoljaca. Procedurom prijemno operativnih karakteristika dokazano je pet značajnih prediktora SDS. Kriterijumske vrednosti za HbA1c, GN, PPG, LDLc i ITM, iznosile su, redom: 6.3%, 6.3 mmol/L, 7.1 mmol/L, 4.39 mmol/L i 25 kg/m^2. Pronađene su značajno veće površine ispod krivih kod svih glikometaboličkih varijabli u odnosu na površinu ispod krive za LDLc, kao i u odnosu na površinu ispod krive za ITM. **Zaključak.** Prevencija SDS kod obolelih pacijenata sa DM, mora da uključi intenziviranje dijetetskih mera uz tretman povišenih vrednosti glikemije našte, postprandijalne glikemije i LDLc i to, pri

Correspondence to: Mira Vuković, Education Center, Sinđelićeva 621, 14 000 Valjevo, Serbia. E-mail: vmira62@gmail.com

njihovim nižim vrednostima u odnosu na aktuelne preporučene vrednosti za pacijente sa DM. Smanjenju telesne mase kod pacijenata sa DM, neophodno je pristupiti još u periodu njihove pre-gojaznosti.

Ključne reči:
diabetes melitus; dijabetesno stopalo; sindrom; hemoglobin, glukozilovan; telesna masa, indeks; prognoza.

Introduction

The term diabetes mellitus (DM) describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. The diabetic foot syndrome (DFS) is a major problem in people suffering from diabetes DM with a tendency for ulcers, infection or damage to the deep soft tissues of the foot [2]. DFS occurs in about 15% of the patients with DM, and the most common risk factors are male gender, long-termed DM, foot deformities and diabetic polyneuropathy (DPN) [3–5]. Despite its high incidence, DFS has not been classified according to the International Classification of Diseases (ICD-10). This may indicate that the incidence of DFS is significantly higher. The patients with DM are at a high risk of developing microvascular complications, especially DPN, which further leads to the DFS development [6]. It occurs in almost 50% of the patients with DM who suffer longer than 10 years and in the patients with diabetic peripheral neuropathy, which is the most common chronic complication in the type 2 diabetes [7]. In some developed countries, DFS is the most common cause of hospitalization, prolonged hospitalization and lower extremity amputation in the patients with DM [8–10].

Former investigations indicated that the level of glycosylated haemoglobin (HbA1c), postprandial glycaemia and dyslipidemia are believed to be of a particular importance for DPN [11]. There are reports that reducing hyperglycaemia decreases the onset and progression of microvascular complications [12, 13]. However, in the above mentioned studies, the examined patients were those with DM (with or without DFS) in the absence of a control group of healthy volunteers, so that the roles of actual and retrograde glucoregulation, lipid metabolism and obesity in DFS have remained insufficiently understood. The elevated HbA1c levels indicate poor chronic glycemic control and are directly related to hypoxemia in the vasa vasorum and microvascular complications in the diabetic patients [14]. Christman et al. [15] reported that glycaemia, as assessed by HbA1c, may be an important biomarker in predicting the wound healing rate in the diabetic patients. Obesity with insulin resistance and hypoadiponectinemia associated with dyslipidemia and the elevated levels of systemic inflammatory markers are also a significant factor in the pathogenesis of DFS. Previous studies showed that the prevalence of diabetic foot ulceration is higher in people with the body mass index (BMI) > 30 kg/m^2 [11]. However, other authors suggest that the BMI is not significantly associated with DFS [16].

The objectives of this study were to assess the discriminant validity of the indicator of the glycemic control, lipoprotein status and BMI in diagnosing DFS.

Methods

Study design, time and place

To estimate the risk factors in the development of DFS, we conducted a comparative observational study. The study group was composed of the patients diagnosed with DM with DFS. DM was diagnosed by the World Health Organization (WHO) criteria. DFS in these patients was observed as the presence of microscopically confirmed lesions (cracks, fissures, clavus) or macrolesions on foot, or following the history of previously diagnosed trophic ulcers. The control group consisted of the healthy volunteers. The study was approved by the Ethics Committee of the Clinical Centre in Kragujevac and was conducted in the period January 2014 – May 2015 at the Centre for Endocrinology, Diabetes and Metabolic Diseases, Department of Internal Medicine, Clinical Centre in Kragujevac. Both groups involved the adult people of both genders who signed the consent form of participation in the research on a voluntary basis.

Variables

The study tracked the demographic variables (gender and age), anthropometric variables (BMI) and metabolic variables (fasting blood glucose, glucose 2h after food intake, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and total triglycerides – all expressed in mmol/L). The levels of HbA1c (%) were also determined.

The measurements of weight and height were obtained for each individual, and the BMI was calculated according to the following formula: BMI (kg/m^2) = body weight (kg) / high^2 (m^2)

Fasting blood glucose (FBG) and postprandial plasma glucose (PPG) were determined spectrophotometrically by the glucose oxidase test (GOD-PAP) with the commercial reagent 'Bioanalitica'and Aeroset Abbott analyzer. The level of HbA1c was determined by using the imunoturbidimetric method of inhibition microparticle agglutination using the reagent produced by the Abbottand Aeroset-Abbott analyzer. The level of the total triglycerides was measured spectrophotometrically by the glycerol phosphate oxidase test (GPO-PAP) using the commercial reagents produced by the Abbott and Aeroset-Abbott analyzer. The level of the total cholesterol was measured spectrophotometrically by the cholesterol oxidase test (CHOD-PAP), using the commercial reagents produced by the Abbott and Aeroset-Abbott analyzer. The level of high density lipoprotein cholesterol (HDLc) was determined by the ultracentrifugation HDLc test, a homogeneous method for directly measuring HDLc, using two commercial reagents and detergent produced by the Abbott and

Aeroset-Abbott. The level of low density lipoprotein cholesterol (LDLc) was calculated by the Friedewald formula: LDLc (mmol/L) = Total cholesterol (mmol/L) – HDLc (mmol/L) – Total triglycerides (mmol/L) / 2.2

Statistical methods

The continuous numerical data sets were represented by the mean and standard deviation. The categorical variables were defined by the relative frequency of outcomes. The independent samples *t*-test was used to assess the differences among continuous numerical variables. The criterion validity of metabolic and anthropometric variables for the detection of DFS was estimated by the Receiver Operating Characteristic (ROC) procedure. The cut-point value, sensitivity, specificity, positive predictive value and negative predictive value were obtained applying the maximum Youden index: $J = max\ [SE_i + SP_i - 1]$, where SE_i denotes sensitivity and SP_i denotes specificity for each potential cut-point value of the resulting variable. Comparison of the areas under the ROC curves was done using the method of Hanley and McNeil. The accepted level of significance was 0.05. The analysis was done with the statistical package IBM SPSS Statistics 20 (NY) and MedCalc 12.5.0 (Belgium).

Results

Among the entire cohort of 130 volunteers, 70 had DFS and other volunteers were in the control group. The study included 80 women and 50 men aged 54.56 ± 14.22 years. In the study group, there were 36 women, and in the control group there were 44 women. Compared to the control group, the patients with DFS were significantly older (t = -12.531, p = 0.000). The patients with DFS were 60.17 ± 12.40 years old on average while in the group of the healthy volunteers the mean age was 47.64 ± 13.31 years. In the group of the patients with DFS, 23 of them had previous ulcer and other patients had the actual micro and/or macro foot lesions.

The student *t*-test showed that the patients with DFS exhibited significantly elevated values of FBG, HbA1c and LDLc in comparison to the control group. In other metabolic parameters no differences were noted between the groups (Table 1). In comparison with the controls, the patients with DFS had a significantly higher BMI (Table 1).

Using the ROC procedure, 5 significant predictors of DFS were proved (FBG, PPG, HbA1c, LDLc and BMI). The ROC procedure parameters are shown in Table 2.

All area under the curve (AUC) values of glycometabolic variables were higher in comparison to the AUC for BMI, and the AUC for LDLc (Table 3 and Figure 1). Also, the AUC for FBG was larger in comparison to the AUC for PPG. Between the AUC for HbA1c related to the AUC for FBG and the AUC for HbA1c related to the AUC for PPG no statistically significant difference was found.

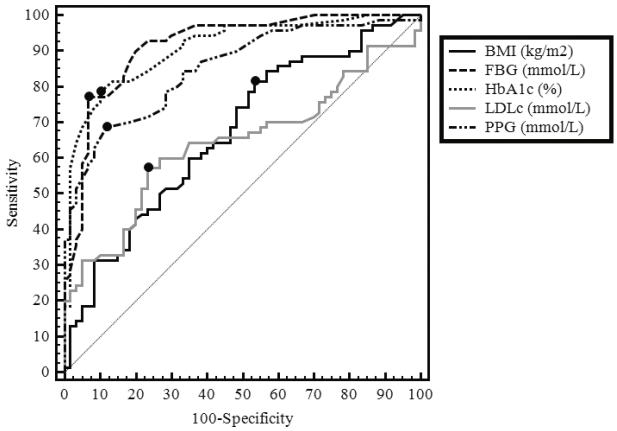

Fig. 1 – Receiver operating characteristic curve (ROC) of the metabolic and anthropometric variables in diabetic foot syndrome detection.
BMI – body mass index; FBG – fasting blood glucose; PPG – 2-h postprandial plasma glucose; HbA1c – glycated hemoglobin; LDLc – low density lipoprotein cholesterol.

Table 1

Descriptive statistics for antrophometric and metabolic variables with the significance of differences between groups (healthy volunteers – HV vs. diabetic foot syndrome – DFS)

Variables	Group		*p* (Student *t*-value)
	HV(n_1=60) Mean ± SD	DFS (n_2=70) Mean ± SD	
BMI (kg/cm^2)	25.89 ± 4.55	28.82 ± 4.66	0.000 (-3.610)
FBG (mmol/L)	5.18 ± 0.94	7.55 ± 1.69	0.000 (-10.056)
PPG (mmol/L)	6.06 ± 1.04	8.54 ± 2.57	0.000 (-7.425)
HbA1c (%)	5.55 ± 0.78	7.48 ± 1.33	0.000 (-10.236)
Total cholesterol (mmol/L)	5.38 ± 0.78	5.95 ± 1.28	0.002 (-3.120)
HDLc (mmol/L)	1.14 ± 0.28	1.16 ± 0.54	0.811 (-0.239)
LDLc (mmol/L)	3.90 ± 0.83	4.41 ± 1.32	0.009 (-2.661)
Triglycerides (mmol/L)	1.68 ± 0.97	1.90 ± 0.80	0.168 (-1.388)

BMI – body mass index; FBG – fasting blood glucose; PPG – 2h post prandial plasma glucose; HbA1c – glycated hemoglobin; HDLc – high density lipoprotein cholesterol; LDLc – low density lipoprotein cholesterol; SD – standard deviation.

Table 2

Receiver operating characteristic (ROC) curve analysis of the significant metabolic and anthropometric variables in the detection of diabetic foot syndrome

Variables	AUC	SE for AUC	95% CI for AUC	*p* (z)	Cut point	SN (%) (95% CI)	SP (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
FBG	0.915	0.025	0.854–0.957	0.000 (16.452)	> 6.3	77.14 (65.6–86.3)	93.33 (83.8 – 98.2)	93.1 (83.3–98.1)	77.8 (66.4–86.7)
HbA1c	0.908	0.026	0.845–0.952	0.000 (15.808)	> 6.3	78.57 (67.1–87.5)	90.00 (79.5–96.2)	88.7 (77.5–95.6)	80.8 (69.8–89.1)
PPG	0.851	0.033	0.779–0.908	0.000 (10.731)	> 7.1	68.57 (56.4–79.1)	88.33 (77.4–95.2)	85.5 (72.9–93.7)	73.8 (62.5–83.1)
LDLc	0.642	0.049	0.553–0.724	0.004 (2.899)	> 4.39	57.14 (44.7–68.9)	76.67 (64.0–86.6)	71.0 (56.8–82.7)	64.1 (52.4–74.8)
BMI	0.669	0.047	0.582–0.750	0.000 (3.574)	> 25	81.43 (70.3–89.7)	46.67 (33.7–60.0)	60.4 (49.4–70.8)	71.5 55.5–84.4

AUC – area under the curve; SE – standard error; CI – confidence interval; z – normal distribution zed value; SN – sensitivity; SP – specificity; PPV – positive predictive value; NPV – negative predictive value; BMI – body mass index; FBG – fasting blood glucose; PPG – 2h postprandial plasma glucose; HbA1c – glycated hemoglobin; LDLc – low density lipoprotein cholesterol.

Table 3

Difference between the area under curves (AUC) pairs with the metabolic and anthropometric variables in the diabetic foot syndrome prediction

	Difference between AUC	SE	95% CI	z	*p*
BMI vs FBG	0.246	0.050	0.147–0.345	4.869	0.000
BMI vs HbA1c	0.239	0.051	0.138–0.339	4.649	0.000
BMI vs LDLc	0.027	0.066	0.102–0.157	0.416	0.678
BMI vs PPG	0.182	0.056	0.072–0.292	3.232	0.001
FBG vs HbA1c	0.007	0.027	0.046–0.060	0.263	0.792
FBG vs LDLc	0.273	0.052	0.172–0.375	5.265	0.000
FBG vs PPG	0.064	0.032	0.001–0.126	2.002	0.045
HbA1c vs LDLc	0.266	0.050	0.168–0.365	5.289	0.000
HbA1c vs PPG	0.057	0.029	0.001–0.115	1.918	0.055
LDLc vs PPG	0.210	0.055	0.102–0.317	3.816	0.000

AUC – area under the curve; SE – standard error; CI – confidence interval; z – normal distribution zed value; BMI – body mass index; FBG – fasting blood glucose; PPG – 2h postprandial plasma glucose; HbA1c – glycated hemoglobin; LDLc – low density lipoprotein cholesterol.

Discussion

In this study, we dealt with the assessment of the validity of metabolic and anthropometric parameters as the predictors of the DFS. DFS is a late-diagnosed DM complication, mainly due to the lack of instruments for a reliable early diagnosis in primary care. Due to the multidisciplinary approach to this problem, the possible risk factors have been selectively investigated in the research so far, and due to the non-standardized instruments no consensus over the prevention of DFS has been achieved within the World Health Organization.

Former studies dealing with DFS were mostly related to the cost-effectiveness of treatment of the patients suffering from foot ulcers, morbidity, mortality and a treatment for the special care patients if compared to those who were the diabetic patients without DFS, while no research with the healthy populations as the control group was undertook [17].

Our results showed that HbA1c is the most important independent predictor of DFS among the metabolic parameters, which is consistent with other studies [18]. According to our analysis, the cut-point for HbA1c is 6.3%. The resulting value is lower than the value according to the American Diabetes Association (ADA) guidelines for HbA1c (threshold ≥ 6.5%) and the recommended values for HbA1c (threshold = 7.0%) according to the European Association for the Study of Diabetes (EASD) criteria [19].

When the HbA1c ≥ 6.5 %, the PPG levels contribute to a large portion of this value. If we take into account that sudden increases in blood glucose cause oxidative stress and induce endothelial dysfunction, which leads to the chronic complications of DM, then special importance is attached to PPG. According to the ADA recommendations, the PPG value was higher and measured at 11.1 mmol/L. Having analyzed the results presented in Diabetes Epidemiology: collaborative analysis in Europe (DECODE) and Diabetes Epidemiology: collaborative analysis in Asia (DECODA) studies, the PPG is given priority over FBG regarding their predictive values in predicting chronic complications [20]. The results of our study showed the lower PPG values if compared

to the recommendations. The cut-point we obtained was at 7.0 mmol/L and closer to the ADA pre-diabetes criteria. Concerning the PPG testing, in this study, we got a larger area under the curve for fasting blood glucose. These three parameters – FBG, HbA1c and PPG – make the three most important therapeutic goals of achieving the optimal glycemic control.

The experts on the diagnosis and classification of DM defined the criteria for impaired fasting glucose (IFG) at 5.6–6.9 mmol/L, and the World Health Organization set IFG cutoff at 6.1 mmol/L. According to the American Diabetes Association (ADA) criteria, the DM value is slightly higher – at 6.5 mmol/L, and the European Association for the Study of Diabaetes (EASD) suggested the value of 7.0 mmol/L. In our results, the FBG cut-point is at 6.3 mmol/L which requires starting the treatment much earlier in order to prevent DFS, even, in accordance with the current FBG criteria, in the pre-diabetes phase [19].

LDLc in our study appeared to be an important metabolic predictor of DFS. The values obtained are consistent with the results of the Framingham Heart Study, the Multiple Risk Factor Intervention Trial (MRFIT), where LDLc was identified as a risk factor of the utmost importance along with FBG and PPG [21]. In the current guidelines for the prevention of coronary heart disease in the diabetic patients, elevated LDLc is the primary target of the lipid-lowering therapy.

In the patients with low cardiovascular risk, the target LDLc value was at 2.6 mmol/L and in those with a high cardiovascular risk, it was at 1.8 mmol/L.

In our results, the validity of LDL in the prediction of DFS is lower than the validity of the BMI. We obtained the cut-point value of 4.39 mmol/L. Following the ADA criteria, some studies showed that only 58.5 % of the patients reached the target LDLc value during treatment, while 7.2% achieved the target values for both LDLc and HbA1c simultaneously [22]. In favor of and in addition to previously stated, the treatment of dyslipidemia in the DM patients should be commenced earlier, from the very onset of the disease.

In our study, the BMI was singled out as an important anthropometric parameter and, according to its validity in relation to the metabolic parameters; it had the smallest area under the curve, the cut-point being at 25 kg/m^2. Previous studies showed that the prevalence of diabetic foot ulceration is higher in the people with BMI > 30 kg/m^2. In the RICH-ARD investigators study conducted on 2,339 patients the mean BMI was 29.9 kg/m^2, and even 42.9% had the BMI ≥ 30 kg/m^2 [23]. Our results showed that body weight regulation is significant in the prevention of DFS which is consistent with the results of Gray et al. [24], who showed that elevated BMI is associated with the progressively higher risk of complications from DM, specifically for DFS, and cardiovascular risk in men when the BMI from 27.5 kg/m^2 to 29.9 kg/m^2, or when 25 kg/m^2 to 27.49 kg/m^2 in women.

Previous studies pointed out the need for better approach to prevent DFS, including the intensive patient education and specialist nurse education in primary health care under the supervision of the diabetologists. Such investment is certainly justified, but one should not ignore the importance of the biochemical markers known as the predictors of DM-related complications.

Our study's limitations are connected to a relatively small study population. Additionally, in regards to the age of the patients in the group with DFS, there was no equity, compared to the control group, considering the fact that the patients with DFS were significantly older.

Conclusion

According to the results of our study, if we are to forestall the emergence of diabetic foot syndrome as a diabetes mellitus related complication, the treatment of the patients with elevated values of fasting blood glucose, postprandial plasma glucose, glycosylated haemoglobin and low density lipoprotein cholesterol should begin much earlier, at the lower values than currently recommended in diagnosing diabetes mellitus, and the reduction of the body mass index should be given even greater emphasis in the period of pre-obesity. Of course, we need and expect additional studies to be conducted on the larger groups of patients.

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Antibacterial effects of new endodontic materials based on calcium silicates

Antibakterijski efekti novih endodontskih materijala na bazi kalcijum silikata

Dijana Trišić*, Bojana Ćetenović†, Nemanja Zdravković‡, Tatjana Marković§, Biljana Dojčinović‖, Vukoman Jokanović†, Dejan Marković*

University of Belgrade, *Faculty of Dental Medicine, †Institute for Nuclear Sciences "Vinča", ‡Faculty of Veterinary Medicine, §Institute for Medicinal Plants Research "Dr Josif Pančić", ‖Institute of Chemistry, Technology and Metallurgy, Belgrade, Serbia

Abstract

Background/Aim. The main task of endodontic treatment is to eliminate pathologically altered tissue, to disinfect root canal space and to obtain its three-dimensional hermetic obturation. The main purpose of this study was to evaluate antimicrobial activity of new endodontic nano-structured highly active calcium silicates based materials albo-mineral plyoxide carbonate aggregate (ALBO-MPCA) and calcium silicates (CS) in comparison to mineral trioxide aggregate (MTA$^+$) and UltraCal XS (CH). **Methods.** The antimicrobial activity of materials was tested against *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 14506) strains, and following clinical isolates: *Rothia dentocariosa, Enterococcus faecalis, Staphylococcus aureus, Streptococcus anginosus* and *Streptococcus vestibularis* using a double layer agar diffusion test. The pH measurements were performed using the pH meter. Total amount of released ions was determined by inductively coupled plasma optical emission spectrometry (ICP-OES). **Results.** All tested materials showed the best antibacterial potential after 1 h of incubation. After 3h and 24h of the incubation period, the antibacterial potential of all tested materials were similar. The Agar diffusion test showed that ALBO-MPCA, CS and MTA$^+$ had similar inhibition zones ($p > 0.05$), except in the activity against *Staphylococcus aureus* where ALBO-MPCA showed better antimicrobial properties than MTA$^+$ in 3h and 24h of the incubation period ($p < 0.05$). Following 24h of the incubation, the inhibition zones were the strongest with CH against *Staphylococcus aureus* (16.67 ± 2.34 mm) followed by ALBO-MPCA (14.67 ± 1.21 mm) and the weakest with CS against *Enterococcus faecalis* (6.50 ± 1.76 mm). CH showed the highest pH, followed by ALBO-MPCA, CS and MTA$^+$. **Conclusion.** The expressed antibacterial effects indicate that materials based on nano-structured highly active calcium silicates represent effective therapeutic agents for root canal obturation in one-visit apexification treatment, therefore they are recommend for further examination and clinical trials as they are proposed for MTA substitution.

Key words:
dental pulp diseases; root canal preparation; calcium silicate; calcium hydroxide; anti-infective agents.

Apstrakt

Uvod/Cilj. Osnovni cilj endodonskog lečenja je eliminacija patološki izmenjenog tkiva, eliminacija infekcije korensko kanala i njegovo hermetičko trodimenzionalno zatvaranje. Cilj istraživanja je bio da se proceni antibakterijska aktivnost novih endodontskih nano-strukturiranih materijala na bazi visoko aktivnih kalcijum silikata *albo-mineral polyoxide carbonate aggregate* (ALBO-MPCA) i *calcium silicates* (CS) u odnosu na *mineral trioxide aggregate* (MTA$^+$) i *UltraCal XS* (CH). **Metode.** Testirana je antibakterijska aktivnost materijala protiv *Staphylococcus aureus* (ATCC 25923) i *Enterococcus faecalis* (ATCC 14506), kao i kliničkih izolata: *Rothia dentocariosa, Enterococcus faecalis, Staphylococcus aureus, Streptococcus anginosus* i *Streptococcus vestibularis* pomoću agar difuzionog testa. Merenja pH vrednosti obavljena su korišćenjem pH metra. Ukupan iznos oslobođenih jona određivan je pomoću ICP-OES. **Rezultati.** Svi testirani materijali pokazali su najbolji antibakterijski efekat nakon 1 h od inkubacije. Nakon 3 h i 24 h od inkubacije, antibakterijski efekat svih testiranih materijala bio je sličan. Agar difuzioni test pokazao je da materijali ALBO-MPCA, CS i MTA$^+$ ispoljavaju slične zone inhibicije rasta ($p > 0.05$) osim u slučaju *Staphylococcus aureus*, gde je materijal ALBO-MPCA pokazao bolje antibakterijsko dejstvo nego MTA$^+$ nakon 3 h i 24 h od inkubacije ($p < 0.05$). Nakon 24 h od inkubacije, zone inhibicije su bile najizraženije u slučaju materijala CS protiv *Staphylococcus aureus* (16.67 ± 2.34 mm), zatim ALBO-MPCA (14.67 ± 1.21 mm), a najslabije u slučaju CS protiv *Enterococcus faecalis* (6.50±1.76 mm). Materijal CH ispoljio je najveću pH vrednost, zatim ALBO-MPCA, CS i MTA$^+$. **Zaključak.** Ispoljeni antibakterijski efekti ukazuju na to da materijali na bazi visoko aktivnih

Correspondence to: Dijana Trišić, Faculty of Dental Medicine, 6 dr Subotića, 11 000 Belgrade, Serbia. E-mail: diana.trisic@hotmail.com

kalcijum silikata mogu da predstavljaju efikasnu zamenu za MTA u terapiji zuba sa nezavršenim rastom korena u jednoj poseti, te se stoga preporučuju za dalja klinička ispitivanja.

Ključne reči:
zub, bolesti pulpe; zub, lečenje korenskog kanala; kalcijum silikat; kalcijum hidroksid; antiinfektivi.

Introduction

The main task of endodontic treatment is to eliminate pathologically altered tissue, to disinfect root canal space and to obtain its three-dimensional hermetic obturation, as residual microorganisms are usually present in apical ramifications and isthmuses that are never completely filled [1]. More than 99.5 % of Gram-positive bacteria, is eliminated by a proper chemo-mechanical root canal treatment [2]. Residual microorganisms, particularly *Enterococcus* and *Streptococcus* species, are considered to be responsible for the treatment failure [3]. Moreover, *Enterococcus faecalis* has the ability to bind with the collagen fibers and survive up to 12 months in the environment without the substrate [3]. Facultative anaerobes and Gram-positive species, revealing a heterogeneous profile of polymicrobial infection are frequently isolated from the root canals following an unsuccessful endodontic treatment [2].

An ideal material for root canal obturation must prevent both, apical and coronal leakage. It has to be biocompatible, noncancerous and nongenotoxic, dimensionally stable and insoluble in tissue fluids. Considering the ability of residual microorganisms to provoke periapical irritations, it is preferable for sealing materials to possess antibacterial activity [4].

So far, the sealers based on calcium hydroxide proved to be the most efficient against a range of pathogenic microorganisms [5]. Their major advantage is a high pH which is toxic to bacterial cells, leading most likely to protein denaturation and damages of cytoplasmic membrane or DNA [6]. However, it is also proved that the calcium hydroxide based sealers have a limited antimicrobial effect on *Enterococcus faecalis* [6].

In the early 1990, different commercial products of mineral trioxide aggregate (ProRoot MTA, WMTA Angelus, GMTA Angelus) were synthesized. Initially, MTA was recommended as a root-end filling material, while today it is used in a number of endodontic procedures, particularly as an apical barrier in teeth with incomplete root development [7]. MTA is composed of hydrophilic particles which, in the presence of water, form a colloidal gel that is transformed into solid cement [8]. When mechanically mixed, MTA based materials achieve better marginal adaptation, and consequently possess better sealing property [9]. The high pH value achieved during the setting suggests a potential antibacterial activity of the material [10]. Due to variations in the chemical composition of MTA based materials, and the grain size, differences in hydration rate, flowability, consistency and setting time can be expected [8]. Incorporation of the hydrosoluble polymer can reduce dry consistency of MTA based materials and thus to improve the material handling [8]. Several attempts were made to improve the MTA manipulation characteristics which complicate its use during the orthograde canal filling procedures [11–13]. Similar to MTA, new nano-structured materials, calcium silicates (CS) and albo-mineral polyoxide carbonate aggregate (ALBO-MPCA), with the reduced setting time and morphology which provides a distinct activity after their placing into vital tissues, were introduced [14, 15].

The aim of this study was to evaluate pH, ion release and the antimicrobial effects of two new endodontic materials based on nano-structured highly active calcium silicates (ALBO-MPCA and CS) in comparison to MTA^{+} and Ultra-Cal XS (CH).

Methods

The study was carried out at the University of Belgrade: Faculty of Dental Medicine, Institute for Nuclear Sciences "Vinča", Faculty of Veterinary Medicine and Institute of Chemistry, Technology and Metallurgy. Prior to conducting this study, informed written consent was obtained from the patients. The study was designed in accordance with the guidelines of the Declaration of Helsinki and approved by the Ethics Committee.

The isolation of microorganisms

All clinical isolates used in the experiment were obtained at the University Clinic, from the patients' infected root canals, using the endodontic needles. The endodontic needle samples were taken in pairs (for aerobic and anaerobic cultivation) and collected in thioglycollate broth (Institute of Virology, Vaccines and Sera-Torlak, Belgrade, Serbia) and brain heart infusion agar (BHI, Becton, Dickinson and Company, Sparks, USA) and left for 24 h at 37°C. The overnight cultures were streaked on the appropriate media for cultivation; aerobic cultures on Columbia agar with 5% sheep blood (COS, bioMérieux, Marcy-l'Étoile, France) and MacConkey agar (Becton, Dickinson and Company, Sparks, USA) and incubated in the aerobic atmosphere overnight, while anaerobic ones on Columbia agar with 5% sheep blood and incubated in a jar under the anaerobic conditions using GasPack (GasPak™ EZ Gas Generating Container Systems, Becton, Dickinson and Company, USA), at 37°C for 2 to 5 days. The grown bacterial colonies from the anaerobic conditions were put on Columbia agar with 5% sheep blood at 37°C overnight to determine demand for obligatory anaerobiosis in such bacteria. Preliminary identification of clinical isolates was done by the Gram stain, hemolysis on chitodigosaccharide (COS), catalase, oxidase (Oxidase Reagent Droppers Becton, Dickinson and Company, USA) and coagulase tests (Rabbit plasma, Veterinary Medicine Institute Inc., Zemun, Serbia). In order to confirm the identification of the Gram positive bacteria, the BD BBL Crystal™ Identification Systems Gram–Positive ID (Becton, Dickinson and Company, Sparks, USA) was conducted.

Materials

For the synthesis of two new nano-structured materials based on the active silicate systems calcium silicates (CS) and albo-mineral polyoxide carbonate aggregate (ALBO-MPCA), mixture components were prepared [14, 15]. Briefly, calcium silicate phases, 2β-$CaSiO_4$ (β-C_2S) and Ca_3SiO_5 (C_3S), were synthesized using stoichiometric quantities of $CaCl_2 \times 5H_2O$ and silica sol by hydrothermal treatment, in the following ratio: $C_3S : C_2S = 2 : 1$. $Al(C_2H_3O_2)$ was added to allow the production of an active C_3A phase. Calcium chloride tetrahydrate was used as the precursor for production of $CaCO_3$, while sulfonyl dodecyl sulfate was added as an antiagglomeration agent. The final mixture was made by mixing $CaCO_3$ with calcium silicate phases (C_3S and β-C_2S) in the case of CS, while the monoclinic Bi_2O_3 was added in case of as ALBO-MPCA as a radiocontrast agent.

As the control materials, mineral trioxide aggregate (MTA^+, Cercamed, Stalowa Wola- Poland), consisting of calcium hydroxide and silicon, iron, aluminium, sodium, potassium, bismuth, magnesium oxides and calcium phosphate as well as calcium hydroxide based paste (UltraCal XS, UltraDent, South Jordan, USA) were used.

Agar diffusion test

The antimicrobial activities were examined against the following bacterial strains: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 14506 and clinical isolates: *Enterococcus faecalis*, *Staphylococcus aureus*, *Rothia dentocariosa*, *Streptococcus anginosus* and *Streptococcus vestibularis*. After activation form the stock culture, microorganisms were maintained as the overnight cultures on Cation Adjusted Mueller-Hinton Broth (CAMHB, Becton, Dickinson and Company, Sparks, USA) and seeded on Cation Adjusted Mueller-Hinton agar (CAMHA, Becton, Dickinson and Company, USA) and COS at 37°C for 24 h before use.

The examination of antimicrobial activity of endodontic materials was conducted by the double layer agar diffusion test (ADT) on the 90 mm sterile Petri plates. The base layer was made of 10 mL sterilized CAMHA. After 24 h, four uniform wells (5 mm in diameter), each one corresponding to a single tested sealer, were made by the sterile plastic tubes and filled with the freshly mixed materials. The seeding layer that was put over the base, consisted of 10 ml sterile CAMHA inoculated to achieve 10^8 (CFU)/mL of tested bacteria, which corresponds to the 0.5 McFarland scale. The plates were left at room temperature for 2 h, in order to allow prediffusion of materials, and after that they were incubated for 1 h, 3 h and 24 h, at 37°C. Aliquots of 5 mL of triphenyltetrazolium chloride (TTC) prepared with 0.05% of TTC and 1% CAMHA were added for optimization. After solidification of CAMHA+TTC, the plates were incubated for 30 min at 37°C. Negative control was conducted using the same method without placing the materials. The diameters of inhibition zones of bacterial growth were measured in above mentioned time intervals. All tests were done in sixtiplicate, except the positive controls which were done in triplicate.

pH measurements

All pH values were repeatedly measured (three times), using the pH-meter (pH-vision Microcomputer 6071, JENCO Electronics Ltd., Linkou Shiang, Taiwan) combined with the HI-type electrode 1131 (Hanna Instruments WTW GmbH, Woonsockets, RI-USA). The calibration of pH-meter was performed using biftalato (pH = 4.01) and phosphate buffer (pH = 7.00) (Carlo Erba Reagents SpA, Rodano, Italy). Suspensions of 50 mg/mL of each tested material into deionized water were prepared, then shaken on vortex for 30 min and centrifuged for 15 min at 4000 rpm. Readouts of the pH measurements were carried out after 1 h, 3 h and 24 h. The solutions of deionized water were used as controls (5.76 ± 0.51).

Inductively coupled plasma-optical emission spectroscopy (ICP-OES) analysis

Investigated materials were prepared according to the manufacturers instruction and placed into the plastic molds (5 mm in diameter and 5 mm high) to set. After the setting, the discs of each investigated materials were placed into 20 mL of deionized water (n = 3). Deionized water was changed after 1 h, 3 h and 24 h and the concentrations of ions were measured using the Thermo Scientific iCAP 6500 Duo ICP (Thermo Fisher Scientific, Cambridge, UK) spectrometer equipped with the RACID86 Charge Injector Device detector, concentric PTFE nebulizer, quartz torch and alumina injector. The ICP-OES measurements for each sample were carried out three times. Quantifications of released ions into deionized water were performed at the adequate emission wavelength of light.

Statistical analysis

Data analysis was performed using the ANOVA Repeated Measures test, and post hoc Tukeys' test. The level of significance was set at $p < 0.05$ and the data were processed using the statistical software IBM SPSS 20.

Results

The data obtained in the ADT for each of the investigated materials are presented in Figures 1–7. The CH had the largest inhibitions zones against all bacterial strains (Figure 8). The inhibition zones of tested materials, 24 h following the incubation, were the largest with the CH against *Staphylococcus aureus* (16.67 ± 2.34 mm) followed by the ALBO-MPCA (14.67 ± 1.21 mm), and the weakest with the CS against *Enterococcus faecalis* (6.50 ± 1.76 mm). *Streptococcus anginosus* did not exhibit any growth after 1 h. The statistically significant differences were observed between the CH and other investigated materials with respect to: *Streptococcus anginosus* and *Enterococcus faecalis*; *Enterococcus faecalis* ATCC and *Streptococcus vestibularis*, except between CH (24 h following the incubation) and ALBO-MPCA (1 h following the incubation). The statistically significant

differences concerning antibacterial activity against *Staphylococcus aureus* were also registered between: the CH and MTA$^+$, in all observation periods; the CH and CS (3 h and 24 h following the incubation); the MTA$^+$ (3 h and 24 h following the incubation) and ALBO-MPCA (1 h and 3 h following the incubation).

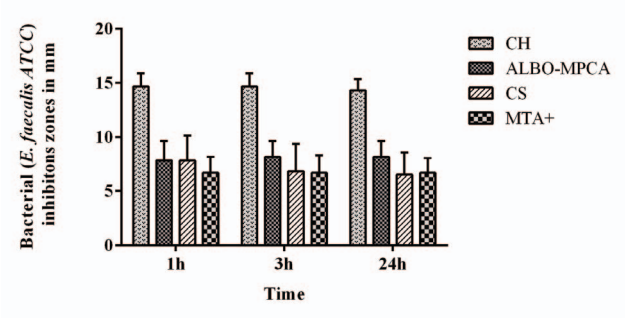

Fig. 1 – Inhibition zones of *Euterococcus faecalis* (ATCC14506) determined by the double layer agar diffusion test in different time periods.
CH- UltraCal XS; MTA- mineral trioxide aggregate, MTA$^+$; ALBO-MPCA- calcium silicate based material with Bi_2O_3; CS- calcium silicate based material without Bi_2O_3.

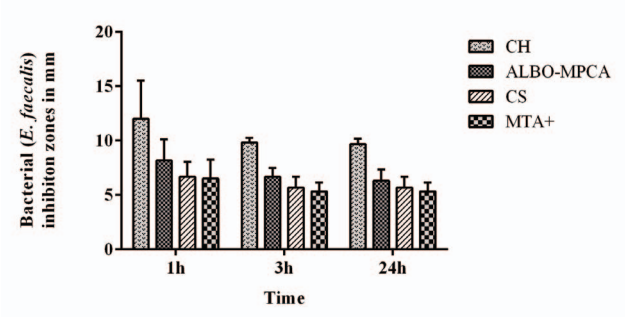

Fig. 2 – Inhibition zones of *Euterococcus faecalis* determined by the double layer agar diffusion test in different time periods.
CH- UltraCal XS; MTA- mineral trioxide aggregate, MTA$^+$; ALBO-MPCA- calcium silicate based material with Bi_2O_3; CS- calcium silicate based material without Bi_2O_3.

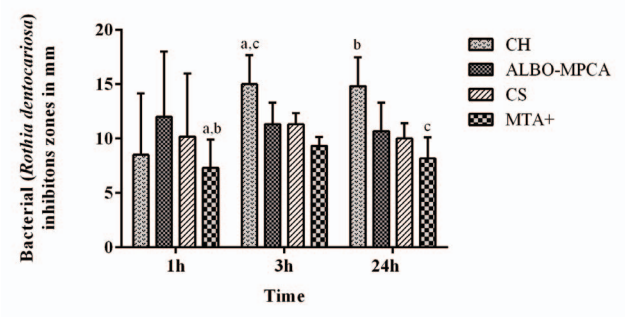

Fig. 3 – Inhibition zones of *Rothia dentocariosa* determined by the double layer agar diffusion test in different time periods.
CH- UltraCal XS; MTA- mineral trioxide aggregate, MTA$^+$; ALBO-MPCA- calcium silicate based material with Bi_2O_3; CS- calcium silicate based material without Bi_2O_3. Different small letters indicate the statistically significant differences between the tested materials ($p < 0.05$).

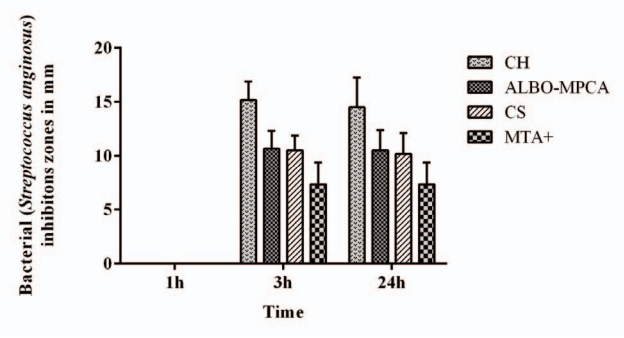

Fig. 4 – Inhibition zones of *Streptococcus anginosus* determined by the double layer agar diffusion test in different time periods.
CH- UltraCal XS; MTA- mineral trioxide aggregate, MTA$^+$; ALBO-MPCA- calcium silicate based material with Bi_2O_3; CS- calcium silicate based material without Bi_2O_3.

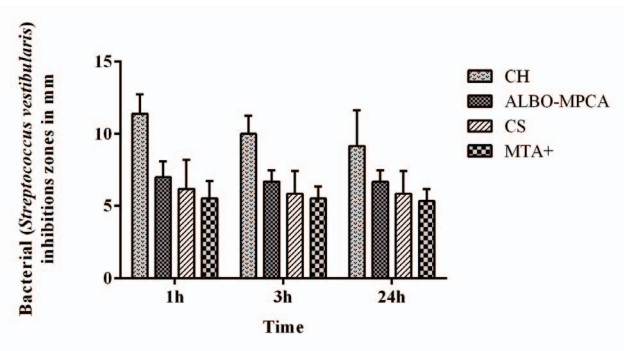

Fig. 5 – Inhibition zones of *Streptococcus vestibularis* determined by the double layer agar diffusion test in different time periods.
CH- UltraCal XS; MTA- mineral trioxide aggregate, MTA$^+$; ALBO-MPCA- calcium silicate based material with Bi_2O_3; CS- calcium silicate based material without Bi_2O_3.

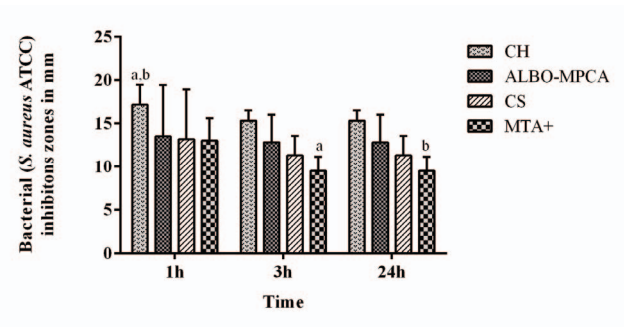

Fig. 6 – Inhibition zones of *Staphylococcus aureus* (ATCC 25923) determined by double layer agar diffusion test in different time periods.
CH- UltraCal XS; MTA- mineral trioxide aggregate, MTA$^+$; ALBO-MPCA- calcium silicate based material with Bi_2O_3; CS- calcium silicate based material without Bi_2O_3. Different small letters indicate the statistically significant differences between the tested materials ($p < 0.05$).

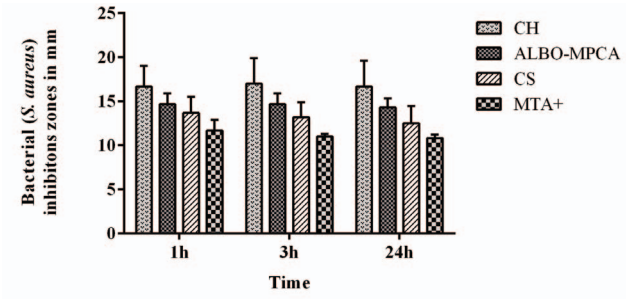

Fig. 7 – Inhibition zones of *Staphylococcus aureus* (in mm) determined by the double layer agar diffusion test in different time periods.
CH- UltraCal XS; MTA- mineral trioxide aggregate, MTA^+; ALBO-MPCA- calcium silicate based material with Bi_2O_3; CS- calcium silicate based material without Bi_2O_3.

The values of inhibitions zones decreased over time in most tested bacterial strains and incubation periods, but increased or remained in size in the certain cases: ALBO-MPCA against *E. faecalis* ATCC 14506 (8.17 ± 1.47); CH (14.83 ± 2.64) and MTA^+ (8.17 ± 1.94) against *Rothia dentocariosa*; and CH (16.67 ±2.94) against *S. aureus*. Although without observed statistical differences, the investigated materials in our study seem to show better antibacterial activity against clinical isolates in comparison to *S. aureus* ATCC 25923 strain, with an exception in case of the CH and the MTA^+ 1h following the incubation. On the contrary, the smaller inhibition zones concerning clinical isolates of *E. faecalis* were observed, then the referent strain.

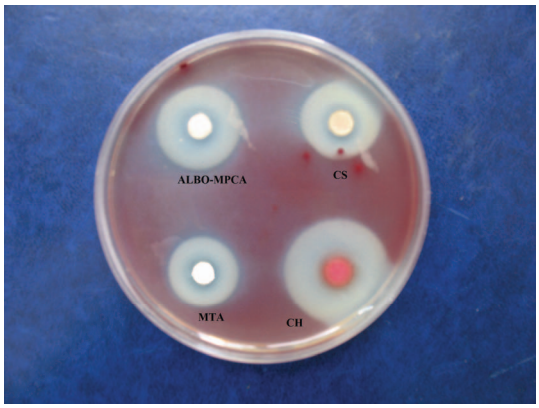

Fig. 8 – Representative inhibitions zones of a clinical isolate *Staphylococcus aureus* determined by the double layer agar diffusion test, after 1 hour.
CH- UltraCal XS; MTA- mineral trioxide aggregate, MTA^+; ALBO-MPCA- calcium silicate based material with Bi_2O_3; CS- calcium silicate based material without Bi_2O_3.

The mean pH values of investigated materials are presented in Table 1. All of them acquired the pH values above 11, with an increasing trend during time, except in the case of the MTA^+. The pH values for the MTA^+ were the lowest (8.23 ± 0.01), but still alkaline.

Table 1

Mean values and standard deviations of pH in different time intervals

Materials	1 h	3 h	24 h
CH	12.42 ± 0.01	12.35 ± 0.06	12.40 ± 0.01
ALBO-MPCA	11.54 ± 0.01	11.70 ± 0.01	12.13 ± 0.15
CS	11.19 ± 0.01	11.30 ± 0.01	11.75 ± 0.01
MTA^+	10.68 ± 0.01	9.04 ± 0.01	8.23 ± 0.01

Note: There are no statistically significant differences among tested materials ($p > 0.05$).
CH – UltraCal XS; ALBO-MPCA – albo-mineral polyoxide carbonate aggregate; CS – calcium silicates; MTA – mineral trioxide aggregate; h – hour.

Table 2

Cumulative ion release (mean value) by the investigated materials into deionized water (ppb)

Materials*	Time	Al	Ca	K	Mg	Na	P	Si
MTA^+	1 h	755	44,570	1,997	161	1,775	5	0
	3 h	2,144	80,900	2,506	338	3,233	8	615
	24 h	4,164	115,900	3,313	473	4,548	11	4,546
CS	1 h	255	25,820	11	423	6,356	5	22
	3 h	1,441	67,870	345	728	7,696	9	104
	24 h	2,101	118,780	702	895	8,452	10	3,520
ALBO-MPCA	1 h	936	25,820	3,859	170	1,657	4	717
	3 h	1,437	62,890	5,354	759	10,605	11	2,792
	24 h	3,644	131,990	7,003	1,510	19,867	12	12,556
CH	1 h	0	46,100	940	207	1,498	0	0
	3 h	0	92,820	1,074	383	2,620	0	0
	24 h	0	145,610	1,333	618	4,437	0	0

***For abbreviations see under Table 1; ppb- parts per billion.**

Table 2 represent ion releases by investigated materials into deionized water. The calcium ion release increased over time with regard to all tested materials, except the MTA^+, where the release kept declining. Unlike the cumulative aluminium ion release (MTA^+>ALBO-MPCA>CS>CH), the values for the cumulative release of calcium were as follows: CH>ALBO-MPCA>CS>MTA^+. Although weaker antibacterial performance, AMBO-MPCA had multiple larger potassium, magnesium and sodium ion release compared to CH.

Discussion

The ADT is a widely used method for the determination of antibacterial activity of soluble materials. The results obtained by this method may depend on solubility of tested materials, their ability to diffuse in agar and cell medium [16]. The diffusion ability of materials may be influenced by numerous factors, such as: agar type, contact between material and agar, molecular mass, size and form of antibacterial agent, load and concentration of tested material, agar viscosity, ion concentration in relation to medium, used microorganisms, agar quantity, incubation time, etc. [16]. One of the major limitations of the ADT method is that it is not capable of determining whether material possesses bacteriostatic or bactericidal effect [17].

So far, many researchers reported conflicting results on the antibacterial effects of a range of sealers and their different forms, weather they were freshly mixed or completely set [16, 18]. Nevertheless, sealers may have the ability to release constituents with the antibacterial effects even after their complete setting [19]. Since the sealing materials are commonly applied freshly mixed in everyday clinical practice, in our study we investigated the antibacterial effects of materials in such a form. We left Petri dishes for 2 h at the room temperature to rest in order to achieve prediffusion of the tested materials, which is an important step in demonstrating the antibacterial effects, as previously observed [20]. Optimization with 0.05% TTC was performed in order to differentiate the exact growth of bacterial colonies [20]. Special attention was paid to pre-diffusion and optimization with the (TTC) 2, 3, 5-triphenyltetre zoliumchloride procedures, which allowed us to precisely determine zones of bacterial growth inhibition, and avoid possible misinterpretation with a diffusion capacity of the materials.

Up to now, little attention has been devoted to investigation of antibacterial efficacy of materials similar to MTA against bacterial clinical isolates [21]. With regard to that, a major part of our experiment was conducted using the clinical isolates collected from the infected teeth of patients. Since similar materials previously showed the highest and the lowest antibacterial effects against *E. faecalis* and *S. aureus* [10, 22], in our experiment, we compared the results of ADT using the clinical and ATCC strains of the same two important bacteria. Though without statistical differences and with exceptions in cases of the CH and MTA^+ following 1h of incubation, the investigated materials in our study appeared to possess better antibacterial activity against clinical isolates in comparison to *S. aureus* ATCC strain(s).

The antimicrobial activity of calcium hydroxide based sealers is linked to the release of hydroxyl ions as strong free radicals, and the capacity to absorb carbon dioxide [23]. The similar mechanism may be proposed for the MTA based materials, taking into account their setting process [8]. It is quite familiar that the pH values above 12 inhibit the growth of many microorganisms, including *E. faecalis* [24]. Despite the high pH, even 7-day period time appears to be insufficient for CH pastes to kill bacterial cells in biofilm [25]. Limited antibacterial efficacy against *E. faecalis* for calcium hydroxide based sealers which have pH beyond 12, put this particular bacteria in closer scope. Evens et al. [6] suggest that the main reason for *E. faecalis* resistance lies in proton pumps that exist in its cell membrane. Our results are in agreement that the material solo property extremely high pH value was not sufficient and that apart from it, some other factors also interfere with bacterial growth.

All materials tested in our study had the highest antibacterial effects against *S. aureus* and the lowest against *E. faecalis,* which is in accordance with results of some previous studies [10, 22]. In addition, the MTA based materials may also fail to inhibit the growth of *E. faecalis* [26], but inhibited the growth of caries-associated bacteria [27, 28]. An increase in inhibition zones exceeding 10%, between 1h and 24h, was observed in the case of CH and MTA^+ against *E. faecalis* ATCC 14506, and in the case of CH, MTA^+ and CS against *Rothia dentocariosa*. Our results obviously do not support previously reported ones stating that an increase in duration of incubation leads to a decrease in effectiveness of tested materials [22], which is probably due to differences in the methodology applied (the authors of this study compared 24 hours and 7 days samples), chemical composition of tested materials and bacterial strains. It is known that the CH is formed during hydration reaction of the MTA based materials, but for the complete maturation of different phases the time should be sufficient [8]. This might be a possible reason for acquiring conflicting results, in addition to the fact that the measured pH values may not necessary match the ones achieved during the complex process of MTA setting and thus do not depict *in vivo* conditions.

Tanomaru-Filho et al. [10] showed that the MTA based materials possess the antimicrobial activity against *S. aureus* and *E. faecalis,* although the sealers based on zinc oxide and eugenol made larger inhibition halos. Asgary and Kamrani [29] also tested antibacterial activity of gray GMTA and WMTA, CH and a new endodontic cement (NEC) on the same bacteria species and confirmed the antibacterial activity of all tested materials, with significant differences observed between the CH and NEC in comparison to the MTAs. The conclusions reported by Holt et al. [30] and Sipert et al. [31] were similar, beside that the antibacterial activity may be increased by the aerobic conditions (created by inducing reactive oxygen species) [32]. In contrast to the previous studies, Yasuda et al. [33] and Miyagak et al. [34] concluded that the ProRoot MTA had no antimicrobial activity against any investigated species (*S. aureus, E. faecalis, C. albicans, S. mutans* and *S. sanguinis*), while the AH plus exhibited the highest antimicrobial activity out of all tested materials.

Previous studies showed that aluminium ions possess antibacterial effects [35]. Investigated material MTA$^+$ showed largest aluminum cumulative ion release. Regarding the correlation between aluminium ion release and antibacterial effects, results of our study seem to be not enough conclusive, meaning that the individual impact of other factors had to be further investigated. The CH showed highest cumulative calcium ion release after 24h (145610 ppb), and though an initial calcium release was high with respect to the MTA$^+$ (44570 ppb), it declined over time, but only in the case of this material. The CH also exhibited the smallest sodium cumulative ion release. The above stated information contributes to understanding their antibacterial efficacy and longevity. While sodium is a vital nutrient for many oral *Streptococci,* calcium is alkaline metal with relatively high atomic mass which diffuses slowly [36].

Conclusion

Calcium hydroxide pastes have been considered as a "golden standard" for the treatment of immature teeth for decades, but the risk of tooth fractures, potential reinfections, incomplete calcifications difficulties and consequently therapy duration remains. Considering the fact that materials based on nano-structured highly active calcium silicates possess the favourable physicochemical properties, biocompatibility and as shown in this study, express the satisfactory antibacterial effects, they are the effective therapeutic agents for root canal obturation in one-visit apexification treatment and thus significantly decrease duration of therapy. The microbiological properties of new-age nano-structured highly-active materials CS and ALBO-MPCA suggest further investigations in clinical aspect and they may substitute the MTA materials in dental medicine of the future.

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Assessment of nursing care-associated predictors of in-hospital mortality in the patients with acute ischemic stroke

Određivanje prediktora intrahospitalnog mortaliteta povezanih sa negom pruženom od strane medicinskog tehničara kod bolesnika sa akutnim ishemijskim moždanim udarom

Angelina Škodrić*, Gorica Marić†‡, Dejana Jovanović*‡, Ljiljana Beslać-Bumbaširević*‡, Darija Kisić-Tepavčević†‡, Tatjana Pekmezović†‡

Note: Angelina Škodrić and Gorica Marić have equal contribution.

Clinical Center of Serbia, Clinic of Neurology, *Department for Emergency Neurology, Belgrade, Serbia; †Institute of Epidemiology, Belgrade, Serbia; University of Belgrade, ‡Faculty of Medicine, Belgrade, Serbia

Abstract

Background/Aim. Stroke remains one of the leading causes of death and disability worldwide. The aim of the study was to determine the nursing care-associated predictors of in-hospital mortality in the patients with acute ischemic stroke (AIS) who were hospitalized at the Department of Emergency Neurology. **Methods.** Prospective cohort study included 59 consecutive patients with AIS admitted to the Department of Emergency Neurology, Neurology Clinic, Clinical Center of Serbia, Belgrade. The patients were followed until discharge or death. For exploring a relationship between the outcome of patients with AIS and different groups of factors, the univariate and multivariate Cox proportionate hazard regression models were used. **Results.** There were 32 male and 27 female patients with AIS. The mean age was 62.5 ± 15.2 years. The average duration of hospitalization was 11.1 ± 9.6 days (median 8 days; range 1–54 days). Almost 80% of patients (47/59; 79.7%) were admitted to the stroke unit, while 12 (20.3%) patients were admitted to the intensive care unit. In the univariate Cox regression analysis the significant variables ($p < 0.05$) were the Morse score ($p = 0.030$) and the type of admission unit ($p = 0.029$). The multivariate predictive model revealed that the type of admission unit (stroke unit *vs* conventional unit) [hazard ratio (HR) = 0.16; $p = 0.032$] was the independent predictor of in-hospital mortality in the patients with AIS. **Conclusion.** The results of this study showed an important role of nursing staff in the recovery of the AIS patients, as well as that admission to the stroke units versus the conventional units is the independent predictor of decreased in-hospital mortality.

Key words:
stroke; mortality; nursing care; nursing; convalescence.

Apstrakt

Uvod/Cilj. Moždani udar i dalje predstavlja jedan od vodećih uzroka umiranja i onesposobljenosti u svetu. Cilj ove studije bio je određivanje prediktora intrahospitalnog mortaliteta povezanih sa negom pruženom od strane medicinskih tehničara kod bolesnika sa akutnim ishemijskim moždanim udarom (AIMU), hospitalizovanih na Odeljenju urgentne neurologije Kliničkog centra Srbije (KCS). **Metode.** Prospektivnom kohortnom studijom obuhvaćeno je 59 konsekutivnih bolesnika sa AIMU primljenih na Odeljenje urgentne neurologije, Klinike za neurologiju, KCS u Beogradu. Bolesnici su praćeni do otpusta, odnosno smrtnog ishoda. U ispitivanju povezanosti različitih grupa faktora sa ishodom bolesnika korišćene su univarijantna i multivarijantna Koksova proporcionalna hazardna regresiona analiza. **Rezultati.** Uzorak je činilo 32 muškaraca i 27 žena sa AIMU. Prosečan uzrast bio je 62,5 ± 15,2 godina. Prosečna dužina hospitalizacije iznosila je 11,1 ± 9,6 dana (medijana 8 dana; opseg 1–54 dana). Blizu 80% bolesnika (47/59; 79,7%) bilo je primljeno u Jedinicu za moždani udar, dok je 12 (20,3%) bilo primljeno u Jedinicu intenzivne nege. Univarijantnom regresionom analizom kao značajne varijable ($p < 0,05$) izdvojile su se Morse skor ($p = 0,030$) i jedinica prijema ($p = 0,029$). Multivarijantni model pokazao je da tip jedinice prijema (Jedinica za moždani udar prema Jedinici konvencionalne nege – [*hazard ratio* (HR) = 0,16; $p = 0,032$] predstavlja nezavisni prediktor intrahospitalnog mortaliteta kod osoba sa AIMU. **Zaključak.** Rezultati studije pokazali su značajnu ulogu

Correspondence to: Tatjana Pekmezović, University of Belgrade, Faculty of Medicine, Clinical Center of Serbia, Institute of Epidemiology, Višegradska 26A, 11 000 Belgrade, Serbia. Tel/Fax: +381 11 3607062. E-mail: pekmezovic@sezampro.rs

medicinskog kadra tokom oporavka bolesnika sa AIMU, kao i da je prijem u Jedinicu za moždani udar prema Jedinici konvencionalne nege, nezavisni prediktor sniženog intrahospitalnog mortaliteta.

Ključne reči:
mozak, infarkt; mortalitet; nega bolesnika; medicinski tehničari; oporavak.

Introduction

Stroke remains one of the leading causes of death and disability worldwide [1]. Recently, it has been shown that in Serbia, during the period 1992–2013, a proportion of deaths due to stroke in overall mortality was 16% [2]. Despite the fact that overall mortality rates decreased, stroke is still the second leading cause of premature death in our country with 314,000 (18.8%) years of life lost (YLL) during the period 1990–2010 [3].

Approximately 4 out of 5 persons with the stroke have the acute ischemic stroke (AIS) subtype[1]. According to the Global Burden of Disease (GBD) 2013 study [4], the worldwide age-standardized mortality rates from AIS have decreased from 1990 to 2013 by 19.6 %. In Serbia, the age-standardized mortality rates per 100,000 persons from this stroke subtype, initially decreased from 2.5 in 1990 to 2.2 in 2003 and afterward slightly increased to 2.3 in 2013 [2].

Although many patient-related predictors of the AIS outcome are well-established, some studies showed that some factors associated with healthcare can influence recovery following AIS as well [5, 6]. There is the emerging evidence that higher level of organized stroke care is associated with the improved outcome [7]. Keeping in mind the fact that the stroke-related mortality differ significantly between health care units, this rate is assumed as one of the most prominent indicators of the stroke-care quality level [8].

The aim of this study was to determine the nursing care-associated predictors of in-hospital mortality in the patients with AIS, at the Department of Emergency Neurology, Neurology Clinic, Clinical Center of Serbia, Belgrade.

Methods

Study design

The prospective cohort study included 59 consecutive patients with AIS admitted to the Department of Emergency Neurology, Neurology Clinic, Clinical Center of Serbia in Belgrade during June and July 2015. The patients were followed until discharge or death. The study was approved by the Institutional Review Board.

Data collection

Data on demographic characteristics, personal and family history, with special emphasis on vascular risk factors, were documented. Information on blood sample analyses and neurological examinations were obtained from medical records. Data on in-hospital complications were also collected. The National Institute of Health Stroke Scale (NIHSS) was used for assessment of stroke severity [9]. Functional dependency was assessed using the modified Rankin Scale (mRS) and the Barthel Index (BI) during first 24 hours after the admission [10]. The Glasgow Coma Scale (GCS) was used for the determination of conscious level [11]. The health care quality level was estimated by the Braden and Morse scales for the assessment of a risk for development of decubital ulcer and a risk of falls, respectively [12, 13].

Statistical analysis

The baseline characteristics of the study sample were presented using descriptive statistics (mean, standard deviation, percentages). Furthermore, for exploring the relationship between the outcome of patients with AIS and different groups of factors, the univariate and the multivariate Cox proportionate hazard regression models were used. In these analyses, the AIS-related death was considered as the dependent variable. The independent variables were separated into two models. The first model ("Scale scores at admission") comprised the baseline scores of NIHSS, BI and mRS, GCS, Morse and Braden scales. The second model ("Nursing factors") comprised the type of admission unit, presence of nasogastric tube, presence of endotracheal tube, mechanical ventilation, presence of urinary tract infection (UTI) and diarrhea during hospitalization. All variables that appeared to be associated ($p < 0.05$) with the endpoint in the univariate analysis were put together to the multivariate Cox proportionate hazard regression analysis in order to determine the independent predictors of in-hospital mortality in the patients with AIS. All analyses were done using the SPSS (Statistical Package for Social Sciences), version 17.0. Probability level of < 0.05 was considered statistically significant.

Results

A total of 59 patients with AIS were enrolled in the study. In 47 (79.7%) patients, it was the first AIS, while 12 (20.3%) patients had the recurrent AIS. The average duration of hospitalization was 11.1 ± 9.6 days (median 8 days, range 1–54 days). Almost 80% of patients [47/59 (79.7%)] were admitted to the stroke unit, while 12 (20.3%) patients were admitted to the conventional unit. The basic demographic characteristics of participants are presented in Table 1. There were 32 male and 27 female patients with AIS. The mean age was 62.5 ± 15.2 years; the youngest patient was 27 years old and the oldest one was 85. A vast majority of patients (61%) had secondary school level. In terms of marital status, more than a half (55.2%) was married. Furthermore, 32.8% of patients were employed.

Table 1

Demographic characteristics of patients with acute ischemic stroke

Variable	Number (%)
Gender	
male	32 (54.2)
female	27 (45.8)
Age (years)	62.5 ±15.2*
Education level	
no education	2 (3.4)
primary school	7 (11.9)
secondary school	36 (61.0)
university	14 (23.7)
Working status	
employed	19 (32.8)
unemployed	15 (25.9)
retired	24 (41.4)
Marital status	
married	32 (55.2)
singled	9 (15.5)
divorced	5 (8.6)
widowed	12 (20.7)

***value is presented as mean ± SD.**

The data on different parameters of the patients at admission are shown in Table 2. The systolic blood pressure values ranged from 104 to 232 mm Hg, while the diastolic blood pressure ranged from 61 to 120 mm Hg. The average value of glycemia was 8.0 ± 4.0 mmol/L. In terms of stroke severity, 33 (55.9%) patients had a mild stroke (NIHSS 0-7), 13 (22.0%) patients had a moderate stroke (NIHSS 8–15), and the same proportion of the patients (22.0%) had a severe stroke (NIHSS > 15). Further, the mean GCS was 13.3 ± 2.9. Functional dependency at admission, measured by mRS and BI was 3.3 ± 1.5 and 47.8 ± 36.5, respectively. The risk of fall (Morse score) at admission was 41.3 ± 15.9. Finally, the risk for decubital ulcer development (Braden score) was 15.4 ± 4.6.

During hospitalization, 6 out of 59 (10.2%) patients died (case-fatality ratio 10.2%). The same proportion (37.3%) of the AIS patients was either discharged or sent to another hospital, while 15.3% was sent to the rehabilitation center. The results of the univariate and multivariate Cox proportionate hazardous regression models are presented in Tables 3 and 4. In the univariate Cox regression analysis, the significant variables ($p < 0.05$) were the Morse score ($p = 0.030$) in the "Scale scores at admission" model and type of admission unit ($p = 0.029$) in the "Nursing care-associated factors" model. The multivariate predictive model revealed that the type of admission unit [stroke unit vs. conventional (HR) = 0.16, $p = 0.032$)] was the independent predictor of in-hospital mortality in the patients with AIS.

Table 2

Clinical characteristics of patients at admission

Variable	Mean ± SD
Systolic blood pressure (mm Hg)	151.6 ± 24.0
Diastolic blood pressure (mm Hg)	89.2 ± 13.7
Temperature (°C)	36.5 ± 0.3
Pulse rate (/min)	85 ± 19
Oxygen saturation (%)	97 ± 2
Glycemia (mmol/L)	8.0 ± 4.0
Platelets ($\times 10^6$/mcL)	219 ± 61
PTT (s)	23.6 ± 4.2
INR	1.2 ± 0.5
NIHSS	9.5 ± 8.8
BI	47.8 ± 36.5
mRS	3.3 ± 1.5
GCS	13.3 ± 2.9
Morse fall scale	41.3 ± 15.9
Braden scale	15.4 ± 4.6

PTT – Partial Thromboplastin Time; INR – International Standardized Ratio; NIHSS – National Institute of Health Stroke Scale; BI – Barthel Index; mRS – modified Rankin Scale; GCS – Glasgow Coma Scale; SD – standard deviation.

Table 3

Univariate Cox proportionate hazard regression model of scale scores at admission

Variable	HR	*p*-value
NIHSS	2.47	0.075
Barthel Index	1.20	0.263
mRS	1.04	0.995
GCS	7.28	0.115
Morse fall scale	1.28	0.030
Braden scale	0.04	0.086

HR – hazard ratio; NIHSS – National Institute of Health Stroke Scale; mRS – Modified Rankin Scale; GCS – Glasgow Coma Scale.

Table 4

Univariate Cox proportional hazard regression of nursing care-associated factors

Variable	HR	*p*-value
Admission unit (stroke unit vs. conventional unit)	0.05	0.031
Nasogastric tube (yes vs. no)	6.67	0.250
Endotracheal tube (yes vs. no)	0.14	0.371
Mechanical ventilation (yes vs. no)	7.60	0.247
UTI during hospitalization (yes vs. no)	1.90	0.718
Diarrhea during hospitalization (yes vs. no)	6.26	0.250

HR – hazard ratio; UTI – urinary tract infection.

Discussion

A numerous studies conducted so far have revealed that the AIS patients admitted to the stroke unit have better survival probabilities, a higher post-stroke level of independency and greater chance to be discharged, compared to the AIS patients admitted to the general wards [14, 15]. There are several potential reasons for such differences which include the 24-hour monitoring, multidisciplinary health care provider teams, involvement of family members, etc. Recently published results of an observational study from Australia revealed that besides all patient-related benefits, the stroke units are also cost-effective [16]. Namely, the authors analyzed data on the patients with a stroke treated before and after establishing the stroke unit in this country, and concluded that although one day of stay in the stroke unit was more expensive compared to the general ward, total costs are lower due to a shorter stay and reduced needs for certain examinations because of their availability in the stroke unit [16]. However, some studies reported different results, i.e., they did not find any differences in the outcome of the AIS patients treated in the stroke unit vs. conventional unit [17]. One of the explanations is a difference in the applied methodological approach. Namely, in clinical trials, the results often showed that the stroke unit was more effective compared to the conventional unit, while in some observational studies, results were contradictory. Consequently, these disparities between the results obtained in the observational and intervention studies could be explained by the artificial conditions seen in clinical trials.

The results of our study also showed that admission to the stroke unit is independent protective factor for the outcome in the AIS patients. The first stroke unit in Serbia was established in 2005 at the Department of Emergency Neurology, Neurology Clinic, Clinical Center of Serbia in Belgrade [18, 19]. The aim was to provide the diagnostic and therapeutic protocols for the stroke patients according to the internationally accepted standards. It comprises six beds and it is equipped with six monitors necessary for the continuous monitoring of electrocardiography (ECG) and vital functions. Furthermore, extracranial color Doppler, transcranial Doppler machine and computed tomography as well as blood analyses and consultative examinations of different specialists are available 24 hours. Also, all-day presence and the work of physiotherapists and speech therapists as well as extended stay of the family with the patient facilitate their rapid recovery. It is worthwhile to mention that the AIS patients treated with intravenous thrombolysis in this stroke unit are included in the prospective, multicenter, observational Serbian Experience with Thrombolysis in Ischemic Stroke Registry (SETIS) [18, 20].

From the time of admission to the stroke unit until discharge, nurses have one of the key functions as health care providers. Well-coordinated stroke care management can result in better outcome of the AIS patients, shorter hospitalization, and reduced costs [21]. One of the most important tasks is monitoring of patients' vital parameters (such as blood pressure, glucose level, heart rate, breathing pattern, temperature) through the regular examinations, bearing in mind that timely response on changes in those parameters can improve patient's outcome [21]. Furthermore, nursing staff have a pivotal role in decreasing a probability of complications occurrence [21]. A prospective cohort study conducted at the Belgrade stroke institutions revealed that the most frequent infections among the AIS patients were urinary tract, intections (UTI) (17.7%) and chest infections (9.7%) [22]. It is well known that complications are associated with worse outcome in those patients [23]. Further, in Korea, the nurses are included in the Stroke Alerts Teams (SAT) organized with the aim to accelerate time to thrombolysis in the patients with AIS [24]. In London, where 8 hyper-acute stroke units were established in 2010, the nursing staff is involved in thrombolysis treatment in terms of the patients' fulfillment of criteria for receiving this therapy, providing accommodation and adequate care [25]. A recent study which compared the stroke care teams in 11 Veterans Affairs medical centers in the USA, concluded that the advanced practice professionals (nurse practitioners and physician assistants) can represent coordinators in a process of stroke care [26]. Beside mentioned duties, early mobilization and education of patients and their families how to prevent reccurrence of AIS are the scope of nursing commitments as well [21]. According to the American Stroke Association (ASA) Guideline for the early management of patients with acute ischemic stroke, the nursing staff represent one of pivotal members of stroke management team including the Emergency Department nurses and inpatient nurses and should be included in all phases of care of stroke patients [27]. The ASA Guidelines for Adult Stroke Rehabilitation and Recovery put emphasis on a role of nursing care during hospitalization, but also after discharge [28]. For example, the authors pointed out that a great proportion of the AIS patients experience dysphagia immediately after the stroke and thus highlighted contribution of nursing staff in adequate nutrition of patients in order to prevent this problem and its consequences [28]. Further, it was reported that the nursing staff has important role in the early supported discharge and that their key tasks in this process include prevention of skin lesions, urinary and gastrointestinal tract disorders, malnourishment, regular use of therapy etc. Finally, the authors emphasized the function of nursing staff in the prevention and management of post-stroke depression [28].

Use of different stroke scales can also be helpful in everyday work of the nursing professionals. They are used for the assessment of stroke severity as well as for monitoring of patient's recovery during and after hospitalization. Based on the scale scores, the nurses can make decisions to which special care the patients should be directed [21]. One of these scales especially important for nursing staff is the Morse Fall scale [13]. It is used as a quick tool for the assessing a patient's chance of falling. The North American Nursing Diagnosis Association (NANDA) classified fall risk as a nursing diagnosis [29]. Although the Morse Fall scale score was significant prognostic factor in univariate regression analysis in our study, it was not shown to be an independent predictor of in-hospital mortality among the AIS patients, probably due to a small sample size.

Some limitations of our study have to be mentioned. Firstly, a total of 59 patients with AIS were enrolled in this

study. It is apparent that our research would benefit from the larger sample size, i.e., these results should be interpreted with caution. However, the consecutive sampling design, in the defined period of 2 months, ensures the representativeness of the sample and the generalizability of the results. Further, 33 out of 59 (55.9%) patients had mild AIS, according to the NIHSS score, and 6 patients died during hospitalization (10.2%). These facts could have affected our results.

Conclusion

The results of this study showed an important role of nursing staff in the recovery of AIS patients, as well as that admission to the stroke units versus the conventional units is the independent predictor of decreased in-hospital mortality.

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Conflict of interest

All authors declare that they have no conflict of interest.

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Morphological characteristics of the myocardial bridges in the level of the anterior interventricular branch of a human fetal heart

Morfološke karakteristike miokardnih mostova u nivou prednje interventrikularne grane srca ljudskih fetusa

Ognjen Čukuranović*, Vladimir Mandarić[†], Vukica Pantović[‡], Dragan Mihailović[§], Ivan Jovanović[‖], Aleksandra Veljković Čukuranović*, Jovana Čukuranović Kokoris[‖], Ljiljana Vasović[‖]

University of Niš, *Faculty of Medicine, [§]Department of Pathology, [‖]Department of Anatomy, Niš, Serbia; Military Medical Academy, [†]Clinic for Cardiosurgery, Belgrade, Serbia; [‡]Public Health Institute, Niš, Serbia

Abstract

Background/Aim. Myocardial bridges (MB) are narrower or wider fascicles of the atrial or ventricular muscle fibres which form a "bridge" either across the main trunks of coronary arteries or their major subepicardial branches. The aim of this research was to determine and present the exact frequency, morphological, morphometric and histological characteristics of the MB in the level of anterior interventricular branch (AIB) in human fetal hearts. **Methods.** The study was performed on 63 human fetal hearts. Images of the analyzed hearts were captured with a digital camera and afterwards morphometrically evaluated with ImageJ. Characteristic cases of the MB were dissected, sampled and further routinely processed for the subsequent histological analysis. Finally, the obtained morphometric data were statistically analyzed. **Results.** The presence of the MB on the AIB was proven histologically and under the magnifying glass. Myocardial bridges were found in 53.97% of the hearts. The percentage of the hearts with only one MB detected on AIB 88.24% was significantly higher than the percentage of the hearts with two MBs on the AIB (11.76%) ($p < 0.001$). **Conclusion.** We suggest that the MBs are just one anatomical variation of the fetal period as well as of adulthood.

Key words:
fetus; heart; coronary vessels; abnormalities; myocardial bridging.

Apstrakt

Uvod/Cilj. Miokardni mostovi *myocardial bridges* (MB) predstavljaju uzane fascikuluse atrijalnih ili ventrikularnih miokardnih vlakana, koja obrazuju "most" bilo preko glavnih stabala koronarnih arterija, ili preko njihovih subepikardnih grana. Cilj ovog istraživanja je bio da se ustanovi učestalost, morfološke, morfometrijske i histološke karakteristike MB prednje interventrikularne grane (*enterior interventricular bronch* – AIB) srca ljudskih fetusa. **Metode.** Studija je sprovedena na uzorku od 63 srca ljudskih fetusa. Digitalne fotografije analiziranih srca su zatim morfometrijski analizirane uz pomoć Image J sistema. Karakteristični slučajevi MB su disecirani, i dalje rutinski procesirani za potrebe histološke analize. Konačno, dobijeni rezultati morfometrijske analize su statistički obrađeni. **Rezultati.** Prisustvo MB na AIB je verifikovano morfološki (pod lupom) i histološki. Miokardni mostovi su ustanovljeni kod 53,97% slučajeva. Procenat slučajeva sa jednim detektovanim MB na AIB (88,24%) je bio statistički značajno viši u odnosu na procenat slučajeva sa dva MB detektovana na AIB (11,76%) ($p < 0,001$). **Zaključak.** Miokardni mostovi najverovatnije predstavljaju anatomsku varijaciju karakterističnu kako za fetalni, tako i za adultni period života čoveka.

Ključne reči:
fetus; srce; koronarni krvni sudovi; anomalije; miokardni mostovi.

Introduction

Recently, morphologists and clinicians have focused their attention on myocardial bridges across the coronary arteries and/or their branches. Šećerov-Zečević [1] described myocardial bridges (MB) as narrower or wider fascicles of the atrial or ventricular muscle fibres which form a "bridge" either across the coronary arteries' main trunks or their major subepicardial branches. Some of these fibres may wrap around the mentioned arteries and form loops in such way.

Correspondence to: Ognjen Čukuranović, University of Niš, Faculty of Medicine, 81 Dr Zoran Djindjic Blvd, 18 000 Niš, Serbia. E-mail: ognjencukuranovic@gmail.com

According to Acunã et al. [2], the MBs were first described by Reyman in the 18th century. Subsequent studies pointed to their numerous variations in frequency, localization, length, thickness, direction of fibres and clinical repercussions.

Results of the studies performed on hearts obtained post-mortem showed that the incidence of the MB varies from 15% to 85% in adults [2–8], and about 50% in human fetal material [9,10]. Clinical studies reported that their incidences varied from 0.5% to 40% [6, 11–13].

Depending on their width, the MBs have been usually classified as superficial and deep [5], with the anterior interventricular branch (AIB) of the left coronary arteries as the most frequent location [7, 8, 14, 15]. Deep MBs are usually symptomatic and result in ischemic disease of varying intensity [16]. According to some authors, the MBs are not only characteristic of the human heart, but also of some mammals such as dogs, camels or gorillas [17].

As previous studies showed, the most common localization of the MBs is the AIB [2, 7, 8, 15]. This research aims to determine and to present their exact frequency, morphological, morphometric and histological characteristics in the human fetal hearts.

Methods

The study was performed on 63 human fetal hearts obtained post-mortem (28 males and 35 females) and fixed in 10% buffered formalin. Gestational age of the fetuses ranged from 13 to 28 weeks [18]. They are the part of the collection of the Institute of Anatomy of the Faculty of Medicine, Niš, Serbia. The coronary arteries of the hearts used during this research were injected with Micropaque or Latex. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Niš (No: 01−9002−4).

The anatomical dissection and morphological analysis of the MBs on the AIB was done under the surgical magnifying glass (5×). Anterior thoracic wall of the fetuses (sternum and costal cartilages with adjacent structures) was removed and afterwards hearts with the large blood vessels of is bases were their pulled out of the pericardial sac and inferior middle mediastinum. The anterior descending branch of the left coronary artery was divided into three segments: the first included its segment at the level of the conus arteriosus top and the second and the third segments relatively fit with the halves of the remaining part of the AIB (Figure 1). Morphometric parameters (outer diameter of the AIB, length of the MB) were measured by the Image J [19] on the digital images of the analyzed hearts captured with a digital camera. The morphology and distribution of the MB on the AIB of each case was analyzed under the magnifying glass (5×) and afterwards schematically presented. The histological analysis was performed on the tissue samples of the fetal hearts whose coronary arteries were injected with Micropaque or Latex. The samples were embedded in paraffin and cut into sections 5 μm thick. The obtained sections were stained with haematoxylin eosin (HE) at the Institute of Pathology at our Faculty of Medicine.

The continuous variables were described by means and standard deviations. Frequencies of categorical data were given by absolute numbers and percentages. Differences between the independent groups were tested with the Student's unpaired *t*-test. The χ^2 test was used to compare proportions of categorical variables among the groups. The Pearson's correlation coefficients were used to analyze associations between the continuous variables. The level of significance was set at 0.05. All analyses were performed by the SPSS software [20].

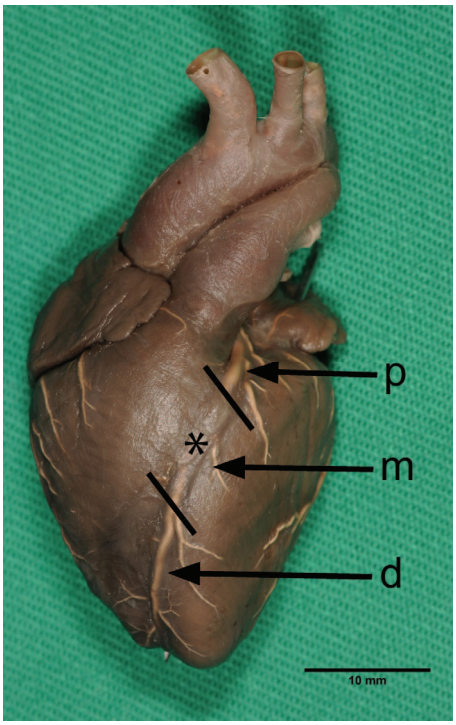

Fig. 1 – The heart of fetus of male gender (gestational age 29 weeks). Sternocostal surface. The coronary arteries were filled with Micropaque. The insert showed anterior interventricular branch (AIB) divided into three segments; proximal (p) (a boundary is a horizontal line to the conus arterios top), middle (m) and distal (d) (horizontal line between the half of the remaining of AIB length to the apex of heart); *myocardial bridge.

Results

The cases were classified into two groups according to the presence of the MBs. The first group included 34 (53.97%) cases with the MBs of which 15 (44.12%) were males and 19 (55.88%) were females. Twenty-nine (46.03%) cases of the second group were without the observed MB across the AIB, 13 (44.83%) were males and 16 (55.17%) were females. The percentage of the male and female cases in the obtained groups were not significantly different ($p > 0.05$). The mean age of the fetuses in the first group was 19.12 ± 3.46 weeks, while in the second group it was 18.00 ± 2.25 weeks of gestation, which was not significantly different ($p > 0.05$).

The total number of the MBs on the AIB observed in the first group was 38. The percentage of the hearts with only one MB detected on the AIB (n = 30 or 88.24%) was significantly higher than the percentage of the hearts with two MB on the mentioned artery (n = 4 or 11.76%) ($p < 0.001$) (Figures 2A and 2B).

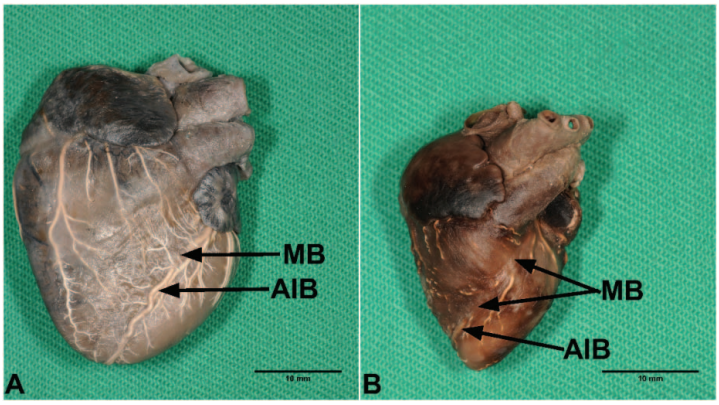

Fig. 2 – Two hearts of fetuses of male gender [gestational age 19 weeks (A) and 17 weeks (B)]. Sternocostal surface. The coronary arteries were filled with Micropaque. The insert A showed one myocardial bridge – MB (arrow) located at the middle segment of the anterior interventricular branch (AIB); The insert B showed double MB (arrows) located at proximal segment, and simultaneously at middle and distal segments of AIB.

Table 1

Myocardial bridges in relation to the arterial localization and gender

Localization	Number of hearts with MB n (%)	Number of hearts with 1 MB n (%)	Number of hearts with 2 MB n (%)	Gender (M/F)
AIB p	6 (17.65)	6 (17.65)	/	3/3
AIB m	9 (26.47)	9 (26.47)	/	3/6
AIB d	1 (2.94)	1 (2.94)	/	0/1
AIB p+m	15 (44.12)*	13 (38.24)	2 (5.88)	7/8
AIB m+d	1 (2.94)	/	1 (2.94)	1/0
AIB p+m+d	2 (5.88)	1 (2.94)	1 (2.94)	1/1
Total	34 (100%)	30 (88.24%)†	4 (11.76%)	15/19

MB – myocardial bridges; AIB – anterior interventricular branch; p – proximal; m – middle; d – distal; p+m – proximal and middle; m+d – middle and distal; p+m+d – proximal, middle and distal; M/F – male/female.
***There is a statistically significance in relation to other localization ($p < 0.05$).**
†There is a statistically significant difference between hearts with one and with double MB ($p < 0.001$).

The coronary dominance of all of the hearts was as follows: 32 (50%) were right dominant, 11 (17.19%) were left dominant and 20 (31.25%) were balanced.

The coronary dominance of the hearts with the MB was as follows: 17 (50%) were right dominant, 5 (14.71%) were left dominant and 12 (35.29%) were balanced.

The morphological analysis showed the presence of 6 morphological types according to the number of the MB present on the different AIB segments. The number of cases included in each morphological type and their percentages are shown on Table 1.

The myocardial bridges were most frequently localized at the proximal and middle segments border and in such way simultaneously covered the parts of both AIB segments (n = 15 or 44.12% of the cases). The percentage of such cases was significantly higher, compared to the cases with localization on the proximal or middle AIB segment separately ($p < 0.05$), as well as the other observed combinations of their localization on the other AIB segments ($p < 0.001$).

The mean length of the MBs in the males (9.67 ± 5.14 mm) was insignificantly ($p > 0.05$) higher than in the females (7.48 ± 3.32 mm).

The presence of MBs on the AIB was proven under the magnifying glass and histologically (Figure 3). Reticular cardiomyocyte as well as numerous lacunae, were noticed in deeper layers of the myocardium.

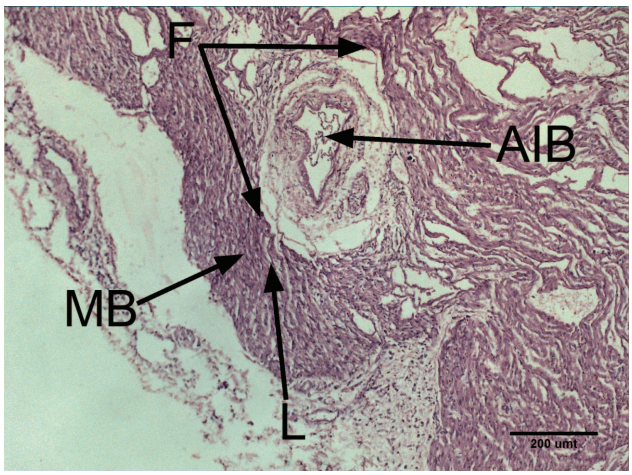

Fig. 3 – Myocardial bridge (MB) seen under the microscope. Obtained section was stained with haematoxylin eosin (HE, x10). The anterior interventricular branch (AIB) over bridged with the MB. The direction of cardiomyocytes was parallel and at a distance from the AIB they were separated into two fascicles (F). Visible parallel flattened lacunae (L).

A relatively irregular direction of cardiomyocytes was observed in some regions of the myocardium. The direction of cardiomyocytes was predominantly parallel and at a certain distance from the AIB they were separated into two fascicles. One fascicle continued posterior and the other travelled in front of the AIB partially enveloping it (visible parallel flattened lacunae). In addition to transversal cardiomyocytes which formed the MB, there were also some oblique and longitudinal cardiomyocytes.

There was a correlation between the length of the MB and gestational age of the fetuses. The length of the MB increased with the gestational age of the fetuses, but this increase was not statistically significant ($\rho = 0.027$; $p > 0.05$).

The mean AIB outer diameter proximally and distally of the MB was compared with the outer diameter of the same segments of the artery in the cases without the MB. Its value was higher in the cases with the MB than in those without the MB, but this difference was not significant ($p > 0.05$).

Discussion

Although the MBs were subjects of various clinical [6, 8, 11–13] and autopsy [3–7, 8, 12] studies, their practical (clinical) significance remained unclear and has not yet been completely resolved.

Some authors reported that segment of the artery under the MB may be protected from atherosclerosis, while proximal segment of a bridged vessel is more susceptible to it [6,13,21,22]. This can be partially supported by an increased axial wall stress just proximal to the MB due to the compression on the over bridged segment [23].

Our study showed that the presence of the MB was not significantly correlated with either of the genders in the evaluated sample. Some authors revealed the presence of insignificant male predominance [2, 7], while in their study, Polacek and Kralove [4] established a significantly higher frequency of the MBs in the male individuals of the Czechoslovakian population. Nevertheless, until today, a rational explanation for differences with respect to gender has never been given.

Incidence of the MBs at the level of the AIB was presented in different ways by many studies. Incidence of the MBs of 53.97%, that we established, is higher than the same of Šećerov [9], who examined the MB on the same artery in fetal hearts (gestational age 3–10 lunar months) and found their incidence of 32.72%. However, the author conducted her research on 55 human fetal hearts among which 28 (50.9%) had one or more MBs in different coronary arteries, and only 18 (32.72%) of them had the MB on the AIB. Concerning the MB location, Cakmak et al. [10] also analyzed their presence in the human fetal hearts (gestational age 6–10 lunar months) and found 50% of all MBs at the level of the AIB. They showed that 18 (46.2%) of the 39 fetal hearts had in total 26 MBs (one or more) on the different branches of coronary arteries. Loukas et al. [7] studied 200 adult hearts and found 81 MB in 34.5% of the hearts, from which 43.2% were on the AIB.

The above cited authors [7, 10] presented incidence of the MBs on the AIB as their percentage in relation to the MBs detected in all investigated branches of coronary arteries and for that reason our results could not be compared to theirs. Nevertheless, Polacek and Krelove [4] reported the presence of the MBs on the AIB in 60% of the adult samples, which coincided with our results. Acunã et al. [2], examined the autopsy material which consisted of 154 hearts (average age 31.5 years) and found the MB in 40.3% of all cases, from which 36.9% were localized on the AIB, which is not in agreement with our findings.

The presence of only one MB on the AIB, was observed in 30 (88.24%) of 34 hearts with the detected MB in our study. Results by Šećerov [9] showed similar frequency of one MB on the same blood vessel. Loukas et al. [7] found that this percentage is 85.5%, but with regard to the number of one MB on all analyzed coronary arteries. Similar to Acunã et al. [2] in their paper, we established that the incidence of only one MB, when all evaluated AIB were taken into the consideration, was 67.74%. Frequency of the double MBs on the AIB detected in this study was 11.76%, which is very similar to the frequency detected by Šećerov [9] (11.11% of the cases) and Loukas et al. [7]. We did not detect the presence of the triple MBs on the AIB.

The angiographic studies suggested that the MBs can be deep and superficial [5] and that they mostly occur in the middle segment of the AIB [7, 8, 14, 15]. Analyzing the distribution of the MBs on the AIB in our study, we determined that their most common localization was in the proximal and middle segment of the AIB simultaneously, with incidence of 44.12% of the samples, which is in agreement with the findings of Reig et al. [24] and Bezerra et al. [25]. Less frequently, we detected the MBs in the middle (26.47%) or in the proximal segment (17.65%) of the vessel.

High variability of the MB length was reported in the literature, ranging from a few up to 50 millimeters [7, 15, 24, 26]. According to our results, the average MB length on the AIB was similar in both sexes (8.57 mm) which corresponded to the findings of Šećerov [9] (3–10 mm). The correlation analysis between the length of the MB and gestational age of the fetuses revealed a weekly increase of the MB length with gestational age, but this increase was not significant. However, an intensity of the increase of the MB length during the postnatal period remains a hypothetical question. Additionally, whether this increase during postnatal period coincides with the frequency of myocardial infarction and other coronary disorders may be the subject of future studies.

In spite of the fact that we established that an outer diameter of the AIB was higher in cases with the MBs than in ones without them, a significant correlation was not statistically confirmed.Taking into consideration that there are no available data in the present literature about this, such findings might be the consequence of a relatively small sample used during this study, and future studies will give definite answer to this dilemma.

Histological organization of the MB in fetal hearts presented by Šećerov [9] did not differ from the one presented in our study. Namely, she showed that muscle fibers of myocardium, after a parallel stream with the AIB, divide into two fascicles stretching, in most cases, perpendicularly upward and beyond the AIB, forming a loop around it, after which they unite again and continue with a parallel course.

Reviewing the literature, it appears that vascular dysfunction at the MBs in adults may cause vasospasm which can lead to the lethal ischemic events, arrhythmias, myocardial infarction and a sudden cardiac death [27, 28]. According to Duygu et al. [28], who conducted their study on 71 patients with the MB (mean age 51 ± 10 years), the myocardial bridge may induce a development of atherosclerotic lesions in a segment of the AIB proximal to MB. They divided the patients into two groups, those with the MB and no atherosclerotic lesions and those with the atherosclerotic coronary artery disease in addition to the MB. After performing the exercise testing of all patients, the authors obtained the following results: stable angina pectoris occurred more frequently in the first group than in the second one (70% *vs* 35% respectively; $p = 0.01$), while acute coronary syndrome appeared more frequently in the second group (65%) than in the first one (30%), ($p = 0.04$). Their treatment depended on length and depth of the MB. The patients with the long and deep MB underwent surgical revascularization, while others received a medical management.

Conclusion

It can be concluded that there was an association between the MBs in the prenatal period and in adulthood. Based on the morphological characteristics of the MBs on the AIB, we suggest that the MBs are just one anatomical variation of the fetal period, as well as of the adulthood. However, since their presence has been noted during adulthood, it is possible that in some cases they could be responsible for some of the vascular dysfunctions and/or genesis of atherosclerosis.

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The application of the local histograms of apparent difusion coefficient in differentiation of brain astrocytomas

Primena lokalnih histograma prividnog difuzionog koeficijenta u diferencijaciji astrocitoma mozga

Jelena Mihailović*, Danica Grujičić*†, Slobodan Lavrnić‡, Marko Daković†§

***National Cancer Research Center, Belgrade, Serbia; Clinical Center of Serbia, †Clinic for Neurosurgery, ‡Center for Radiology and Magnetic Resonance, Belgrade, Serbia; University of Belgrade, §Faculty of Physical Chemistry, Belgrade, Serbia**

Abstract

Background/Aim. Microstructural diversity of brain astrocytomas makes their diagnostics and differentiation by using the diffusion weighted imaging (DWI) difficult. In this study we used the histogram-based positioning of regions of interests on the apparent diffusion coefficient (ADC) maps in order to restrict the determination of diffusion parameters to regions of interest (ROI) corresponding to maximum cellularity. Success of ADC standard deviation (ΔADC) and kurtosis (K) in differentiation of brain astrocytomas was evaluated. **Methods.** The thirtyone patients (16 women and 15 men, median age 37 years, age range 6–72 years) with suspected supratentorial astrocytomas were included in the retrospective study. The magnetic resonance imaging (MRI) examinations were performed using the 1.5 T MR system (Avanto; Siemens, Erlangen, Germany) and 8-channel phased array head coil. The DWI images were acquired in three orthogonal directions for the b-values 0, 500 and 1000 s mm^{-2}. The histogram calculations and determination of diffusion parameters were performed using the MIPAV software package and the statistical analysis was done in the Openstat software. **Results.** The ADC values enabled differentiation of diffuse astrocytomas (DA) from a high-grade astrocytoma (HGA), but not between the classes of HGA. In addition, the ΔADC value provided discrimination between the anaplastic astrocytoma (AA) and glioblastoma multiforme (GBM) with 100% of sensitivity and 89% of specificity . The kurtosis value can also differentiate between the grades AA and GBM although with the lower sensitivity and specificity. **Conclusion.** The histogram analysis of tumor region on the ADC maps can provide a guidance for an appropriate choice of the ROIs. The parameters which characterize diffusion of such defined ROIs, as well as their combination can improve differentiation of brain astrocytomas.

Key words:
astrocytoma; neoplasm staging; histology; diffusion magnetic resonance imaging.

Apstrakt

Uvod/Cilj. Razlikovanje atrocitoma mozga zasnovano na analizi mapa difuzijski naglašenog snimanja (DWI) predstavlja težak zadatak, što je posledica mikrostrukturne heterogenosti ovih entiteta. U ovoj studiji primenjena je histogramska analiza mapa prividnog difuzionog koeficijenta (ADC) da bi se lokalizovali regioni (ROI) koji odgovaraju maksimalnoj celularnosti tumora. Testirana je mogućnost korišćenja parametara dobijenih iz tih regiona [ADC, standardna devijacija ADC (ΔADC) i kurtozis (K)] za razkovanje različitih gradusa astrocitoma. **Metode.** U retrospektivnu studiju bio je uključen 31 bolesnik (16 žena i 15 muškaraca, prosečna starost 37 godina; raspon 6–72 godina) sa suspektnim supratentorijalnim astrocitomima. Pregledi magnetno rezoanantnim snimanjem (MRI) urađeni su na uređaju jačine magnetnog polja 1,5 T (Avanto; Siemens, Erlangen, Nemačka). Korišćena je 8-kanalna zavojnica za glavu. DWI snimci dobijeni su u tri međusobno normalna pravca i tri vrednosti difuzijske ostetljivosti b (0, 500 and 1000 s mm^{-2}). Histogramska analiza i određivanje difizionih parametara izvršeno je korišćenjem MIPAV sofvera. Statistička analiza urađena je u Openstat programskom paketu. **Rezultati.** Ustanovljena je statistički značajna razlika između ADC vrednosti za difuzne astrocitome (DA) i visokogradusne astrocitome (HGA), ali ne i između HGA podklasa. Statistički značajne razlike nađene su i između ΔADC vrednosti za anaplastične astrocitome (AA) i glioblastome mulitforme (GBM) za 100% osetljivošću i 89% specifičnosti. K parametar takođe može poslužiti u razlikovanju AA i GBM ali sa manjom osetljivošću i specifičnošću. **Zaključak**. Histogramska analiza regiona tumora na ADC mapama može po-

Correspondence to: Marko Daković, University of Belgrade, Faculty of Physical Chemistry, Studentski trg 12, 11 000 Belgrade, Serbia. E-mail: marko@ffh.bg.ac.rs

služiti kao vodič za pravilno pozicioniranje ROI. Parametri koji karakterišu difuziju na tako definisanim ROI, pojedinačno i kombinovano, mogu pomoći u diferencijaciji astrocitoma mozga.

Ključne reči:
astrocitom; neoplazme, odredjivanje stadijuma; histologija; magnetna rezonanca, difuziona.

Introduction

Astrocytic tumors are the most frequent brain neoplasmas which comprise about two- thirds of brain gliomas. The choice of appropriate therapeutic strategy largely relies on the assessment of histopatological grade of tumor. Stereotactic biopsy is an option, but the pronounced structural heterogeneity of astrocytomas, which even may manifest in coexistence of two distinct histological types within tumor bulk [1], can lead to sampling errors. The method for the non-invasive assessment of grade and localization of the most active segments of tumors would significantly improve diagnostics and treatment of these tumors.

The conventional magnetic resonance imaging (MRI) presents the backbone of the brain tumor imaging. Although the techniques of T1 weighted (T1W), T2 weighted (T2W), the fluid attenuated inversion recovery (FLAIR) and gadolinium enhanced T1W imaging provide a good qualitative interpretation, they lack in specificity in tumor grading [2]. It has been generally agreed that the dynamic contrast enhanced (DCE) imaging tracing regions of increased capillarity and neovascularization can distinguish between the low and high grade gliomas, but not within the groups (although there are some different opinions [3, 4]). This could be attributed to the fact that the differences in cellularity may not always coincide with the differences in capillarity.

Diffusion weighted imaging (DWI) is an advanced MRI technique which provides unique information about microstructure of tissues by probing self-diffusion of water molecules. Microscopic movements of water molecules are mostly affected by the presence of cell membranes, which, due to low permeability for water as compared to the free diffusion, represent a barrier for unhindered movement of water molecules [5–7]. Therefore, any alteration in cell density or size will affect water diffusion in tissue and the extent of hindrance can be quantified by the apparent diffusion coefficient (ADC) as measured on the DWI.

The differentiation of brain astrocytomas (BA) using the DWI represents challenge from a viewpoint of the DWI because of their microstructural diversity. Despite the well-established correlation between the ADC values and tumor cellularity [8–12], this, apparently straightforward approach, produced a variety of results. Some authors found that the ADCs could not be used to separate different tumor grades [13–15] while others managed to differentiate a low- from a high-grade tumors, but not between the subtypes [8, 9, 16–20]. The discrepancies in results of these studies can be explained by two inter-related factors: a placement of the region of interest (ROI) for the ADC measurement and structural peculiarities of astrocytoma. Namely, the results were obtained by placing the ROI on a selected representative section of the tumor based on their appearance on the selected images. Such approach relies on *a priori* knowledge about the tumor structure introducing subjectivity and effects of sampling in analysis. To overcome that problem, some authors [21, 22] opted to place the ROI over the entire tumor and analyze obtained histograms of ADC values within the tumor. By using this approach, a successful differentiation was achieved within the group of a low grade as well as between a low- and a high-grade astrocytoma (HGA).

Diffusion MR imaging offers other potentially useful techniques, besides measurements of the ADC, in characterization of the brain lesions which were attempted in grading of BA. The diffusion tensor imaging (DTI) depicts the degree of directionality of water molecules movements. The fractional anisotropy (FA) maps of water diffusion confirmed that these pathologies in general show the lower FA values compared to normal white matter, because of displacement and/or destruction of axonal tracts [23–25]. However, only a coarse separation of the low- from high-grade tumors has been achieved [15, 26]. Recently introduced technique, the diffusion kurtosis imaging (DKI) evaluates structurallity of tissue by quantifying of departure of water molecules displacements from the Gaussian distribution. The measure of this is dimensionless parameter named excessive diffusion kurtosis (EDK) [27, 28]. In general, the technique showed the remarkable success in neural tissue characterization [27, 29]. The initial results in the differentiation of BA grades based on the mean EDK are promising [30].

In this paper, we used both advanced strategies in better differentiation of brain astrocytomas: analysis of the texture of tumor on the ADC maps by histograms as the guidance for placing the ROIs in the areas of the minimal ADCs; beside using just the ADCs, we determined parameters which describe microheterogeneity within the selected ROIs – standard deviation of ADC (ΔADC) and kurtosis (K) to evaluate the added values of the DWI. The principal aim of this study was differentiation between subtypes of HGA.

Methods

Patients

Thirty-one patients (16 women and 15 men, median age 37 years, age range 6–72 years) with the suspected supratentorial glial tumors were included in this retrospective study. The study was approved by the Ethics boards of our institutions. Informed consent was obtained from all examined patients. The subjects with the recurrent gliomas were excluded from the evaluation. After the MRI examination, all patients underwent surgery and the histological diagnosis was provided by the analysis of post-operative specimens. The analysis revealed the presence of 13

diffuse astrocytoma [World Health Organization (WHO) grade II], 9 anaplastic astrocytoma (AA) (WHO grade III), and 9 glioblastoma multiforme (GBM, WHO grade IV).

Magnetic resonance imaging methods

The MRI examinations were performed using the 1.5 T MR system (Avanto; Siemens, Erlangen, Germany) and 8-channel phased array head coil. Conventional MR images were obtained using the T_1 weighted [spinecho (SE), TR = 550, TE = 10 ms] and turbo T_2 weighted [turbo spin echo (TSE), 4800/94], both with 5 mm slice thickness and 1 average. The DWIs were acquired using a single-shot echo-planar imaging sequence (TR = 3800, TE = 89 ms); twenty-five sections of 5 mm thickness were obtained (field of view 230 × 230, matrix size 191 × 192) in three orthogonal direction for b-values 0, 500 and 1000 $s \cdot mm^{-2}$. In cases where the presence of blood/blood derivates were suspected, the gradient echo (T2*) sequence (TR = 847, TE = 25 ms and flip angle 20°) was conducted. This was observed in one case of anaplastic astrocytoma (11%) and in four cases of GBM (45%).

Afterwards, the paramagnetic contrast agent (0.1 $mmol \cdot kg^{-1}$) was administered and the T_1W sagital sections were acquired using the magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence (TR/TE = 2000/4.7, slice thickness 0.9 mm). The post-contrast coronal and axial 5 mm thick sections were obtained from these images using a reconstruction algorithm included in the software of MRI workstation.

Determination of parameters on apparent diffusion coeficient images

Upon the completion of the MR exam, all data were exported to the personal computer workstation with the Ubuntu 14.04 operating system. The MIPAV software (National Institute of Health, Bethesda, USA) [31] was used for determination of the ADC, ΔADC, and K on the ROI. In the first step, the T2W images were matched with the size and resolution of the DWI/ADC images ("Match images" alghoritm in the Mipav). The regions of hyperintensity on three consecutive T_2W slices (the middle one matching to maximal tumor diameter) were manually delineated (primary ROIs) and transferred by copying to the corresponding ADC maps. The areas that coincide to the presence of blood/blood products as seen on the T_2^*W images were excluded. Second, a histogram of ADC was obtained and histogram-based color maps were generated for the primary ROI (Figure 1C). The total of six ROIs of equal size (12 pixels) were placed in the areas which coincide with the blue regions (hypointensities) on those maps (Figure 1, right). The values of parameters (ADC, ΔADC, K) were determined by averaging those from individual ROIs. The histogram analysis of corresponding color coding of tumor was presented in Figure 1B. The kurtosis parameter, which defines the "peakedness" of distribution of measured values, were closely connected to the standard deviation by the equation $K = \frac{\mu_4}{\sigma^4}$ (1) where μ_4 is the fourth moment about the mean and σ is the standard deviation.

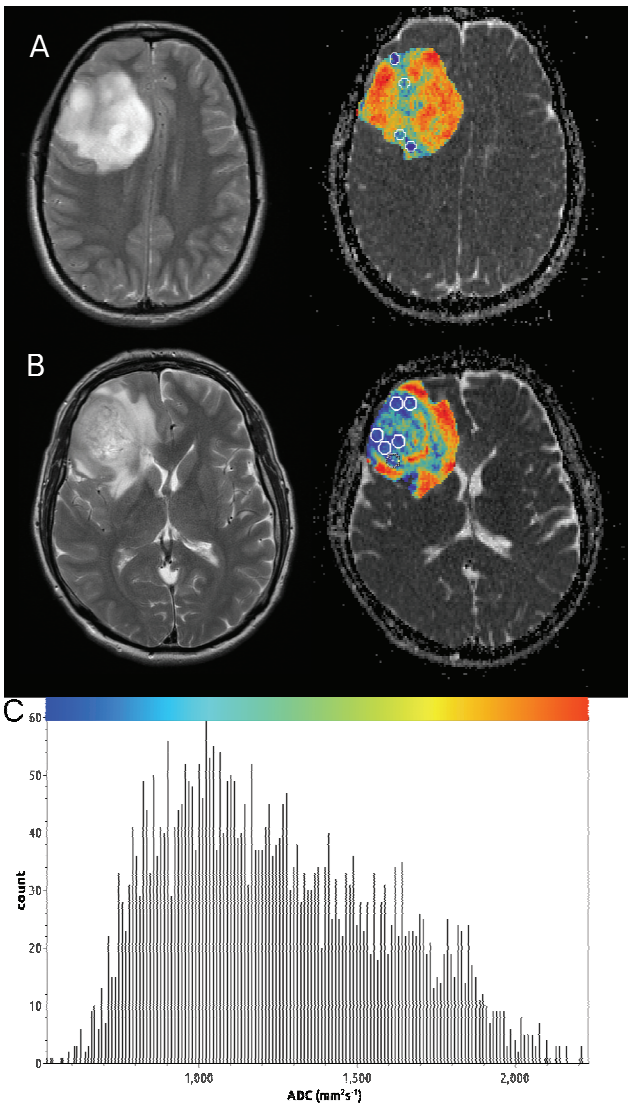

Fig. 1 – The examples of positioning of the regions of interest based on the histogram-based color maps of tumor region in: A) diffuse astrocytoma; B) glioblastoma multiforme; C) The histogram analysis of corresponding color coding of tumor shown in B. ADC – apparent diffusion coefficient.

Statistical analysis

Statistical analysis of the data obtained was performed using the OpenStat software [32]. Normality and homogeneity of the data were tested using the Shapiro-Wilk and Levine's tests. The differences among parameters for astrocytoma groups were assessed using the *t*-test and one-way ANOVA with Bonfferoni correction. The findings were considered significant if $p < 0.05$. The receiver operating characteristic (ROC) curves were generated in order to obtain pairwise comparison between the astrocytoma grades.

Results

The box-whiskers plot in Figure 2 shows the distribution of the ADC values for different astrocytoma grades. The grade II tumors showed the higher ADCs compared with the

grades III and IV, which in turn, had close values (Table I). The significant differences were found between grades II and III ($p < 0.01$) and between grades II and IV ($p < 0.02$), but not between the grades III and IV. A significant difference ($p < 0.001$) was also found when the ADC values of DA were compared to the groups of the HGA (III and IV).

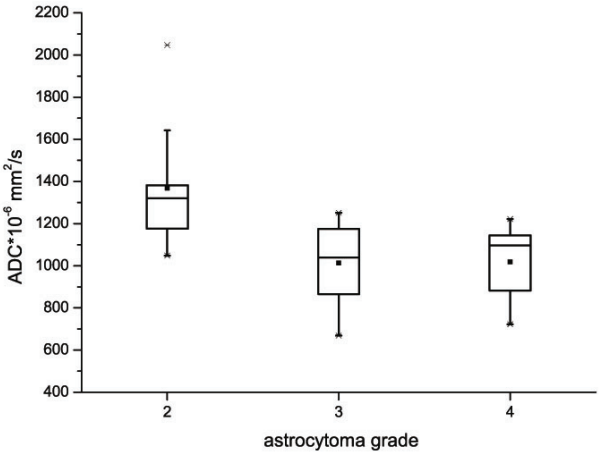

Fig. 2 – Box whiskers plot of the apparent diffusion coefficinet (ADC) values for different astrocytoma grades. The ADC values were measured on the regions of interest placed in regions of the minimal ADC values.

The values of ΔADC, presented by a box-whiskers plot in Figure 3, decreased from grade II to grade III and then rose to grade IV for which the highest values were observed (Table 1). The significant differences were found between the values for the grades II and III, $p < 0.05$), III and IV ($p < 0.001$), but not for II and IV, although there was a trend towards significance. The distribution of kurtosis values for different astrocytoma grades, given in Figure 3, showed the similar trend as for the ΔADC values. The significant differences were found only between the grades III and IV ($p < 0.05$).

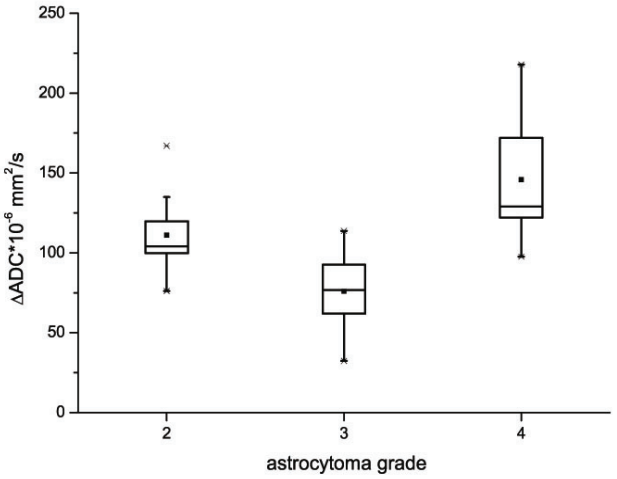

Fig. 3 – Box whiskers plot of standard deviation of the apparent diffusion coefficient (ΔADC) values for different astrocytoma grades measured on the regions of interest placed in regions of the minimal ADC values.

Table 2 shows the results of the ROC analysis of the parameters obtained from the ADC maps in the pairwise comparisons of astrocytic grades. The ΔADC value had the largest area under curve (AUC) among the other analyzed parameters for distinguishing grade 2 from grade 3.

These grades could be distinguished with 78% sensitivity and 89% specificity at the cutoff value of $0.1 \cdot 10^{-3}$ mm^2/s. The ADC value could differentiate between the grades II and III with the same sensitivity, but with somewhat lower specificity (78%) (Figure 4).

Table 1

Average values of ADC, ΔADC and K determined for different astrocytoma grades

Type of astrocytome	ADC·10^{-3} mm^{2}s^{-1}	ΔADC·10^{-3} mm^{2}s^{-1}	K
Astrocytoma grade II	1308 ± 275	111 ± 33	2.8 ± 0.7
Anaplastic astrocytoma (grade III)	1013 ± 217	76 ± 24	2.4 ± 0.4
Glioblastoma multiforme (grade IV)	1019 ± 183	145 ± 41	3.1 ± 0.9

ADC – apparent diffusion coefficient; ΔADC – standard deviation of ADC; K – kurtosis.

Table 2

Results of pairwise ROC analysis for combinations of astrocytoma grades

Parameter	AUC	*p* value	Cutoff	Sensitivity (%)	Specificity (%)
Differentiation between grade 2 *versus* grade 3 astrocytoma					
ADC	0.88	0.01	$1.211 \cdot 10^{-3}$	78	78
ΔADC	0.85	0.007	$0.1 \cdot 10^{-3}$	78	89
K	0.78	0.047	2.76	57	78
Differentiation between grade 3 *versus* grade 4 astrocytoma					
ADC	0.469	> 0.9	$1.100 \cdot 10^{-3}$	67	56
ΔADC	0.975	0.001	$0.17 \cdot 10^{-3}$	100	89
K	0.784	0.042	2.71	78	78

AUC – area under curve; ROC – receiver operating characteristic.
For other abbreviations see under Table 1.

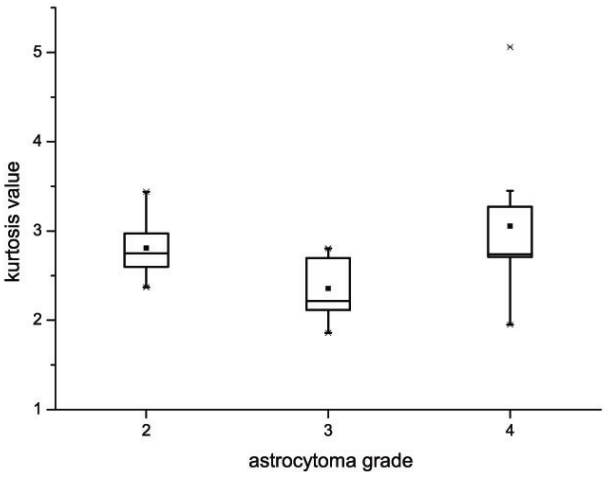

Fig. 4 – Box whiskers plot of the kurtosis values for different astrocytoma grades measured on the regions of interest placed in regions of the minimal apparent diffusion coefficient values.

When distinguishing between the grades III and IV, the ΔADC value had largest AUC (0.975) with sensitivity 100% and specificity 89% and the cutoff value of $0.17 \cdot 10^{-3}$ mm^2/s (Table 2). The kurtosis value could also differentiate between the grades III and IV although with sensitivity and specificity of 78% (AUC = 0.784).

Discussion

Diffusion weighted imaging enables insight into the microstructure of tissues, thus representing an attractive tool for characterization of brain tumors. Here we report the application of DWI in classification of BA. The main accomplishment of the study was the ability to differentiate the gradus III from gradus IV (AA from GBM) using two DWI parameters. The importance of this finding is a potential impact on therapy planning. Treatments are virtually the same for both tumor types – surgery and concomitant radiotherapy and chemotherapy. However, the choice of chemotherapeutic agent for the treatment of primary glioblastoma is narrowed to moderately efficient temozolomide [33], while multiple choices are available for the treatment of AA.

The ADC values of BA obtained in our study are within the range of values reported in a majority of studies [9, 16, 20], but slightly higher than the values obtained by Kitis et al. [17] and Fan et al. [34]. The data analysis showed that ADC values can be used in distinguishing between the astrocytoma grades II and grades III and IV, but not between the last two. This is in agreement with a majority of studies [9, 16–20, 34]. However, there are studies that question the ability of differentiating a low- from a high-grade BA [13–15]. These discrepancies are the most probably the consequence of the procedure of the ROI selection which appears to be extremely important in such highly heterogeneous tumors. For example, in studies where the ROI encompassed the whole tumor no differentiation between the astrocytoma grades was achieved on the basis of mean diffusivity [15, 22]. This can be attributed to masking of cellularity contribution to the average tumor ADC value by contribution of other tumor components such as microcysts, necrosis, bleeding, calcifications, etc. Hence, various other strategies of the ROI selection were employed since the ROIs should reliably represent the most active part of the tumor. In a number of studies, the ROIs were placed on the tumor tissue which appeared as "solid" on the T2W/FLAIR images or enhanced on the T1W images [9, 16, 18, 34, 35]. Some studies used the DWI to select the ROIs [8, 17, 36] under assumption that the minimal ADC values should correlate with the high tumor cellularity and proliferative indexes. The outcome of both approaches was that it was possible to distinguish the low- from high-grade tumors, but not to differentiate the high-grade tumors, i.e., AA from GBM.

The alternative approach, similar to ours, employed the histogram analysis of the whole tumor region in the ADC maps (whole tumor in single ROI) and use only the ADC value of the low edge of the histogram in discriminating grades [22]. This enabled differentiation between the grade II and grade III astrocytoma, but not between the grades III and IV. In our study, we used combined strategy which included an initial histogram analysis of ADC values in whole tumor followed by generation of the histogram-based color maps and placement of ROI in the regions which corresponded to the lowest ADC values. Such approach diminished the influence of subjectivity in the ROI positioning, and more reliable localizations of zones that corresponded to the highest cellularity can be achieved. Nevertheless, we also did not succeed in distinguishing the AA from GBM based on the ADC values.

There are, however, papers where the authors claim that is possible to distinguish the AA from GBM using the ADC data [18, 36, 37]. However, findings of Yang et al. [36] are flawed by the fact that the orientation independent DW image was calculated as an arithmetic mean instead of correct geometric mean. Higano et al. [37] used the method of elimination of the ROIs to the one with a minimal ADC value. The risk of using a single ROI to characterize tumor lies in the fact that such region of the minimal ADC values in the high-grade tumors, besides high cell density, may contain micro bleedings and micro calcifications which could lead to underestimation of the minimal ADCs: these features are more frequent in glioblastomas. Yamasaki et al. [18] used the logistic discriminant analysis to construct classifiers containing both the ADC values of tumors and patient related data (age and gender). This involved the parameters other than those obtained by the MRI and the selection of patients appropriate for inclusion in the analysis.

The lack of difference between the ADCs for the astrocytoma grades III and IV in our study can be explained by similar histopathology of the tumors in the regions that corresponded to high cellularity. Both types are characterized by cell atypia, high mitotic activity and pleomorphism. In addition to this, grade IV is characterized by the presence of ischemic regions and vascular proliferation. However, because of domination of cell component and effects of averaging, these features might not reflect in the ADCs.

Although being the major factor, cell density does not give enough information for histopathological characterization of the brain tumors. Other determinants are cell polymorphism, number of mitoses, density of capillary, presence of micronecrosis and microcalcifications, etc. They have influence on intravoxel and heterogeneity within the ROI, their contribution to tumor ADC values should be considered.

The values of ΔADC obtained in our study enabled differentiation between both the astrocytoma of grades II and III, and also between the grades III and IV. The distribution of the parameter values showed that anaplastic astrocytoma exhibited the lowest heterogeneity in series, followed by the diffuse astrocytoma and glioblastoma multiforme what was in agreement with the microstructure of these tumors [1]. To our knowledge, there are only two studies considering application of this parameter in characterization of the brain pathologies. Bosma et al. [38] found that the ΔADC could be used to discriminate between systemic lupus erythematosus, but surprisingly did not discuss this result. In their study, Kang et al. [22] measured the values of ΔADC of astrocytoma, but did not evaluate the use of this parameter in their differentiation.

The values of kurtosis parameter determined in this study enabled distinguishing the astrocytoma grades III and IV, although with somewhat lower sensitivity and specificity. Kang et al. [22] did not find the significant differences among the kurtosis values of astrocytoma grades which could be explained by the large errors in determination of kurtosis when whole tumor was considered as the ROI. Raab et al. [30] reported ability of excessive kurtosis, obtained from the DTI at the multiple b-values, to differentiate between the astrocytoma grades. However, this procedure (diffusion kurtosis imaging DKI) requires a considerable amount of imaging time and the use of the MRI devices operating at the magnetic fields equal to or higher than 3T [27]. This questions suitability of the DKI in the routine clinical examinations.

Our results suggest that the parameters which characterize heterogeneity of diffusion within defined ROI have advantage over the mean ADC value in differentiation of BA grades III and IV, where the ΔADC value showed the highest sensitivity and specificity. However, it showed similar ability as the mean ADC (although higher specificity) in differentiation grades II and III. The sensitivity and specificity of kurtosis factor in differentiation of these tumor types were somewhat lower compared to that of the ΔADC, but still showed higher performance compared to the mean ADC. This suggests that all determined diffusion parameters should be considered in differentiation of the brain astrocytoma.

The main limitation of this study is a small number of brain astrocytomas included. Analysis of a larger group of tumors may improve the achievements of combination of diffusion parameters in differentiation of the BA. The alternative approach could use the ROI placement based on chemical shift imaging (maps of choline concentrations within lesion) and/or MRI perfusion images, as better indicators of regions of increased cellularity and/or vascularity. We have not included this method in our study, because only two patients had these procedures included in the exam. Further, in this study we did not evaluate diffusion in the peritumoral area or performed normalization to the corresponding values for normal appearing white matter. The reason for such approach was a comparison of successfulness of other parameters derived from the ADC maps measured from the same ROI placed in a lesion; use of normalization would obscure inherent information they contain.

Conclusion

The results presented in this work pinpoint to importance of histogram analysis of the ADC maps in adequate positioning of ROI. Using this approach, it is possible to distinguish the astrocytomas grade II and III using the ADC values. Further analysis of heterogeneity of the ADC values in tumor using the values of ΔADC and kurtosis yielded to even more successful differentiation among astrocytoma grades. Therefore, for overall grading of these tumor types, all three parameters should be used for successful diagnostics.

Acknowledgements

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Risk factors for recurrent *Clostridium difficile* infection among patients in the Clinical Centre of Vojvodina, Serbia: a retrospective clinical trial

Faktori rizika od pojave relapsa *Clostridium difficile* infekcije u Kliničkom centru Vojvodina, Srbija: retrospektivna klinička studija

Nadica Kovačević, Radoslava Doder, Tomislav Preveden, Maria Pete

Clinical Center of Vojvodina, Clinic for Infectious Disease, Novi Sad, Serbia; Univerity of Novi Sad, Faculty of Medicine, Novi Sad, Serbia

Abstract

Background/Aim. In the last two decades the incidence of recurrent *Clostridium difficile* infection (CDI) has risen. The aim of this study was to determine the risk factors for the recurrent CDI among patients hospitalized with the initial CDI. **Methods.** We conducted a retrospective clinical trial at the Clinic for Infectious Diseases, Clinical Center of Vojvodina, Serbia, between January 2010 and January 2016. We enrolled 488 patients with the initial CDI who were treated with oral vancomycin (125 mg, 4 times per day) or oral metronidazole (400 mg, 3 times per day) for 10 days. After the completion of therapy, there was 60 days of the follow-up period for the assessment of the rates of relapse. To determine the risk factors for the CDI relapse, we compared the demographics, clinical and laboratory characteristics of the patients who had a relapse with the patients who had a stable clinical response. **Results.** Of the 488 cases, 29.09% recurred. The relapse occured in 22.72% patients who received vancomycin and in 36.60% patients treated with metronidazole (p = 0.038). A statistically significant effect on the CDI relapse had the comorbidities such as a malignancies (19.52% *vs* 8.82%, p = 0.023) and the postoperative CDI (25.67% *vs* 10.29%, p = 0.035), hipoalbuminemia (< 25 g/L) (70.27% *vs* 41.94%; p = 0.034) and the concomitant antibiotic therapy (50.67% *vs* 20.29%; p = 0.031). The persistence of *C. difficile* toxin in the stool at the end of treatment was registered in 22.32% of patients treated with metronidazole *vs* 9.09% of patients given vancomycin (p = 0.03). **Conclusion.** Our data suggest that the important risk factors for the CDI relapse are comorbidities (surgery within a month before developing CDI and malignancy), hipoalbuminemia (< 25g/L) and concomitant non-CDI antibiotics therapy. Vancomycin is more effective than metronidazole in the elimination of *C. difficile* toxins. The presence of *C. difficile* toxins in the stool after the successful completion of the initial CDI therapy does not affect significantly the occurrence of relapse.

Key words:
clostridium difficile; infection; recurrence; risk factors.

Apstrakt

Uvod/Cilj: Incidenca relapsne *Clostridium difficile* infekcije (CDI) je u poslednje dve decenije u porastu. Cilj rada bio je utvrđivanje faktora rizika od relapsa kod bolesnika sa inicijalnom CDI. **Metode.** Na Klinici za infektivne bolesti Kliničog centra Vojvodine u Novom Sadu sprovedena je retrospektivna studija u period od januara 2010. do januara 2016. Studijom je obuhvaćeno 488 bolesnika sa inicijalnom CDI koji su lečeni peroralnim vankomicinom (125 mg, četiri puta dnevno) ili peroralnim metronidazolom (400 mg, tri puta dnevno) 10 dana. Nakon završene terapije bolesnici su praćeni 60 dana u cilju utvrđivanja pojave relapsa. U cilju identifikacije faktora rizika od relapsa CDI upoređivane su demografske, kliničke i laboratorijske karakteristike bolesnika sa relapsom u odnosu na bolesnike sa stabilnim kliničkim odgovorom. **Rezultati.** Relaps CDI je registrovan kod 142/488 (29,09%) bolesnika od kojih je 22.72% lečeno vankomicinom i 36.60% lečeno metronidazolom. Statistički značajan uticaj na relaps CDI su imali komorbiditet kao što su maligna oboljenja (19,52% *vs* 8,82%, p = 0,023) i postoperativna CDI (25,67% *vs* 10,29%, p = 0,035), hipoalbuminemija (< 25 g/L) (70,27% *vs* 41,94%, p = 0,034), konkomitantna antibiotska terapija (50.67% *vs* 20,29%, p = 0,031). Perzistencija *C. difficile* toksina u stolici po završenoj terapiji je registrovana kod 22,32% bolesnika lečenih metronidazolom i 9,09% bolesnika lečenih vankomicinom (p = 0,03). Prisustvo toksina *C. difficile* u stolici nakon uspešno završene terapije inicijalne CDI nije uticalo signifikantno na pojavu relapsa. **Zaključak.** Naši rezultati pokazuju da faktore rizika od relapsa CDI predstavljaju komorbiditeti (postoperativna CDI, maligniteti), hipoalbuminemija i konkomitantna primena antibiotika. Vankomicin je efikasniji u eliminaciji toksina *C. difficile* iz kolona.

Correspondence to: Nadica Kovačević, Clinic for Infectious Disease, Clinical Center of Vojvodina, Hajduk Veljkova 1–7, 21 000 Novi Sad, Serbia. E-mail: nadica.kovacevic@mf.uns.ac.rs

Prisustvo toksina *C. difficile* u stolici nakon uspešno završene terapije ne utiče signifikantno na pojavu relapsa.

Ključne reči:
clostridium difficile; infekcija; recidiv; faktori rizika.

Introduction

The appearance of modern broad-spectrum antibiotics and other therapeutic agents has led, in the second half of the 20th century, to an increase in the incidence of their adverse effects. This ascertainment especially refers to *Clostridium difficile* infection (CDI), which is today the most common form of nosocomial diarrhea due to favorable conditions for the transmission of the disease in hospitals and the presence of vulnerable population in them [1].

Particularly serious clinical and therapeutic problem is a recurrent CDI. Although the patients with an initial episode of CDI in most cases show a good response to therapy, 15% –55% of them develop the recurrent form of the disease [2, 3]. The problem of the recurrent CDI has increased because of the fact that the first relapse is a significant predictor for new relapses. In pathophysiological terms, CDI involves complex interaction between factors of the host, antibiotic activity and virulence of the pathogen. The cause of recurrent infections lies in the fact that no antibiotic eliminates the *C. difficile* (CD) spores from the intestinal tract. After a successful treatment response in the initial episode of disease, the endogenous spores in a reduced protective bacterial flora of the intestinal tract, transform themselves by germination into the vegetative forms that produce toxins and again lead to the development of diarrhea [2–4]. The severity and frequency of CDI increased rapidly in the last two decades, especially in the population of patients over 65 years. This is due to the fact that most people in this age category are immunocompromised as well as that intestinal microflora of bifidobacteria, which is considered protective, naturally declines in old age [1, 5]. Relevant studies also showed that certain comorbid diseases, leukocytosis, hypoalbuminemia, the degree of renal insufficiency, concomitant use of antibiotics, immunosuppressants and proton pump inhibitors carry with them an increased risk of the CDI relapse [2, 3, 5]. The impact of the recurrent CDI on the whole health system becomes increasingly important because repeated episodes of the disease extend the average duration of hospitalization and significantly increase the costs of treating the patients.

As the clinical trials in recorded an increase in incidence of CDI relapse, there comes to the need for clearer defining the predictors that would indicate a possible occurrence of relapse, and, accordingly, an application of the appropriate therapy for the high risk population of patients.

The aim of this study was to identify risk factors (RF) associated with relapse of CDI among the patients hospitalized with the initial CDI.

Methods

CDI was defined as diarrhea (defined as three or more unformed stools per day for at least 2 consecutive days) with positive CD toxin assay from faeces. Relapse was defined as a new episode of CD toxin positive diarrhea within 60 days after completion of therapy. Toxin was confirmed by the ELISA, RIDASCREEN CD Toxin A and B (C0801), R-Biopharm AG, Germany. Stool samples were taken for analysis of CD toxins within 48 hours after hospitalization, after completion of the CDI treatment and any time of suspected recurrence of CD diarrhea. All stool specimens from our study patients were cultured for *Salmonella, Shigella, Yersinia enterocolitica and Campylobacter* species to exclude other infectious causes of diarrhea.

The criteria for inclusion in the study were: age > 18 years, a history of ongoing diarrhea, positive CD toxin assay from stool samples within 3 days prior to hospitalization or positive stool sample collected for testing within 48 h after hospitalization. The patients with diarrhea due to another known cause unrelated to CDI were excluded from the study. Data abstracted from the medical records included demographics information (age, gender), clinical information (dates of diarrhea onset and resolution, stooling frequency, fever), the presence of a chronic underlying illness (diabetes mellitus, chronic respiratory disease, chronic renal failure, liver disease, cardiovascular disease, malignancy, neurological disease and surgery within a month before developing CDI), history of concomitant medications of importance (antibiotics and proton pump inhibitorsduring the treatment of initial CDI). Laboratory parameters of the initial CDI episode (peripheral leucocyte count, serum creatinine levels, albumin levels, serum C-reactive protein), were obtained within 48 hours of hospitalization. A follow-up period was 60 days after the completion of therapy. In order to monitor the occurrence of relapses after discharge from the clinic, the follow-up visits were carried out 20, 30 and 60 days after the completion of therapy. During those visits, the anamnestic data and physical examination were performed and stool samples were taken for analysis of CD toxins any time of suspected CDI relapse.

Statistical analyses

Statistical analysis was performed using the statistical package SPSS version 13.0. The descriptive statistical parameters were shown in the standard statistical variables, arithmetic mean ($\overline{x}$), standard deviation (SD), interval values (maximum and minimum). Testing a statistical significance was determined for the parametric data by the ANOVA test (analysis of variance), and for non-parametric data by the χ^2 test, Fisher's or Mann-Whitney test. For all tests the level of statistical significance, was $p < 0.05$.

Results

During the study period, we diagnosed 142/488 (29.09%) of patients with the first relapse of CDI. The re-

lapse occured in 60/264 (22.72%) patients who received vancomycin, and in 82/224 (36.60%) patients treated with metronidazole ($p = 0.038$) (Table 1).

Risk factors for recurrence

In our analysis, age ($p = 0.26$) and sex ($p = 0.40$) did not have statistically significant effect on the CDI relapse occurrence. Most of the patients were of the age category of over 65 years in both groups of patients (69.34% of patients with the stable clinical response and 75.22% of patients with the relapse) (Table 2). With regard to the clinical characteristics of the patients in the first CDI episode, the case and control patients did not differ significantly in terms of presence of fever ($p = 0.69$) and maximum stooling frequency ($p = 0.34$), but the duration of diarrhea during the treatment of the first episode of CDI had a statistically significant effect on the relapse occurrence ($p = 0.016$). The patients had a stable clinical response if the average duration of diarrhea after initiation of therapy was 4.45 [± standard deviation (SD) = 3.14] days, while the relaps was registered in the patients with the average duration of diarrhea of 8.32 (± SD = 6.21) days ($p = 0.016$) (Table 2).

The analysis of the comorbid conditions at the occurrence of CDI relapse showed that malignant diseases and surgery within a month before developing CDI had a statistically significant effect on the CDI relapse. We found that 32/346 (8.82%) patients with malignancies had a stable clinical response compared to 29/142 (19.52%) patients with a relapse ($p = 0.023$). Total of 36/346 (10.29%) patients with postoperative CDI had a stable clinical response versus 38/142 (25.67%) patients with a relapse ($p = 0.035$). Other comorbid conditions did not have a statistically significant effect on the relapse occurrence. The laboratory parameter with a statistically significant impact on occurrence of the CDI relapse was low albumin level (< 25 g/L). Total 146/346 (41.94%) of patients had a stable clinical response and 104/142 (70.27%) patients got a relapse ($p = 0.034$). High leucocyte count ($p = 0.37$) and creatinine level ($p = 0.28$) did not statistically significantly affected the occurrence of CDI relapse.

It is known that concomitantly applied therapy during the first episode of CDI can have a statistically significant effect on the relapse occurrence. We found that 69/346 (20.29%) patients on antibiotic therapy for concomitant disease had a stable clinical response, and 75/142 (50.67%) patients had the relapse ($p = 0.031$). Concomitant use of proton pump inhibitors had no statistically significant effect on the CDI relapse (Table 2).

Table 1

Treatment outcome in patients treated by different antibiotics

Treatment outcome	Metronidazole (n = 224)	Vancomycin (n = 264)	Total (n = 488)	*p*
	n (%)	n (%)	n (%)	
Stabile clinical response	142 (63.39)	204 (77.27)	346 (70.90)	0.038
Relapse	82 (36.60)	60 (22.73)	142 (29.10)	

Table 2

Characteristics of patients and treatment outcomes

Patients characteristic	Stable clinical response (n = 346)	Relapse (n = 142)	*p*
Demographics			
age (≥ 65 years), %	69.34	75.22	0.26
gender (male / female), n	61 / 39	58 / 42	0.40
Comorbidities, n (%)			
malignancy	32 (8.82)	29 (19.52)	0.023
postoperative CDI	36 (10.29)	38 (25.67)	0.035
Clinical characteristic			
no. of bowel movements ≥ 10/24 h, n (%)	139 (40.98)	56 (37.83)	0.34
temperature ≥ 38°C, n (%)	755 (22.05)	28 (18.91)	0.69
durations of diarrhea during treatment (days), mean ± SD	4.45 ± 3.14	8.32 ± 6.21	0.016
Laboratory data			
albumin level < 25 g/L, n (%)	146 (41.94)	104 (70.27)	0.024
leukocytosis ≥ 15,000/mm³, n (%)	132 (38.82)	62 (41.89)	0.37
serum creatinine level ≥ 200 μg/L, n (%)	48 (14.11)	29 (19.59)	0.28
C- reactive protein (μg L) mean ± SD	108.99 ± 80.92	157.13 ± 75.13	0.14
Concomitant medications, n (%)			
antibiotics	69 (20.29)	75 (50.67)	0.031
proton pump inhibitors	58 (17.05)	22 (14.86)	0.22
Microbiological data after treatment, n (%)			
clearance of CD toxins	298 (86.13)	108 (76.05)	0.12
persistence of CD toxins	48 (13.87)	34 (23.94)	0.09

***CDI – *Clostridium difficile* infection; SD – standard deviation.**

Table 3

Elimination of CD toxins after treatment and treatment outcomes

Microbiological effect after treatment	Metronidazole, n (%)			Vancomycin, n (%)		
	stabile clinical response (n = 142)	relapse (n = 82)	*p*	stabile clinical response (n = 204)	relapse (n = 60)	*p*
Clearance of CD toxins	115 (80.98)	56 (68.51)	0.08	183 (89.71)	52 (86.67)	0.16
Persistence of CD toxins	27 (19.01)	26 (31.71)		21 (9.82)	8 (13.33)	

CD – *Clostridium difficile.

Microbiological data after treatment

Through the study, we also investigated the presence of CD toxins in the stool after the successfully completed initial CDI treatment, and the impact of the toxins persistence on a CDI relapse. We found that vancomycin is significantly better than metronidazole in clearing CD toxins. After the completion of therapy, the persistence of CD toxins was registered with 50/224 (22.32%) of patients treated in metronidazole and in 24/264 (9.09%) of patients treated with vancomycin ($p = 0.003$).

The patients treated with metronidazole, after successful elimination of CD toxins in stool had a stable clinical response in 115/142 (80.98%) of cases while relapse developed in 56/82 patients (68.51%). The patients with persistence of CD toxins in stool treated with metronidazole, developed a relapse in 26/82 (31.71%) cases *vs* 27/142 (19.01%) patients with a stable clinical response ($p = 0.08$). After the successful elimination of CD toxins in stool, the vancomycin therapy led to a stable clinical response in 183/204 (89.71%) patients, and to a relapse in 52/60 (86.67%) patients. When the CD toxins in the stool persisted after the completed vancomycin therapy, the relapse was registered in 8/60 (13.33%) patients and 21/204 (9.82%) patients had a stable clinical response ($p = 0.16$). The results showed that the presence of CD toxins in the stool after the successful completion of the initial CDI therapy did not affect significantly the occurrence of relapse (Table 3).

Discussion

Due to the fact that the relapse occurs after the successfully completed therapy in 10%–20% of patients, but when the patients had one recurrence, a rate of further recurrences increase to 40%–65%, i.e., each relapse is a potential predictor for the development of new relapses, there appears a need for a clearer identification of specific RF associated with the CDI relapse [5]. In our study, the occurrence of the CDI relapse was observed in 29.09% of patients. Numerous studies demonstrated the existence of a link between age and the CDI relapse occurrence [1–3, 6, 7]. Our study did not confirm this fact, probably because most of the patients were of the age category of over 65 years (69.34% of patients with a stable clinical response and 75.22% of patients with a relapse). Various therapeutic regimes applied in the treatment of the first episode of CDI had a different impact on occurrence of CDI relapse. The frequency of relapses in the patients who were treated with metronidazole in the initial episode of CDI was 36.60% *vs* 22.72% of the patients who had a relapse after the treatment with vancomycin ($p = 0.038$). Contrary to our research, Lupşe et al. [6] did not record a statistically significant difference in the occurrence of relapses in these treatment groups. Scheurer and Ross [8] demonstrated, similarly to our research, that the patients treated with metronidazole were more likely to develop relapse compared to the patients treated with vancomycin (14% *vs* 7%, $p < 0.025$). Contrary to these results, Kim et al. [9] found a higher incidence of relapse after the therapy of vancomycin compared to metronidazole (41.2% *vs* 18.7%, $p = 0.054$), but they stressed that significantly more patients with severe forms of CDI (52.9% *vs* 21.1%, $p = 0.009$) were treated with vancomycin.

According to the results of our research, specific comorbid states have a statistically significant impact on the occurrence of CDI relapse. After surgeries, the relapse developed in 25.67% of our patients while the stable clinical response was found only in 10.29% of patients ($p = 0.035$). Similarly to our research, the study of Jung et al. [10] showed that the surgical procedures are statistically significant predictors of CDI relapse after the treatment with metronidazole ($p = 0.032$), and the research of Hsu et al. [11] showed that postoperative CDI after organ transplantation had a statistically significant impact on the CDI relapse after the vancomycin therapy ($p = 0.011$). An increased risk of relapse in the operated patients is mostly dependant on the state of malnutrition and immune deficiency occurring in the postoperative period as well as the frequent use of antibiotics both before and during the postoperative period [12]. The results of our study confirmed a statistically significant impact of malignancies on the occurrence of CDI relapse. The relapse developed in 19.52% of patients with malignant tumors, while the stable clinical response had only 8.82% of patients ($p = 0.023$). Some studies showed that the patients with malignancies which require chemotherapy more often got the CDI relapse independently of the use of antibiotics, almost with each cycle of chemotherapy. Several factors may contributed to this and they are such as: alteration of the intestinal microflora, severe inflammatory lesions of mucosa of the colon, chemotherapy, intestinal necrosis, decreased degradation of CD toxin and the inability to regenerate normal intestinal flora. Taking into account the occurrence of oral-gastrointestinal mucositis and nausea caused by chemotherapy, these patients often tolerate metronidazole poorly and it is considered justified the initial implementation of vancomycin even in the easier forms of CDI in the patients with malignant disease which requires the use of chemotherapy [13–15].

In our findings, a high peripheral leucocyte count (> 15,000/mm³) at onset of the initial CDI episode was not predictive of recurrent CDI, which is contrary to results of Rodrigues-Pardi et al. [16]. These authors suggest that the patients with high leucocyte count had the more severe initial CDI episode which may leave the bowel more vulnerable to subsequent CDI. In our study, hypoalbuminemia had a statistically significant effect on the occurrence of CDI relapse. In the patients with the albumin level of < 25 g/L, the relapse developed in 70.27% vs 41.94% of patients with the stable clinical response ($p = 0.034$). Similar to our results, Rotramel et al. [17] and Shakov et al. [18] also proved a statistically significant effect of hypoalbuminemia on the occurrence of CDI relapse. These results are attributable to the fact that CD toxin-A increases vascular and mucosal permeability of intestinal tract resulting in intraluminal accumulation of fluid rich with serum albumin. Hypoalbuminemia is a marker of poor underlying health condition, a protracted associated chronic diseases, poor nutritional status and poor immune function of the host, and therefore the lack of production of toxin-neutralizing IgA antibodies to CD which may increase the risk for CDI [5, 13, 19].

We found that concomitant use of non-CDI antimicrobials during the first episode of CDI raise the risk for recurrent CDI. Frequency of CDI relapses compared to the stable clinical response was 50.67% *vs* 20.29% ($p = 0.031$). Kelly's [20] research also demonstrated that the concomitant use of non-CDI antibiotics significantly affects the occurrence of CDI relapse ($p = 0.0012$). The authors of other studies came to the same conclusion [21–23]. The antimicrobial therapy for the concomitant infections may result in altering bowel microflora, favoring CD growth. More frequent occurrence of relapses due to the concomitant use of antibiotics for conditions not related to CDI was due to higher level of additional continuous disruption of the intestinal flora, which allows persistence of CD [3, 24]. In our study, a statistically significant effect of the use of proton pump inhibitors on the occurrence of CDI relapse was not observed. Studies published by the Lupşe et al. [6] and Rodrigues-Pardo et al. [16] demonstrated a statistically significant effect of proton pump inhibitors on the occurrence of CDI relapse, while Rotramel et al. [17] did not find that connection. Whether the gastric acid suppression is truly an independent RF for recurrent CDI remains unknown.

The results of our study showed that vancomycin was more effective than metronidazole in the elimination of CD toxins from the intestinal tract. After the completion of the CDI therapy, the persistence of CD toxins was found in 22.32% of patients treated with metronidazole compared to 9.09% of patients who were given vancomycin ($p = 0.003$). Similarly to our results, McFarland et al. [25] found that vancomycin was significantly more efficient than metronidazole in the elimination of CD toxins from the colon. In this study, 11% of patients treated with vancomycin *vs* 41.2% of patients given metronidazole ($p = 0.0004$) were positive for CD toxins in the stool after the completion of therapy. Wullt and Odenholt [26] registered the persistence of CD toxins after the treatment with metronidazole in 23% of patients, and the study of de Lalla et al. [27] showed that after the treatment with vancomycin, the persistence of CD toxins was found in 25% of patients. The literature data indicate that the post-therapy bacterial persistence is not conditioned only by the applied therapy regime but also by a strain of CD, its capacity of toxin production and sporulation but also by factors of the host such as the presence of nutritional contents in the intestines which are crucial for the toxin production and the state of the immune system, that is, the production toxin-neutralizing Ig A antibodies [28, 29].

Previous studies showed greater efficacy of vancomycin compared to metronidazole in the elimination of CD toxins from the colon, but the studies did not prove the higher frequency of relapse in patients with the persistence of CD toxins [25, 29]. McFarland et al. [25] showed that after metronidazole treatment, the patients who had recurrences did not have a significantly higher frequency of the CD persistence (53.5%) than those who did not have the relapse (31.6%) [26]. Noren et al. [28] analyzed the link between microbial efficiency and clinical outcomes after the CDI therapy with metronidazole and concluded that in the case of persistence of CD toxins, the stable clinical response is achieved in 57% of patients *vs* 74% of patients with the stable clinical response. In accordance with the previous research, the results of our study also showed that the presence of CD toxins in the stool after the completion of the CDI therapy, did not affect significantly the occurrence of relapse. In our study, the persistence of CD toxins was registered in 13.87% of patients with the stable clinical response versus 23.94% of patients with the relapse ($p > 0.05$). After the treatment with metronidazole, this ratio was 19.01% *vs* 31.71% ($p = 0.08$), and after the treatment with vancomycin it was 9.82% *vs* 13.33% ($p = 0.16$). This finding could be interpreted by the previously proven fact that asymptomatic carriers, after the successful completion of the initial episode of CDI, developed a sufficient level of toxin-neutralizing A antibodies to CD, by which this population of patients acquire low risk of the CDI relapse. Therefore, in daily practice, after the successfully completed treatment, it is not recommended the routine testing of stool samples on the presence of CD toxins as a "control test of treatment success" [29].

Our findings confirm that CDI is present in our settings with a significant rate of relapse. The primary strength of our study is its ability to point out the population of patients at the highest risk of CDI relapse in our settings, because almost all important RF of relapse mentioned in the current literature were taken into consideration. Besides, the study encompassed a significant number of patients and therefore we believe that our findings may be of a great importance for the creation of future therapeutic strategies in the CDI treatment. However, this study has several limitations. Firstly, it was a retrospective clinical trial. Secondly, although we are aware that the host immune system plays a crucial role in a CDI relapse, we were not able to measure the anti-toxin IgG levels in our patients. Furthermore, because of the rising prevalence of the epidemic CD strains that produce more severe disease and cause more frequent CDI relapse, the aim of some future investigation could also be to determine and analyze strains of CD in our settings.

Conclusion

Our data suggest that important RF for the CDI relapse are comorbidities such as a recent surgery (within a month before developing CDI) and malignancy, low albumin level (< 25 g/L) as well as concomitant non-CDI antibiotics treatment. Future treatment strategies for the CDI relapse should emphasize those group of patients.

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Six-month success of radiofrequency ablation in cardiac arrhythmias treatment – experience of our centre

Šestomesečni uspeh radiofrekventne ablacije u lečenju poremećaja srčanog ritma – iskustvo našeg centra

Ružica Jurčević Mudrić, Lazar Angelkov, Milosav Tomović, Dejan Kojić, Predrag Milojević

Institute for Cardiovascular Diseases "Dedinje", Belgrade, Serbia

Abstract

Background/Aim. Numerous trials have shown a high success of radiofrequency ablation (RFA) in the treatment of the patients with cardiac arrhythmias. We aimed to examine the RFA initial success in treatment of different cardiac arrhythmias and the RFA success after 6 months of follow-up. Second aim was to evaluate influence of all clinical and echocardiography parameters on initial and 6-month success and failure of RFA. **Methods.** The present study included 320 consecutive patients with atrial and ventricular arrhythmias in which RFA was performed during 2014 in the Institute for Cardiovascular Diseases "Dedinje", Belgrade, Serbia. We evaluated the initial RFA success and success of this procedure after 6-month follow-up. We also investigated the prognostic role of clinical and echocardiography parameters on initial and 6-month success and failure of RFA. **Results.** The RFA initial success for RFA of atrioventriculas (AV) node and AV nodal reentrant tachycardia (AVNRT) was 100%, RFA of pulmonary veins 99%, RFA of atrial flutter 92%, RFA of premature ventricular complexes (PVC) and the Wolf-Parkinson-White (WPW) syndrome 87%, RFA of ventricular tachycardia 85% and RFA of atrial tachycardia 78%. The success of RFA after 6 months of follow-up for RFA of the AV node was 100%, RFA of AVNRT 94%, RFA of atrial flutter 90%, RFA of WPW syndrome 86%, RFA of pulmonary veins 79% (paroxysmal atrial fibrillation 88% and persistent atrial fibrillation 63% with a significant difference $p < 0.05$), RFA of PVC 78%, RFA of ventricular tachycardia 77% and RFA of atrial tachycardia 67%. **Conclusion.** This study proved a very high RFA initial success in treatment of cardiac arrhythmias and a satisfactory RFA success after 6 months of follow-up. Only the prognostic value had the type of atrial fibrillation in the group with catheter ablated pulmonary veins: after 6-month follow-up, the patients with paroxysmal atrial fibrillation had a significantly better outcome than those with persistent form.

Key words:
arrhythmias, cardiac; catheter ablation; electrocardiography; recurrence; serbia.

Apstrakt

Uvod/Cilj. Veliki broj studija dokazao je visok uspeh radiofrekventne ablacije (RFA) u izlečenju bolesnika sa različitim srčanim aritmijama. Primarni cilj rada bio je da odredi neposredni uspeh RFA u lečenju različitih poremećaja srčanog ritma i uspeh RFA nakon šest meseci praćenja bolesnika. Sekundarni cilj je bio evaluacija uticaja svih kliničkih i ehokardiografskih parametara na neposredni i šestomesečni uspeh i neuspeh RFA. **Metode.** Istraživanje je obuhvatilo 320 uzastopnih bolesnika kod kojih je urađena RFA tokom 2014. godine na Institutu za kardiovaskularne bolesti "Dedinje", Beograd, Srbija. Procenjivan je neposredni uspeh RFA i uspeh RFA nakon šest meseci praćenja bolesnika. Određivan je uticaj kliničkih i ehokardiografskih parametara na neposredni, šestomesečni uspeh i neuspeh RFA. **Rezultati.** Neposredni uspeh iznosio je za RFA atrioventrikularnog (AV) čvora i RFA AV nodalne *reentrant* tahikardije (AVNRT) 100%, RFA plućnih vena 99%, RFA atrijalnog flatera 92%, RFA komorskih ekstrasistola i Wolf-Parkinson-White (WPW) sindroma 87%, RFA komorske tahikardije 85% i RFA atrijalne tahikardije 78%. Uspeh RFA nakon šest meseci praćenja ovih bolesnika bio je za RFA AV čvora 100%, RFA AVNRT 94%, RFA atrijalnog flatera 90%, RFA WPW sindroma 86%, RFA plućnih vena 79% (paroksizmalna atrijalna fibrilacija 88% i perzistentna atrijalna fibrilacija 63% sa značajnom razlikom, $p < 0.05$), RFA komorskih ekstrasistola 78%, RFA komorske tahikardije 77% i kod RFA atrijalne tahikardije 67%. **Zaključak.** Postignut je veoma visok neposredni uspeh RFA u izlečenju srčanih aritmija i zadovoljavajući uspeh RFA nakon šest meseci praćenja bolesnika. Prognostički značaj jedino je imao tip atri-

Correspondence to: Ružica Jurčević Mudrić, Institute for Cardiovascular Diseases Dedinje, Heroja Milana Tepića 1, 11000 Belgrade, Serbia. E-mail: ruzicajurcevic@hotmail.com

jalne fibrilacije u grupi bolesnika kojima je urađena RFA plućnih vena: nakon šest meseci praćenja bolesnici sa paroksizmalnom atrijalnom fibrilacijom imali su značajno bolji uspeh RFA nego oni sa perzistentnom formom.

Ključne reči:
aritmija; ablacija preko katetera; elektrokardiografija; ehokardiografija; recidiv; srbija.

Introduction

Importance of radiofrequency ablation (RFA) in the treatment of atrial and ventricular arrhythmias is high and the success of procedure increases every day due to the new methods and techniques [1]. RFA is the most successful therapy for typical atrial flutter (AF), atrioventricular (AV) nodal reentrant tachycardia (AVNRT), the Wolf-Parkinson-White (WPW) syndrome and idiopathic ventricular tachycardia (VT), effective in over 95% of the patients [2]. These patients do not need the antiarrhythmic drugs after successful RFA.

The success rate of RFA is lower in the management of premature ventricular complexes (PVC) and VT in structural heart diseases, 75%–85% after the follow-up of the patients [3]. RFA of pulmonary veins (PV) is the newest field in therapy of atrial fibrillation (AFib) with the greatest results in the paroxysmal form of this disease and about 80% of success after 5 years of following up the patients [4].

The aim of this study was to examine the initial success of RFA in treatment of atrial and ventricular arrhythmias and to evaluate the RFA success after 6 months of the patients' follow-up. We also aimed to investigate the influence of all clinical and echocardiography parameters on the initial and 6-month success of RFA and the initial and 6-month failure of RFA.

Methods

The present study included 320 consecutive patients with different cardiac arrhythmias in which RFA was performed during 2014 at the Institute for Cardiovascular Diseases "Dedinje", Belgrade, Serbia. We divided patients into the following 8 groups: RFA of AF, RFA of atrial tachycardia (AT), RFA of PV, RFA of AV node, RFA of AVNRT, RFA of PVC, RFA of VT and RFA of WPW syndrome. We examined the following in each group: initial success of RFA and success after 6 months of the patients' follow-up; prognostic role of all clinical and echocardiography parameters on the initial and 6-month RFA success; prognostic influence of clinical and echocardiography parameters on initial and 6-month failure of RFA.

We evaluated the clinical symptoms and signs, electrocardiogram (ECG), 24 h ambulatory monitoring, echocardiography and performed the electrophysiological exam and RFA in all patients. Transoesophageal echocardiography (TEE) was done in all patients in the groups for RFA of AF, AT and PV with paroxysmal and persistent arrhythmias; we examined the presence of thrombus in left auricula and left atrium, anatomy of left, right, superior and inferior PV because of a significant inter- and intra-patient variability in the number, size and bifurcation of the PVs. The patients with thrombus were excluded from the study because of a risk of cerebral insult. Multislice computed tomography (MSCT) of PV was performed in all patients in the group for RFA of atrial fibrillation and together with the images from TEE which provided better visualization of PVs.

An expert with a manipulation skill, introduced catheter for ablation in pathoanatomic substrate which caused arrhythmia and delivering the RF energy made a limited lesion, a few millimeters wide and deep in the endocard. The RF energy was applied at a target temperature of 50°C with a power limit to 30–35 W for 30–60 s.

This trial determined the RFA initial success, the RFA success after 6 months of the follow-up and the influence of all clinical and invasive parameters on the procedural success and failure. We defined the initial success as termination and inability to induce arrhythmia after catheter ablation. The success of RFA of AF means the achievement bidirectional block in cavotricuspidal isthmus. The endpoint for RFA of PV was establishment of bidirectional block between left atrium and PV. The failure of RFA means that after the procedure we can induce identical tachycardia like before RFA.

After 6 months from the ablation procedure, a recurrence of arrhythmias was evaluated based on the present symptoms, clinical signs, ECG, 24 h ambulatory monitoring. The patients who had symptoms without documentation of arrhythmia recidivism were examined by 7-day ambulatory monitoring. Recurrence was defined as episodes of arrhythmias lasting for more than 30 s after a 30-day blanking period. None of the patient with the successful RFA received antiarrhythmic drugs after the ablation procedure – except the group with RFA of PV (6 weeks antiarrhytmic therapy and 3 months anticoagulation). It means that the 6-month success for RFA of PV was evaluated in drug-free patients. A repetition of RFA was recommended for the patients with recurrence of their arrhythmias and for initially failed RFA.

The continuous variables are expressed as means ± standard deviation (SD). The initial successes of RFA and that after 6 months of follow-up were shown in percentage. Comparison between the groups was performed using the Student`s *t*-test (unpaired) and the proportions were compared by using χ^2 analysis with an aim to evaluate the influence of all clinical and echocardiography parameters on the initial and 6-month success and failure of RFA. The values of $p < 0.05$ were considered significant.

Results

During 2014, at the Institute for Cardiovascular Diseases "Dedinje", RFA was performed in 320 patients with the initial success in 93% and failure in 7%, respectively, and was effective in 296 patients and unsuccessful in 24 cases (Table 1). The RFA success rate after 6 months of follow-up

was 83% (267 cases) because 29 patients had recurrence of arrhythmia (10%). A total number of patients who appeared to have the ineffective RFA after 6 months of follow-up was 53 (17%) and they were indicated to repeat the intervention.

RFA of AF was done in 38 patients (36 with typical AF and 2 with atypical AF) which baseline characteristics prior to the initial procedure are shown in Table 2. The RFA initial success rate in AF was 92%, 35 patients had the successful RFA and in 8% (3 cases) was ineffectual. After 6 months of follow-up, only 1 patient had a recurrence of AF and we calculated the success of RFA of AF in 34 (90%) cases and failure in 4 (10%) patients who were indicated for the repeat procedure. RFA of typical AF in 36 patients had the initial success in 97% (effectual in 35 patients), and after 6 months of follow-up, it was successful in 94% (recidivism in 1 patient). Both cases with atypical AF had unsuccessful RFA initially. Six patients with ablated AF and accompanied paroxysmal AFib were indicated for RFA of PV. The clinical and echocardiography parameters did not have influence on the initial and 6-month success and failure of RFA in the group with AF.

The initial success rate of RFA in 9 patients with AT was 78%. RFA was effective in 7 cases and in 22% (2 cases) was unsuccessful. After 6 months of follow-up 1 patient had recidivism of AT, and RFA of AT had good results in 67% (6 patients) and failure in 33% (3 patients).

RFA of PV was performed in 76 patients (65 males and 11 females mean age of 55 ± 8 years) with the AFib diagnosis and-systolic diameter (Table 2). The average duration of the symptoms was 5 ± 4 years and 8 (11%) patients had syncope. Echocardiography found the mean, values for ejection fraction (EF) 55 ± 8% (range 20%–65%), endodiastoli diameter (EDD) 54 ± 4 mm, and-systolic diameter (ESD) 36 ± 5 mm and diameter of left atrium (LA) 42 ± 5 mm (between 30–53 mm). According to the duration, AFib was divided into two types: paroxysmal AFib in 49 (65%) patients and persistent AFib in 27 (35%) cases.

The initial success rate for RFA of PV in 76 patients with AFib was 99%, which means that RFA was effective in 75 patients and in 1% (1 patient) was unsuccessful. After 6 months of follow-up, RFA of PV was efficient in 79% (60 patients) and unsuccessful in 21% (16 patients) who were indicated for the repeat procedure. It means that 20% (15) of the initially successfully ablated patients had the recurrence of AFib. A significant difference and prognostic influence was proved for the type of AFib ($p < 0.05$), the patients with paroxysmal AFib had better outcome after 6 months of follow-up with RFA success in 88% (of 49 cases ablated, 43 cured) than those with persistent AFib and the RFA success in 63% (of 27 cases ablated, 17 cured).

Table 1

Radiofrequency ablation (RFA) the initial success and RFA success after 6 months of the follow-up of 320 patients with cardiac arrhythmias

Arrhythmia type (number of patients)	Initial success, number (%) of patients	6 months follow-up success number (%) of patients
Atrial flutter (38)	35 (92)	34 (90)
Atrial tachycardia (9)	7 (78)	6 (67)
Atrial fibrillation (76)	75 (99)	60 (79)
RFA of AV node (11)	11 (100)	11 (100)
AVNRT (50)	50 (100)	47 (94)
PVC (45)	39 (87)	35 (78)
VT (47)	40 (85)	36 (77)
WPW syndrome (44)	39 (87)	38 (86)
Total (320)	296 (93)	267 83)

AVNRT – atrioventricular nodal reentrant tachycardia; PVC – premature ventricular complexes; VT – ventricular tachycardia; WPW – Wolf-Parkinson-White.

Table 2

Baseline characteristics of patients (n = 320) with cardiac arrhythias prior to the radiofrequency ablation (RFA)

Arrhythmia type (number of patients)	Age (years)	Gender (M/F)	Symptoms duration (years)	Syncopa n (%)	Ejection fraction (%)	LA diameter (mm)	Hypertension n (%)	Structural heart disease n (%)
AF (38)	58 ± 11	29/9	3 ± 2	3 (8)	52 ± 11	41 ± 5	5 (13)	27 (71)
AT (9)	41 ± 19	5/4	6 ± 4	2 (22)	52 ± 13	37 ± 6	0 (0)	1 (11)
Afib (76)	55 ± 8	65/11	5 ± 4	8 (11)	55 ± 8	42 ± 5	33 (43)	14 (18)
AV node (11)	68 ± 6	8/3	4 ± 3	3 (27)	28 ± 14	49 ± 5	1 (9)	8 (73)
AVNRT (50)	48 ± 11	22/28	12 ± 11	9 (18)	59 ± 4	36 ± 5	7 (14)	3 (6)
PVC (45)	46 ± 15	17/28	6 ± 5	8 (18)	55 ± 8	38 ± 6	5 (11)	14 (31)
VT (47)	54 ± 16	37/10	3 ± 2	18 (38)	35 ± 17	42 ± 6	1 (2)	45 (96)
WPW syndrome (44)	32 ± 15	29/15	9 ± 9	5 (11)	57 ± 6	35 ± 4	6 (14)	1 (2)

AF – Atrial flutter; AT – atrial tachycardia; Afib – atrial fibrillation; AVNRT – atrioventricular nodal reentrant tachycardia; PVC – premature ventricular complexes; AVnode – atrioneutricular node; VT – ventricular tachycardia; WPW – Wolf-Parkinson-White; M/F – male/female; LA – left atrium.

Complications for RFA of PV were reported in 6 patients: 1 patient had pericardial effusion and 4 patients had hematoma on the place of punction (Table 3). These complications were cured conservatively, except in one case of jugular vein hematoma when vascular surgery had to be done. In one patient an ablation catheter clenched in chordaes of myocardium and a cardiac surgery solved the problem.

Table 3

Significant complications of radiofrequency ablation (RFA) in 12 patients out of 320 (3.75%)

Complications	Frequency, number (%)
Inguinal hematoma	5 (1.56)
Jugular Vein hematoma	1 (0.31)
Pericardial effusion	1 (0.31)
Cardiac tamponade	2 (0.63)
Complete AV block	2 (0.63)
Catheter clenched in myocardium chordaes	1 (0.31)
Total	12 (3.75)

AV – atrioventricular.

The initial success rate for RFA of AV node in 11 patients with permanent tachyarrhythmia absolute was 100% which means that in all patients RFA was effective. After 6 months of follow-up, there was no relapse of arrhythmia and the success rate for RFA of AV node remained 100%.

RFA of AVNRT was efficient in all 50 patients with the initial success of 100%. After 6 months of follow-up RFA of AVNRT had a success in 94% (47 patients) and the recurrence of tachycardia in 6% (3 patients) which were indicated for the repeat intervention. The electrophysiology study prior to RFA diagnosed a typical slow-fast AVNRT in all 50 patients.

RFA of PVC was performed in 45 patients (17 males and 28 females) with the mean age 46 ± 15 years (range 18–77 years). Average duration of the symptoms was 6 ± 5 years (range 1–20 years) and syncope had 8 (18%) patients. On admission to hospital, the changes were found in 27 (60%) cases using the standard ECG: PVC in 26 (58%) patients and rhythm of pacemaker in 1 (2%) case. Echocardiography found average EF 55% ± 8% (range 25–60%), EDD 54 ± 5 mm, ESD 34 ± 6 mm and the LA dimension 38 ± 6 mm (range 28–53 mm).

The RFA initial success in 45 patients with PVC was 87%, i.e. in 39 cases RFA was effective and it was unsuccessful in 6 cases 13%). After 6 months of follow-up, RFA had good results in 78% (35 patients) and failure in 22% (10 patients) because arrhythmias recurrence occurred in 4 cases. The localization of ablated PVC was determined in right ventricle in 61% (27 patients) and in left ventricle in 39% (18 patients). The right ventricular outflow tract was presented in 55% (24 cases) and left ventricle outflow tract in 26% (12 cases). In our results, there were no any good or bad predictor for the initial or 6-months prognosis in this group of patients found.

RFA of VT was done in 47 patients (37 men and 10 women) with the mean age of 54 ± 16 years (range 17–80 years). The average duration of symptoms was 3 ± 2 years (between 1–8 years) and 18 (38%) patients experienced syncope. Fifteen (33%) patients had implantable cardioverter defibrillators. On admission to hospital, the abnormal electrocardiogram was found in 10 (20%) patients: PVC in 3 (6%) patients, VT in 3 patients, AFib in 1 (2%) patient, left bundle branch block in 2 (4%) patients and rhythm of pacemaker in 1 patient. Echocardiography found the mean values for EF 35% ± 17% (range 10–60%), EDD 61 ± 8 mm, ESD 45 ± 10 mm and the LA diameter 42 ± 6mm (range 30–60 mm). Five patients had a significant mitral valve regurgitation (3+) and indication for a surgery of mitral valve.

The initial success rate for RFA of VT in 47 patients was 85%, which means that RFA was efficient in 40 patients and failure happened in 7 (15%) cases. After 6 months of follow-up, 4 (8%) patients had the recurrence of VT, and a success rate was 77% (36 patients) and failure of ablation of VT, after the follow-up was 23% (11 patients). The location of ablated VT was described in right ventricle in 27% (13 patients), left ventricle in 49% (22 patients), epicardial in 18% (9 patients) and fascicular VT in 6% (3 patients). In right ventricle, the VT form outflow tract was presented in 23% (11 cases) and from tricuspid annulus in 4% (2 cases). In left ventricle, VT form outflow tract was found in 4% (2 cases), mitral annulus in 4% and the most common free wall was noted in 34% (16 patients). One patient had VT origin from post infarct psudoaneurysm in the posteroseptal part of left ventricle and in one case VT was from the papillary muscle. The approach to RFA depended of the VT site of origin: retroaortal in 32% (14 cases), through femoral vein in 27% (13 patients), transseptalis access in 23% (11 patients) and epicardial approach in 18% (9 patients). After RFA of VT, an implantable cardioverter defibrillator was indicated in 10 patients because the control electrophysiological study induced fast VT or ventricular fibrillation. We did not prove an influence of VT localization, the clinical and echocardiography parameters on the initial and 6-monthsuccess or failure of RFA.

There were 4 complications of VT catheter ablation: 2 inguinal hematomas were managed conservatively, 1 cardiac tamponade was cured with pericardiocentesis and 1 complete AV block was treated with implantation of pacemaker (Table 3).

Initial success for RFA of WPW syndrome in 44 patients was 87%; in 39 patients the results were good and in 13% (5 patients) they were ineffective. After 6 months of follow-up, recurrence happened in 1 (1%) case, and the RFA success rate was 86% (efficient in 38 cases) and failure in 14% (6 patients). According to the localization, the accessory pathways (AP) were classified: right ventricular free wall in 5 (13%) cases, left ventricular free wall in 19 (43%) cases, septal position in 19 (43%) cases and epicardial location in 1 (1%) case. We did not find any prognostic role of parameters on the initial and 6-months success or failure of RFA. We described two complications of catheter ablation of AP: in one case the complete AV block was managed with implantation of pacemaker, and in another case, the cardiac tamponade was treated with pericardiocentesis (Table 3).

Significant complications of RFA were reported in 12 (3.75%) patients. There were not procedure related deaths.

Discussion

The present study includes the largest number of patients with cardiac arrhythmias that were analyzed at the Institute for Cardiovascular Diseases "Dedinje", Belgrade, Serbia. Our results proved to be of a high importance and capability of RFA in the treatment of atrial and ventricular arrhythmias, and can be compared with results recorded in the eminent electrophysiology laboratories [1, 4]. This trial showed an excellent initial success of RFA in 93% of 320 patients and failure in 7% of cases which were indicated for the repeat procedure. After 6 months of follow-up, 10% had the recurrence of arrhythmias and the RFA success was satisfactory in 83%.

This study found the initial success of 97%, and after 6 months of follow-up, good results were achieved in 94% of patients. Atypical AF was found only in 2 cases and we did not analyze them. In relevant literature, a success of catheter ablation of typical AF is over 95%, and smaller for atypical AF [5].

The group with ablated AT had the small number of patients, so we cannot compare our results with other studis where the success rate was 93% for right atrium AT and smaller for left atrium AT [6].

The present study showed the eminent initial success of 99% in the patients with ablated PV. We proved the RFA success after 6 months of follow-up in 79% and significantly better outcome ($p < 0.05$) in the patients with paroxysmal AFib (success rate of 88%) than those with persistent AFib (success rate 63%). Sohns et al. [7] found the initial success for catheter ablation of PV in 92%, and after 2 years of follow-up it was 81% for paroxysmal AFib and 76% for the persistent form. A large number of studies identified the predictors of a poor outcome following RFA of PV: non-paroxysmal AFib and particulary longstanding persistent AFib; increased atrial size; sleep apnea and obesity; elderly patients; decreased left ventricular function; left atrial fibrosis as detected by cardiac magnetic resonance imaging (MRI) [8]. We proved only one predictor (paroxysmal AFib) of a good 6-month prognosis in our patients with ablated PV. The limitations of our study were small number of cases and only 6 months follow-up after RFA of PV comparing to other trials with a larger number of patients and longer follow-up [4, 7, 8].

Numerous trials reported the recurrence of AFib after RFA of PV in 20%–40% of patients, while recidivism of AFib was 20% in our patients after 6 months of follow-up [4, 6]. Early relapse of AFib and/or AT is frequent during first 3 months after the catheter ablation of PV and can spontaneously vanish, so experts made consensus that repeat RFA was not recommended during this time [4]. They also concluded that, in some patients who had highly symptomatic atrial arrhythmias resistant to the treatment with antiarrhythmic drugs, it was recommended to ablate PV within 3 months after first intervention. Most of the studies confirmed that the patients who had recurrence and underwent repeat RFA had the most frequently origin of AFib from already ablated PV and rarely from a new focus in non-ablated PV and another location in atrium [4, 7, 8].

The treatment of drug resistant permanent absolute tachyarrhythmia in our patients with RFA of AV node had the excellent initial success in 100% without the recurrence after 6 months of follow-up. Numerous trials showed the success rate of 99%–100% for RFA of AV node [2].

The present study reported a great initial success of 100% for RFA of AVNRT, and after 6 months of following up period, the success was 94%. Stern et al. [9] revealed the initial success in 98% of cases for RFA of AVNRT, and after 3 months of follow-up, recurrence was 2%. All our patients had the typical slow-fast form of AVNRT, while in literature this form was described in 90% of cases [6].

RFA of PVC in our patients had the initial success of 87% and after 6 months of follow-up success is 78%. Numerous studies revealed the initial success for RFA of PVC to be 80%–90% [10, 11].

RFA of VT had good initial results in 85% of our patients, and after 6 months of follow-up, the success was 77%. Famous electrophysiology laboratories had 17% of epicardial mapping while our results for RFA of epicardial VT were distinguished by 18% [12]. Pederson et al. [1] revealed the biggest success of RFA for idiopathic VT from right and left ventricular outflow tracts (over 90%), while from other sites of origin the results of VT catheter ablation were modest (71%–79%) [1, 3]. Our results do not prove any good or bad predictor of initial or 6-month success or failure in the groups with RFA of PVC and VT. Numerous trials identified the predictors of a poor prognosis for RFA of PVC and RFA of VT: structural heart disease, decreased left ventricular EF, older patients and presence of the RFA major complications [1].

This study found the modest initial success of RFA of WPW syndrome in 87% of cases, and after 6 months of follow-up, the success in 86% of cases. In literature, the initial good results for RFA of AP was 99%, and after 3 months of follow-up, recidivism was 2% [13]. We did not find a prognostic role of AP location, the clinical and echocardiography parameters in the group of patients with RFA of AP. In the largest trial conducted on 1,050 patients, Calkins et al. [14] reported that the success rate for catheter ablation of left free wall APs was slightly higher than for RFA of right-sided APs (95% *vs* 90%, $p = 0.03$). After an initially successful procedure in 93% of patients, the recurrence of AP conduction was found in approximately 5% of cases. The recurrence-free interval after ablation was also the best with left-sided pathways.

Limitations of the present study were 8 groups of different arrhythmias with the small number of patients and a short 6-month follow-up. These facts influenced our finding of only one prognostic parameter – the type of atrial fibrillation in the group of patients with ablated PV. The data from present trial will be included in a future study with a bigger number of cases and longer follow-up.

This study showed significant RFA complications in 12 of 320 (3.75%) patients, which is acceptable and can compare with result (3%–4.2%) from the other eminent electrophysiology laboratories [2]. There were no deaths during RFA in our patients which are excellent results.

According to our results and the data from literature, we conclude that RFA is a safe procedure that is evolving with a

great success in treatment of cardiac arrhythmias. Catheter ablation is a therapy of choice for the management of typical AF, AVNRT, WPW syndrome and idiopathic VT.

Conclusion

The present study proved a very high RFA initial success in treatment of atrial and ventricular arrhythmias and the satisfactory RFA success after 6 months of follow-up. Only the prognostic value had a type of atrial fibrillation in the group with catheter ablated pulmonary veins: after 6 months of the follow-up, the patients with paroxysmal atrial fibrillation had a significantly better outcome than those with the persistent form.

This trial showed a great success of RFA, high in therapy of cardiac arrhythmias, which can be compared with the results in famous electrophysiology laboratories.

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Mitchell and Golden metatarsal osteotomies for the treatment of moderate hallux valgus deformity: A comparative analysis

Mičelova i Goldenova metatarzalna osteotomija u lečenju umerenih deformiteta čukljeva: uporedna analiza

Nadan Mikić*, Dušanka Grujoska-Veta[†‡], Goran Čobeljić[§], Ismet Gavrankapetanović[‖¶], Zoran Vukašinović[§], Ivan Soldatović, Miroslava Popović***

Zvezdara University Medical Center, *Clinical Department of Orthopaedics and Traumatology, Belgrade, Serbia; [†]University Clinic for Orthopaedic Surgery, Skoplje, Republic of North Macedonia; University "Cyril and Methodias", [‡]Faculty of Medicine, Skoplje, Republic of North Macedonia; University of Belgrade, Faculty of Medicine, [§]Institute for Orthopaedic Surgery "Banjica", **Institute of Medical Statistics and Informatics, Belgrade, Serbia; Clinical Center, [‖]Clinic of Orthopaedics and Traumatology, Sarajevo, Bosnia and Hercegovina; University of Sarajevo, [¶]Faculty of Medicine, Sarajevo, Bosnia and Hercegovina

Abstract

Background/Aim. Despite bunion surgery having been performed for more than 100 years, there has yet to be a technique considered as the "Gold Standard". The aim of the study was to compare postoperative results of Mitchell vs. Golden methods of treating moderate hallux valgus deformity. **Methods.**This observational case control study included 49 patients (81 feet) who had the Mitchell distal metatarsal osteotomy performed, and 49 patients (77 feet) that had the Golden proximal metatarsal osteotomy performed. The results of treatment were evaluated using Hellal's modification of the Bonney and McNab classification and the Hallux Metatarsophalangeal Interphalangeal Score (HMIS). The statistical analysis of the results was done, thus the values $p < 0.05$ were considered statistically significant. **Results.** Both operative procedures showed successful and statistically significant postoperative results compared to the preoperative status ($p < 0.001$). Comparative analysis of the results from the Mitchell and Golden procedures, according to the Hellal's modification of Bonney and McNab classification, proved that there was a high statistically significant difference in favor of the Mitchell method ($p < 0.001$), whereas the comparison based on the HMIS showed no statistically significant difference ($p = 0.123$) between the two methods. The estimated results analysis of both procedures, based on the values of hallux valgus angle, intermetatarsal angle, sesamoid position, length of immobilization, treatment duration and complications demonstrated that there was a highly significant difference in favor of the Mitchell method ($p < 0.001$), whereas the value of the shortening of the first metatarsal bone indicated that the shortening was greater in the Mitchell method ($p < 0.001$), which goes in favor of the Golden method. Regarding the flexion of the thumb of the feet operated on, there was no statistically significant difference ($p = 0.723$). **Conclusion.** The examinations performed indicated that both methods showed good postoperative results, but applying the Mitchell method they were better.

Key words:
hallux valgus; osteotomy; surgical procedures, operative; treatment outcome.

Apstrakt

Uvod/Cilj. Mada se operativno lečenje čuklja primenjuje duže od 100 godina, još uvek nije ustanovljena tehnika koja bi se mogla smatrati takozvanim zlatnim standardom. Cilj rada bio je poređenje postoperativnih rezultata postupaka po Mitchell-u i Golden-u u lečenju umerenih deformiteta *hallux valgus*-a. **Metode**. U opservacionoj studiji kontrole slučajeva (*case control*) učestvovalo je 49 pacijenata (81 stopalo) operisanih distalnom osteotomijom I metatarzalne kosti po Mitchell-u i 49 pacijenata (77 stopala) operisanih proksimalnom osteotomijom I metatarzalne kosti po Golden-u. Za procenu rezultata lečenja korišćene su: Klasifikacija po Boney i McNab-u modifikovana po Hellal-u i *Hallux Meta-*

Correspondence to: Nadan Mikić, Clinical Department of Orthopedics and Traumatology, Zvezdara University Medical Center, Dimitrija Tucovića 161, 11 000 Belgrade, Serbia. E-mail: nadanmikic@gmail.com

tarsophalangeal Interphalangeal Score (HMIS). Rezultati su statistički obrađeni, a vrednosti $p < 0,05$ uzete su kao statistički značajne. **Rezultati.** Oba hirurška postupka su pokazala uspešne statistički visoko značajno bolje postoperativne rezultate u odnosu na preoperativne ($p < 0,001$). Poređenjem rezultata Mitchell-ovog i Golden-ovog postupka prema klasifikaciji po Bonney i McNab-u modifikovanoj po Hellal-u pokazano je da postoji visoko statistički značajna razlika u korist postupka po Mitchell-u ($p < 0,001$), a poređenjem na osnovu HMIS-a da nema statistički značajne razlike ($p = 0,123$). Analiza procene rezultata oba postupka na osnovu vrednosti hallus-valgus ugla, intermetatarzalnog ugla, pozicije sezamoida, dužine imobilizacije, trajanja lečenja i komplikacija pokazala je da postoji visoko statistički značajna razlika u korist postupka po Mitchell-u ($p < 0,001$), dok je procena vrednosti skraćenja prve metatarzalne kosti pokazala da je skraćenje izraženije kod postupka po Mitchell-u ($p < 0,001$), što ide u prilog Golden-ovoj metodi. U pogledu pokreta palca operisanih stopala nije postojala statistički značajna razlika ($p = 0,723$) između dva postupka. **Zaključak**. Oba postupka su pokazala dobre postoperativne rezultate. Poređenjem rezultata oba postupka došlo se do zaključka da su rezultati Mitchell-ovog postupka bolji u odnosu na Golden-ov postupak.

Ključne reči:
halkus valgus; osteotomija; hirurgija, operativne procedure; lečenje, ishod.

Introduction

Hallux valgus deformity shows high prevalence among general population. Depending on the age, the incidence rate of the deformity goes from 7.8% among youth under the age of 18, to 35.7% among those over 65 years old. As for the population between the ages of 18 and 65 years, the deformity incidence rate is 27% [1]. The hallux valgus deformity progression can lead to a gradual loss of function of the forefoot, resulting in the decreased quality of a patient's life. The treatment of hallux valgus deformity can be carried out by nonoperative and operative procedures. For a lack of effectiveness of nonoperative procedures, in the majority of cases the treating of deformity concludes with an operative procedure [2]. The objective of the operative treatment of the deformity is to get a pain-free, functioning and aesthetically pleasing foot. A surgical correction of the deformity can relieve the pain, prevent further progression of the condition and restore the function of the foot.

There is a vast number of operative procedures for treating bunions, which clearly supports the observation that there is no single method that could resolve all clinical varieties of this deformity. The osteotomy that is commonly used is made in the distal first metatarsal bone. Distal metatarsal osteotomy is recommended, primarily, to correct the mild to moderate deformities when there are no arthritic malformations of the first metatarsophalangeal joint. This osteotomy manages to correct most of the components of the deformity: hallux valgus angle (HVA), intermetatarsal angle (IMA), sesamoid position and the length of the first metatarsal bone [2, 3]. Proximal metatarsal osteotomy is used in the operative treatment of the moderate and severe hallux valgus deformity [4].

In medical literature, there is no a definite opinion on whether it is better to perform distal or proximal metatarsal osteotomy with the distal soft tissue procedure [5] for treating moderate deformities (HVA of 25° to 40° and IMA of 13° to 20°).

The aim of the study was to estimate the effects of the Mitchell and Golden procedures in order to determine which procedure should be given the advantage in correcting the moderate hallux valgus deformities.

Methods

An observational case control study was conducted in patients with moderate hallux valgus deformity who underwent the procedure at the Clinical Department of Orthopaedics of Clinical Hospital Center, University Medical Center "Zvezdara", during the period from January, 2007 to February, 2013. Two directions were applied: corrective distal metatarsal osteotomy by Mitchell [6] and corrective proximal metatarsal osteotomy by Golden [7] (Figures 1 and 2).

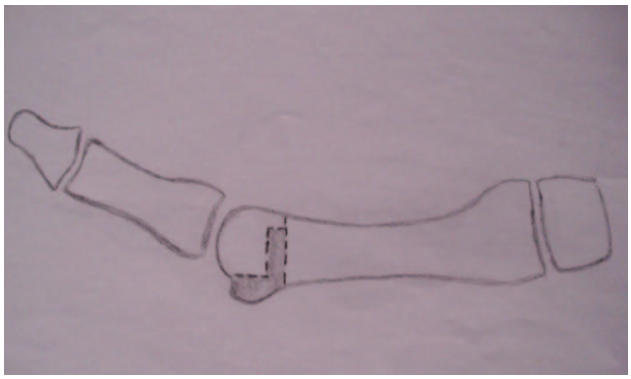

Fig. 1 – Operative technique described by Mitchell (a sketch in the horizontal plane).

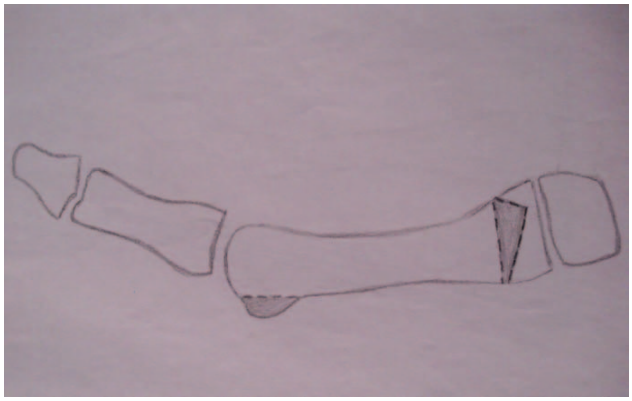

Fig. 2 – Operative technique described by Golden (a sketch in the horizontal plane).

Mitchell method

Distal metatarsal osteotomy was performed according to the Mitchell procedure which includes subcapital osteotomy of the first metatarsal bone with medial translation of the distal fragment with thread fixation in the corrected position. In the postoperative period the operated foot is immobilized with a short walking cast shoe, with an opening in the wound region for the postoperative wound care and check-ups. Decision on removing the cast immobilization is made after the X-ray scan and clinical assessment, in about 5 weeks. After the cast immobilization is removed, the physical therapy may begin.

Golden method

Golden osteotomy includes wedge osteotomy of the base of the first metatarsal bone using wedge base laterally and on the plantar side, where the fragment fixation is enabled by a single Kirschner wire. In addition, a thumb adductor tenotomy of the foot was performed through another approach using a different cut (Figure 3). Postoperatively, a walking cast shoe is placed onto the operated foot and it is removed after 5 to 7 weeks and physical therapy begins (Figure 4).

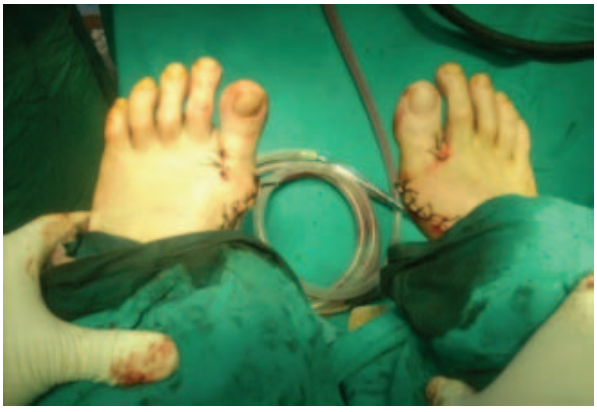

Fig. 3 – Bilateral Golden osteotomy after the completed procedure.

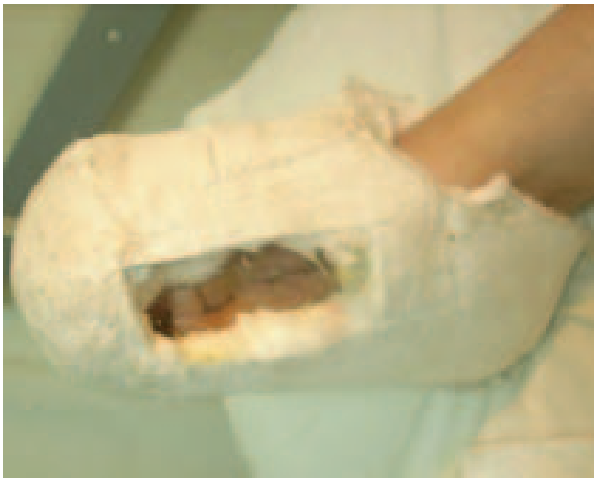

Fig. 4 – Walking cast shoe after Golden osteotomy (postoperative wound check-up).

The number of patients who were operated on was 110, but 12 of them were not part of the study. Furthermore, 5 of those 12 patients did not get regular check-ups, 4 did not wish to join the study and 3 patients were excluded from the study due to the presence of comorbidity (2 patients with rheumatoid arthritis and 1 patient with neuromuscular disorder).

The research involved two study groups. One study group included 49 patients (81 feet) who had the Mitchell procedure. The other study group included 49 patients (77 feet) who had the Golden procedure applied.

In 49 patients who were operated on by the Mitchell method, 43 (87.8%) patients were females and 6 (12.2%) were males. Mean age was 52 years (range 26–67 years). In 32 (65.3%) patients both feet were operated on. The surgery was performed on the left foot in 43 (53.1%) cases and in 38 (46.9%) cases on the right foot.

In 49 patients who had the Golden procedure performed, 46 (93.9%) were females, and 3 (6.1%) were males. Mean age was 51 years (range 23–77 years).). In 28 (57.1%) patients, both feet were operated on. The surgery was performed on the left foot in 33 (42.9%) cases, and on the right foot in 44 (57.1%) cases (Table 1).

The mean follow-up for all patients was 3 years and 4 months (1.5–6.2 years).

The study was approved by the Ethics Committee of the University Clinical Center "Zvezdara" in Belgrade. The ethical principles of the Declaration of Helsinki were respected in the conduct of this research. Each foot was treated as an independent case. Data were obtained before, during and after the treatment by the recommendations of the American Orthopaedic Foot and Ankle Society (AOFAS) [8].

Inclusion criteria that qualified patients to be the part of the study were determined: the patients with moderate hallux valgus deformity, the presence of hallux valgus deformity which had been previously nonoperatively treated and the patients with no degenerative changes in the first metatarsophalangeal joint. Exclusion criteria were: the patients who had prior hallux valgus deformity procedure, the patients with neuromuscular disorders and rheumatoid arthritis and the patients under the age of 18 years.

The assessment of results was performed according to the Hellal's modification of the Bonney and McNab classification criteria [9] and the Hallux Metatarsophalangeal Interphalangeal Score (HMIS) [10].

The analysis of results was based on the preoperative and postoperative assessment of HVA, IMA, sesamoid position and the first metatarsal bone length, using the lateral and dorsoplantar weightbearing foot radiograph projection. Furthermore, these factors were taken into account: movements in the first metatarsophalangeal joint, length of postoperative immobilization, treatment duration and complications (infections, relapses, pain i.e. – metatarsalgia).

The sesamoid position was determined by the position of the medial sesamoid in relation to a line drawn through the mid-longitudinal axis of the first metatarsal bone on the dorsoplantar radiography (0 normal position, 1 less than 50% overlap, 2 more than 50% overpalo, lateral luxation) [11].

Table 1

General characteristics of the analyzed patients and feet and values of the radiographic outcomes before and after the surgery: Hallux valgus angle (HVA), Intermetatarsal angle (IMTA), sesamoid position, and shortening of the first metatarsal

Parameters	Type of surgery		*p*
	Mitchell	Golden	
Patients, n	49	49	
Bilateral operations, n (%)	32 (65.3)	28 (57.1)	0.407[c]
Feet, n	81	77	
Age at surgery (years), mean ± SD	50.5 ± 11.4	52.4 ± 14.1	0.470[a]
Number fender female, n (%)	43 (87.8)	46 (93.9)	0.487[c]
Number left side, n (%)	43 (53.1)	33 (42.9)	0.980[c]
Number both sides, n (%)	32 (65.3)	28 (57.1)	0.407[c]
Before surgery, mean ± SD	33.99 ± 3.60	34.47 ± 3.71	0.410[a]
After surgery, mean ± SD	12.14 ± 1.80	14.81 ± 1.58	< 0.001[a]
Difference (after – before), mean ± SD	21.85 ± 2.15*	19.66 ± 3.31*	< 0.001[a]
IMA (degrees)			
before surgery	15.10 ± 1.66	15.47 ± 2.08	0.223[a]
after surgery	7.27 ± 1.17	8.61 ± 1.16	< 0.001[a]
difference (after – before)	7.83 ± 1.07*	6.86 ± 1.69*	< 0.001[a]
Sesamoid position (values 0–3)			
before surgery	2.19 ± .17	2.30 ± .19	< 0.001[a]
after surgery	0.90 ± .14	1.09 ± .12	< 0.001[a]
difference (after – before)	1.30 ± .06*	1.20 ± .11*	< 0.001[a]
Shortening of the first metatarsal (mm)			
before surgery, mean ± SD	67.18 ± 6.80	66.90 ± 6.20	< 0.001[b]
after surgery, mean ± SD	62.31 ± 5.00	63.93 ± 6.90	< 0.001[a]
difference (after–before), mean ± SD	4.87 ± 7.20*	2.97 ± 7.70*	< 0.001[a]
Dorsi plantar move. of the thumb (degrees)			
before, mean ± SD	40.41 ± 6.13	39.01 ± 4.64	0.111[a]
after, mean ± SD	76.56 ± 9.64	74.71 ± 6.12	0.017[a]
difference (after-before), mean ± SD	36.15 ± 9.32*	35.70 ± 6.06*	0.723[a]

SD – standard deviation; [a]T-test; [b]Mann-Whitney *U* test; [c]χ^2 test; *Significant difference before-after.

Dorsoplantar movement in the first metatarsophalangeal joint was measured using a goniometer before and after the operative treatment.

The assessment of the subjective well-being of the patients in the postoperative period was conducted by 3 orthopaedic surgeons with more than 6 years of specialist experience in practice, who were not involved in the operative treatment. The aforesaid physicians were not acquainted with the strategy and the results of the study.

The data are presented as a count (%) or mean (± SD), depending on the data type. The χ^2 test, *t*-test and Mann-Whitney *U* test were used to compare the nominal and numerical variables between the groups. Changes in HVA, IMA, sesamoid position, shortening of the first metatarsal bone, movements of the metatarsophalangeal joint were calculated as the difference between the first and the last measurement and then compared to the *t*-test and Mann-Whitney *U* test between the groups, depending on the data distribution. All values of $p < 0.05$ were considered significant. The statistical analysis was performed using the SPSS 20.0 (IBM corp.).

Results

All of the preoperative values classified according to the Hellal's modification of the Bonney and McNab criteria, showed poor results in the patients from both groups. Poor results were indicated as well in the preoperative values classified using the HMIS.

The analysis of results based on the two mentioned scoring systems showed that an improvement was made with both applied procedures compared to the preoperative state/condition. There was a high, statistically significant difference in the scores before and after the procedure in favor of the postoperative findings for both operative procedures ($p < 0.001$).

According to the Hellal's modification of the Bonney and McNab classification, in the patients who underwent the Mitchell procedure, 78 (96%) feet showed improvement postoperatively. Twenty-nine (36%) of that number of feet revealed excellent results, 49 (60%) showed good, and in 3 (4%) feet poor results were noted. The patients who underwent the Golden procedure demonstrated improvement in 71 (92%) feet. Thirty-two (30%) feet showed excellent, 48 (62%) good, and 6 (8%) poor results. The statistical analysis of the significant difference classified by the Hellal's modification of the Bonney and McNab criteria before and after the operation, indicated that when the two surgical procedures were compared, there was a high statistically significant difference in favor of the Mitchell compared to the Golden procedure ($p < 0.001$).

The preoperative score according to the AOFAS scale was 48.46 ± 4.91 points in the Mitchell group, and 48.47 ± 5.08 points in the Golden group ($p = 0.989$). The postoperative results according to the HMIS were improved to 95.85 ± 8.08 in the Mitchell group, whereas in the Golden group, they increased to 93.44 ± 11.48 points ($p = 0.123$). The results in the Mitchell group were excellent in 59 (72.8%) feet, good in 16 (19.8%), satisfactory in 4 (4.9%) and poor in 2 (2.5%). Good and excellent results were revealed in 75 (92.6%) of the operated feet. The results observed in the Golden group were excellent in 53 (68.8%) feet, good in 15 (19.5%), satisfactory in 3 (3.9%), and poor in 6 (7.8%).

The statistical analysis of a significant difference classified by the HMIS score scale before and after the operation, indicated that when the two directions of the procedure were compared, there was no statistically significant difference in the results of both procedures ($p = 0.123$) (Table 2).

The assessment of a significance of the differences in HVA, IMA, sesamoid position, shortening of the first metatarsal bone before and after the operations demonstrated a high statistically significant difference in favor of the obtained postoperative values in both operative procedures ($p < 0.001$).

The analysis of dorsoplantar flexion of the thumb was proven to have statistically significant results in both methods postoperatively ($p < 0.001$).

A bigger correction in the HVA was found in the Mitchell group (21.85 ± 2.15) compared to the Golden group (19.66 ± 3.31) ($p < 0.001$). IMA was more reduced in the Mitchell group (7.83 ± 1.07) in relation to the Golden group (6.86 ± 1.690) ($p < 0.001$). The sesamoid position correction was more improved by the Mitchell method (1.30 ± 0.06) versus Golden method (1.20 ± 0.11) ($p < 0.001$). Shortening of the first metatarsal bone was bigger in the Mitchell group (4.87 ± 0.729) compared to the Golden group (2.97 ± 0.77) ($p < 0.001$).

Using the Mitchell method, dorsoplantar flexion of the thumb was increased from 40.41 ± 6.13° to 76.56 ± 9.64°, and by using the Golden method, it was increased from 39.01 ± 4.64° to 74.71 ± 6.12°. Dorsoplantar flexion of the thumb was more improved by the Mitchell method (36.15 ± 9.32°) compared to the Golden (35.70 ± 6.06°); the difference in results was not statistically significant ($p = 0.723$) (Table 1).

The cast removal and physical therapy in the Mitchell group followed 37.1 ± 1.3 days after the procedure and in the Golden group it was 44.1 ± 1.3 days after the procedure ($p < 0.001$). In the Mitchell group, the patients returned to their regular life activities (the end of the treatment) within the period of 57.1 ± 1.3 days after the beginning of the treatment and in the Golden group, within 68.1 ± 1.3 days ($p < 0.001$) (Table 2).

In the Mitchell group, 2 patients developed superficial wound infections, whereas in the Golden group it was the case with 5 feet ($p = 0.268$).

One patient in the Mitchell group showed a total relapse of their deformity, while in the Golden group, it happened in the case of 3 feet ($p = 0.358$) (Table 3).

The preoperative symptoms of metatarsalgia were observed in 41 (51%) feet in the Mitchell group and postoperatively in 19 (16%) feet ($p = 0.741$). In the Golden group, the preoperative metatarsalgia was observed in 41 (53%) feet and postoperatively in 18 (23%) feet ($p = 0.246$) (Table 4).

The X-ray findings of 2 successfully operated patients, whether by the Mitchell asteotomy or Golden osteotomy, are shown in Figures 5 and 6, respectively.

Table 2

The analysis of preoperative and postoperative results of the Mitchell and Golden osteotomies according to the Hellal's modification of Bonney and McNab classification in percentage, and HMIS in points

Parameters	Type of surgery		*p*
	Mitchell	Golden	
Feet, n	81	77	
Good results (Bonney and McNab), n (%)			
preop	0	0	
postop	78 (96.3)	71 (92.2)	< 0.001[c]
HMIS (points), mean ± SD			
preop	48.47 ± 5.08	48.46 ± 4.91	0.989[a]
postop	95.85 ± 8.08	93.44 ± 11.48	0.123[a]
Postoperative results (Bonney and McNab), n (%)			
excellent	29 (35.8)	23 (29.9)	
good	49 (60.5)	48 (62.3)	0.320[b]
poor	3 (3.7)	6 (7.8)	
HMIS, n (%)			
excellent	59 (72.8)	53 (68.8)	
good	16 (19.8)	15 (19.5)	
satisfactory	4 (4.9)	3 (3.9)	0.323[b]
poor	2 (2.5)	6 (7.8)	
Duration of imobilizzation and beginning of physical therapy (days), mean ± SD	37.1 ± 1.3	44.1 ± 1.3	< 0.001[a]
Returning to regular life activities (days), mean ± SD	57.1 ± 1.3	68.1 ± 1.3	< 0.001[a]

SD – standard deviation; [a]T-test; [b]χ^2 test.
HMIS – Hallux Metotarsophalangeal Interphalangeal Score.

Table 3

Complications after surgery

Parameters	Type of surgery		*p*
	Mitchell	Golden	
Feet, n	81	77	
Superficial infections, n (%)	2 (2.5)	5 (6.5)	0.268 [a]
Recurrence of hallux valgus, n (%)	1 (1.2)	3 (3.9)	0.358 [a]

[a]χ^2 **test.**

Table 4

Pre- and postoperative metatarsalgia comparison

Type of surgery	Total (n)	Preoperative, n (%)		Postoperative, n (%)		
		no metatarsalgia	with metatarsalgia	no metatarsalgia	with metatarsalgia	*p*
Mitchell	49	40 (49)	41 (51)	68 (84)	13 (16)	0.741[a]
Golden	49	36 (47)	41 (53)	59 (77)	18 (23)	0.246[a]

[a]**McNemar test.**

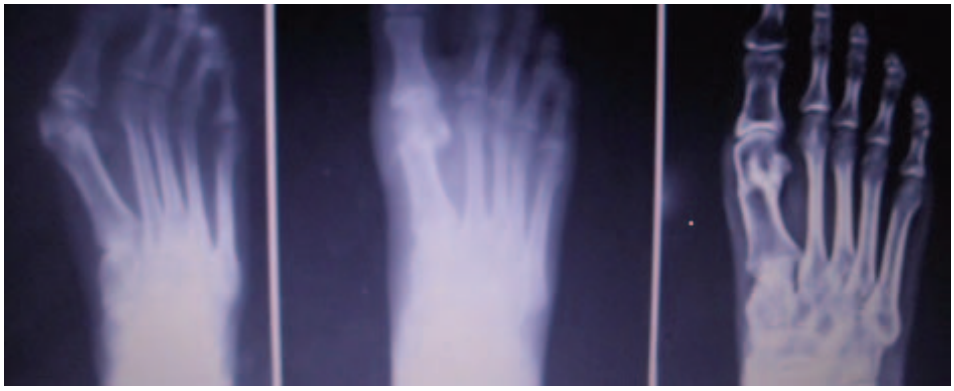

Fig. 5 – X-ray scan of a 53-year-old female patient: left – hallux valgus before surgery; center – postoperative scan of Mitchell osteotomy; right – two years after surgery scan.

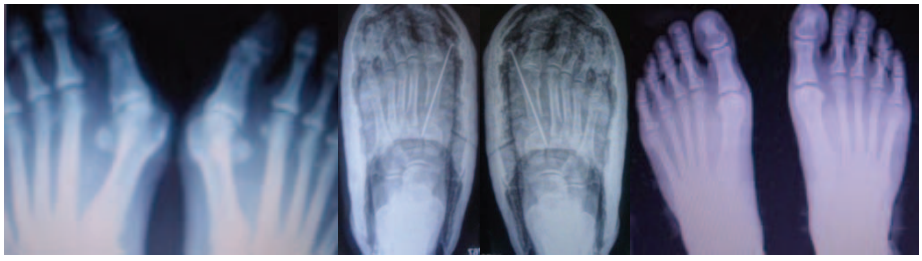

Fig. 6 – X-ray scan of a 49-year-old patient: left – hallux valgus before surgery; center – postoperative scan of Golden osteotomy; right – two years after surgery scan.

Discussion

Hallux valgus is the result of a complex deviation in the structure and the function of the foot. In the operative treatment of moderate hallux valgus deformity, the distal and proximal metatarsal osteotomies are performed, and those procedures enable correction of a majority of components of feet deformities. The purpose of the study was to compare the distal metatarsal osteotomy by the Mitchell to the proximal metatarsal osteotomy by the Golden within roughly similar groups of patients.

Treating moderate and severe hallux valgus using nonoperative methods is, predominantly insufficient. The effects of the treatment of hallux valgus deformity are that much better if the patient undergoes surgery at the early stages of the disease, that is, before any arthritic changes appear in the first metatarsophalangeal joint.

Regardless of the fact that both were introduced the same year (1958.), the Golden method never became widely popular in treating hallux valgus like the method by Mitchel [12]. In scientific literature, we did not find a study that compares these two methods.

Some limitations of this study pertained to the multiple surgeons having performed the procedures as well as the fact that two procedures performed in different rays of the first metatarsal bone were compared.

The general preoperative status evaluation score was poor in all of the feet. Postoperatively, according to the Hellal's modification of the Bonney and McNab classification, the general status was improved in 78 (96%) feet in the Mitchell group. The observed improvement represents the sum of excellent and good outcomes which is in correlation with the results provided by other authors. According to the HMIS, improvement was demonstrated in 79 (97.5%) feet. Other authors indicated that in the patients who had the Mitchell method performed, in 92 % of the cases, good and excellent results were recorded [4, 13].

Using the Hellal's modification of the Bonney and McNab classification, it was observed that general status in the Golden group improved in 71 (92.2%) feet, whereas according to the HMIS, it was the case with 79 (97.4%) feet. Excellent results were obtained in 68 (88.3%) feet. In a study of his own, Golden reported good and excellent outcomes in 78% of cases [7].

In the patients who underwent the procedure by the Mitchell method, the mean HVA correction of 21.85 ± 2.15° was obtained, whereas in the IMA the mean correction was 7.83 ± 1.07° which coincides with the findings of a majority of the authors who reported the correction in the HVA from 10 to 25° and IMA from 5 to 10° [14–17]. In the patients who had the Golden method applied, the mean correction of HVA and IMA was 19.66 ± 3.31°, that is 6.86 ± 1.69°, respectively, which is by 2.19°, that is, by 0,9° less than what was observed in the Mitchell method. The values of obtained results are approximate results of other authors [18, 19].

Malik and Methieson [20] indicated the importance of a role that the sesamoid position played, along with radiographic images and IMA, in estimating hallux valgus deformity. The sesamoid position in the patients who underwent the procedure in the Mitchell group changed from position 2 to position 0, and in Golden group it changed from position 2 to position 1.

Some authors indicated the importance of shortening of the first metatarsal bone in the deformity correction procedure [21]. Shortening of the first metatarsal bone in the Mitchell procedure was 4.87 ± 7.20 mm, whereas in the Golden method, it was 2.97 ± 7.70 mm. As some authors suggested, the shortening of the first metatarsal bone to a greater extent could be one of the main causes of metatarsalgia. Merkel et al. [22] suggested that shortening the bone by more than 10 mm could cause metatarsalgia. Baba et al. [12] observed metatarsalgia in the patients who had the bone shortened by more than 8 mm and chronic pain in the patients who had their metatarsal bone shortened by more than 10 mm. A number of authors do not support a concept that there is a correlation between metatarsalgia and the shortening of the first metatarsal bone [23, 24]. In our study, the preoperative metatarsalgia in the Mitchell group was detected in 51% of feet, whereas in the Golden group, it was found in 53% of feet. Other authors reported the incidence of the preoperative metatarsalgia in 33% of the patients and 40% of the patients, respectively [2, 8]. In the patients who underwent the Mitchell procedure, metatarsalgia was found postoperatively in 13 (16%) feet, and in the patients who underwent the Golden procedure, it was the case with 18 (23%) feet. Some authors indicated the postoperative incidence of metatarsalgia in 27% of cases [25]. During this research, no correlation between the shortening of the first metatarsal bone and the incidence of metatarsalgia was found.

Removal of the cast immobilization and the beginning of physical therapy in the Mitchell group was performed after the mean time of 37.1 ± 1.3 days. The reported outcome was in correlation with the results obtained by other authors who removed the cast in the patients who underwent the Mitchell procedure 5 to 7 weeks after the surgery [10]. Removal of the cast immobilization and the beginning of physical therapy in the Golden group followed after the mean time of 44.1 ± 1.3 days. In his study, Golden indicated that he preferred to remove the cast immobilization and start the physical therapy 6 weeks after the procedure [7]. Longer cast immobilization in the Golden group is required for the relatively poorer fixation stability of the bone fragments secured by the Kirschner wire.

Pain that occurs as a consequence of deformity affects regular life activities of the patients [26], is the main reason they decide to undergo the operative treatment. In 90.1% of the patients who had the Mitchell procedure, the pain disappeared completely. These results are comparable to findings of the other authors who reported the loss of pain in 80%–95% of the operated patients [27–29]. In the patients who underwent the procedure by the Mitchell approach, the chronic pain was observed in 3 cases, while in the patients who had Golden method performed, the chronic pain occurred in 6 feet.

Conclusion

The study indicated that two different osteotomy, by Mitchell and by Golden, can achieve good clinical and radiographic results. The estimated treatment outcomes measured by two scoring systems as well as other observed parameters of HVA, IMA, sesamoid position, metatarsalgia, complications and length of treatment, suggest that the operative treatment of moderate hallux valgus deformity by the Mitchell method provides better results. Whereas the value of the shortening of the first metatarsal bone indicates that the shortening is greater in the Mitchell method, which goes in favor of the Golden method. Regarding the flexion of the thumb of the feet operated on, there is no statistically significant difference between two methods.

Using sutures for maintaining position after Mitchell osteotomy, can eliminate the need for the second surgery (removal of foreign body) which decreases the incidence of infections. In the Golden method, stabilizing the Kirschner wire requires removal at 5 weeks postoperatively, which entails prolonged treatment and additional intervention, thus increased risk of infection.

Incidence of an infection in the Mitchell method was also decreased by using a single-incision in correcting deformity (Golden method uses two–incision technique).

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Impact of the hyperbaric oxygen therapy on the redox status in the patients with systemic lupus erythematosus

Uticaj hiperbarične oksigenoterapije na redoks status bolesnika sa sistemskim eritemskim lupusom

Milorad Rabrenović*, Tamara Nikolić†, Violeta Rabrenović‡§, Jovana Bradić†, Saša Trešnjić*, Anica Petković†, Biljana Jakovljević‖, Siniša Mašić‖, Dubravko Bokonjić§¶

Military Medical Academy, *Center of Hyperbaric Medicine, ‡Clinic of Nephrology, ‖Institute of Hygine, Military Medical Academy, ¶Poisoning Control Center, Belgrade, Serbia; University of Kragujevac, Faculty of Medical Science, †Department of Pharmacy, Kragujevac, Serbia; University of Defence, §Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. Hyperbaric oxygen therapy (HBOT) is a method which increases oxygen solubility in plasma up to 20 times. This effect is very important in the treatment of circulatory disorders, which reduces oxygenation and leads to increased production of inflammatory mediators and free oxygen radicals. The aim of this study was to examine the impact of HBOT on the oxidative stress parameters in the patients with systemic lupus erythematosus (SLE). **Methods.** This prospective study included 18 females with SLE [American College of Rheumatology (ACR) criteria], average age 52.2 ± 8.82 years, treated with HBOT for 60 min/day, with average partial oxygen pressure of 2.2 atmospheres absolute (ATA), during 10 days, in combination with appropriate medication therapy for SLE. The following parameters were determined in the serum: C-reactive protein (CRP), hemoglobin, creatinine, albumin, complement 3 (C3), antinuclear antibodies (ANA), glomerular filtration rate (GFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. In the urine, parameters of oxidative stress were spectrofotometrically determined: levels of superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), nitrites (NO_2^-) and concentration of thiobarbituric acid reactive substances (TBARS). In hemolysate, the parameters of antioxidant protection: superoxide dismutase (SOD), catalase (CAT) and reduced gluthatione (GSH), were measured. The samples for the analysis were collected three times: before HBOT (initial values), after 10 days of HBOT and 1 month after beginning the treatment in relation to the initial value. **Results.** We noticed a statistically significant ($p < 0.05$) decrease in a level of $O_2^{\bullet-}$, both after 10 days and after 1 month of HBOT (8.26 ± 13.62; 8.39 ± 4.94; 11.92 ± 6.86 nmol/mL, respectively). Values of other parameters of oxidative stress such as NO_2^-, TBARS and H_2O_2 showed no significant difference during the monitored period. Regarding the parameters of antioxidant the protection, we revealed slightly higher value of GSH after treatment (initial value: 66.34 ± 16.31; after 10 days of HBOT 79.43 ± 36.77; after 1 month of HBOT69.72 ± 22.32 μmol/mL red blood cells) which was held after a month, but it was not statistically significant. Activity of SOD and CAT, before and after HBOT, did not change significantly. **Conclusion.** Our results suggested the potential beneficial effects of HBOT on redox status in the patients with SLE by decreasing the levels of $O_2^{\bullet-}$.

Key words:
hyperbaric oxygenation; lupus erythematosus; oxidation-reduction; oxidative stress.

Apstrakt

Uvod/Cilj. Hiperbarična oksigenoterapija (HBOT) je metoda kojom se rastvorljivost kiseonika u plazmi povećava i do 20 puta. Taj efekat je veoma značajan u terapiji poremećaja cirkulacije koji smanjuju oksigenaciju i dovode do povećanja produkcije medijatora zapaljenja i slobodnih kiseoničkih radikala. Cilj ove studije bio je da se ispita uticaj HBOT na parametre oksidativnog stresa kod bolesnika sa sistemskim eritemskim lupusom (SLE). **Metode.** Prospektivnom studijom obuhvaćeno je 18 bolesnica sa SLE (kriterijumi Američkog koledža za reumatologiju) prosečne starosti 52,2 ± 8,82 godina, koje su tretirane HBOT u trajanju od 60 min/dan, pri pritisku od 2,2 apsolutne atmosfere (ATA),

Correspondence to: Rabrenović Milorad, Military Medical Academy, Center of Hyperbaric Medicine, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: milorad.rabrenovic@vma.mod.gov.rs

ukupno 10 dana, u kombinaciji sa odgovarajućom terapijom za SLE. U serumu su određivani sledeći parametri: C-reaktivni protein (CRP), hemoglobin, kreatinin, albumin, komplement 3 (C3), antinuklearna antitela (ANA), stopa glomerularne filtracije (GFR korišćenjem *Chronic Kidney Disease Epidemiology Collaboration* (CKD-EPI) formule. U urinu su spektrofotometrijski određivani parametri oksidacionog stresa: nivo superoksid anjon radikala ($O_2^{\bullet-}$), vodonik peroksida (H_2O_2), nitrita (NO_2^-) i koncentracija reaktivnih produkata tiobarbituratne kiseline (TBARS). U hemolizatu, određivani su parametri antioksidativne zaštite: aktivnost superoksid dismutaza (SOD), katalaze (CAT) i redukovani glutation (GSH). Uzorci za analize su sakupljani tri puta: pre HBOT (inicijalne vrednosti), nakon 10 dana HBOT i nakon mesec dana terapije. **Rezultati.** Uočili smo statistički značajno ($p < 0,05$) smanjenje nivoa $O_2^{\bullet-}$ nakon 10 dana, kao i nakon mesec dana od početka HBOT u odnosu na inicijalnu vrednost (8,26 ± 13,62; 8,39 ± 4,94 11,92 ± 6,86; nmol/mL, redom). Nisu pokazane značajne razlike u vrednostima ostalih parametara oksidativnog stresa kao što su NO_2^-, TBARS i H_2O_2 tokom posmatranog perioda. Što se tiče parametara antioksidativne zaštite, otkrili smo nešto veću vrednost GSH nakon tretmana (inicijalno 66,34 ± 16,31; posle 10 daba 79,43 ± 36,77; posle mesec dana tretmana 69,72 ± 22,32 μmol/mL eritrocita), koja se održala nakon mesec dana, ali nije bila statistički značajna. Aktivnost SOD i CAT, pre i posle HBOT, nije se statistički značajno menjala. **Zaključak.** Naši rezultati ukazuju na povoljan efekat HBOT na redoks ravnotežu kod bolesnika sa SLE zbog sniženja nivoa $O_2^{\bullet-}$.

Ključne reči:
hiperbarična oksigenacija; lupus, eritematozni, sistemski; oksidoredukcija; stres, oksidativni.

Introduction

Systemic lupus erythematosus (SLE) is a very serious autoimmune inflammatory disease, with an unpredictable course and outcome, whose etiology remains largely unknown and the effects of conservative treatment are limited [1]. Human and animal studies indicate that oxidative stress is involved in the pathogenesis of SLE. Excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), including peroxynitrite- $ONOO^-$, can damage lipids, proteins and DNA and products of oxidative modification can be detected in biological fluids [2]. The abundance of those products correlates with disease activity in the SLE patients, suggesting oxidative modification acts as a biomarker [3–5]. While several studies implicate nitric oxide as an important mediator of disease in the SLE [6, 7], there is a lack of data revealing the association between the level of urine nitrite and citrulline levels, as surrogate markers of nitrogen monooxide (NO) production, and disease activity among the patients with SLE [8]. Also, previous data suggested that lipid peroxidation could be a risk factor for endothelial dysfunction in some autoimmune diseases [9].

Hyperbaric oxygen therapy (HBOT) is a treatment modality in which a person breathes 100% O_2 intermittently while exposed to increased atmospheric pressure, greater than 1 atmosphere, absolute (ATA) usually 2 to 2.5 ATA [10]. The primary mechanisms of action include hyperoxygenation and a decrease in bubble size, or vasoconstriction, angiogenesis, fibroblast proliferation, oxidative leukocyte degradation, toxin inhibition and antibiotic synergy [11, 12]. Hyperbaric oxygen may be used as the primary therapy intervention in some conditions, such as carbon monoxide poisoning, decompression sickness and arterial gas embolism, arterial insufficiencies, cardiovascular diseases, osteomyelitis and as an adjunctive therapy for wound healing [13–15]. The HBOT showed to have the beneficial effects on hypoxic diabetic ulcers that result in severe wound-healing problems and osteoradionecrosis and is frequently used for necrotic soft tissues and bone that fails to heal. The HBOT also induces significant angiogenesis, which in one study was measurable after the eight HBOT sessions. Previous clinical studies revealed that vasculitis skin ulcers in the patient suffering from SLE was treated successfully with the HBOT [16–18]. There are not many studies that examined effects of HBOT on redox homeostasis and inflammation in the patients with SLE.

Given the fact that the HBOT can modify oxidation-reduction reactions, the aim of our study was to establish an influence of hyperbaric oxygenation on the oxidative stress parameters and antioxidant enzymes activity in the patients with SLE.

Methods

This prospective study included 18 females with SLE, treated with the HBOT once a day for 60 min (total 10 days) with the average partial oxygen pressure of 2.2 ATA, in combination with an appropriate therapy for SLE. The study protocol was approved by the Institutional Ethics Committee (Military Medical Academy Belgrade, Serbia) and the study was conducted in accordance with the Declaration of Helsinki. All the participants were informed about the research protocol before giving their written consent to participate in the study.

All patients were admitted to the Military Medical Academy, Belgrade, Serbia from October 2011 to December 2014, with: a diagnosis of SLE. In order to define a severity of the disease course in this study, the original 1997 American College of Rheumatology (ACR) classification of SLE was used [18, 19]. At the beginning of study, all participants were in the similar stage of the disease, in remission [Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score 0.30 ± 0.47]. The exclusion criteria were: the pregnant women with SLE, the patients with urinary infection (positive urine culture), with renal insufficiency (creatinine clearance < 60 mL/min), the presence of malignancy, the patients with any other ongoing inflammatory process, or under 18 years of age. The patients who were on immunosuppressive therapy such as mycophenolate mofetil, cyclophosphamide and other cytotoxic agents, were excluded. The only therapy, that the patients took, was the corticosteroids (the maintenance dose of 5 mg).

All patients with any contraindication for the HBOT were also excluded. All participants were non-smokers and did not take any antioxidant dietary supplement for 1 month before the study. Before beginning the HBOT, all participants passed a standard medical and physical revision at the hospital. During the study period no patient was eliminated.

Hyperbaric oxygen therapy

The HBOT was performed at The Center for Hyperbaric Medicine, Military Medical Academy in Belgrade, Serbia. The HBOT consisted of 10 sessions (1 session a day/5 days a week) in a multiplace (10-person) hyperbaric chamber**.** In total 60 min of 100% medical oxygen was administered to the patients under the increased pressure of 2.2 ATA during a 70-min hyperbaric session. At this pressure, 100% oxygen was delivered via an oronasal mask in two episodes of 30 min, each interrupted by 5 min of air breathing. During the pressure changes, great care was taken to avoid barotraumas, particularly of the middle ear, which is the most common side-effect of a hyperbaric treatment. All patients tolerated the treatment well without any complications.

Biochemical analysis

The samples for biochemical analysis were collected three times: before the HBOT (initial values), after 10 days of the HBOT (2 hours after the last HBOT session) and after 1 month. The following parameters were determined in the blood serum samples: C-reactive protein (CRP), hemoglobin (Hb), creatinine, albumin, complement 3 (C3), antinuclear antibodies (ANA), glomerular filtration rate (GFR using the CKD-EPI formula). In the urine samples, the following parameters of the redox status were spectrofotometrically determined: levels of superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), nitrites (NO_2^-) and concentration of thiobarbituric acid reactive substances (TBARS). The parameters of antioxidant protection were measured in the blood samples: activity of superoxide dismutase (SOD) and catalase (CAT) and level of reduced glutathione (GSH).

Superoxide anion radical determination

The level of $O_2^{\bullet-}$ was measured using nitro blue tetrazolium (NBT) reaction in TRIS-buffer combined with the urine samples and read at 530 nm [20].

Hydrogen peroxide determination

The protocol for measurement of H_2O_2 is based on oxidation of phenol red in the presence of horseradish peroxidase [21]. The 200 μL sample with 800 μL of phenol red solution (PRS) and 10 μL of Horseradish Peroxidase (HRP) were combined (1 : 20). The level of H_2O_2 was measured at 610 nm.

Nitric oxide determination

Nitric oxide (NO) decomposes rapidly to form the stable metabolite nitrite/nitrate products. NO_2^- was determined as an index of nitric oxide production with the Griess reagent [22]. 0.1 mL of 3 N Perchloride acid (PCA), 0.4 mL 20 mM ethylenediaminetetraacetic acid (EDTA), and 0.2 mL urine were put on ice for 15 min, then centrifuged 15 min at 6,000 rpm. After pouring off the supernatant, 220 μl of K_2CO_3 was added. Nitrites were measured at 550 nm. Distilled water was used as a blank probe.

Determination of concentration of thiobarbituric acid reactive substances

The degree of lipid peroxidation in urine was estimated by measuring concentration of TBARS using 1% thiobarbituric acid (TBA) in 0.05 NaOH, incubated with urine at 100 °C for 15 min and read at 530 nm. Distilled water was used as a blank probe. The TBA extract was obtained by combining 0.8 mL of urine and 0.4 mL of trichloro-acetic acid (TCA), then the samples were put on ice for 10 min and centrifuged for 15 min at 6,000 rpm. This method was described previously [23].

Preparation of hemolysate

The blood samples were taken from the antecubital vein into vacutainer test tube containing sodium citrate anticoagulant. Blood was centrifuged to separate plasma and red blood cells (RBCs). Isolated RBCs were washed 3 times with 3 vol. of ice cold 0.9 mmol/L NaCL. The blood samples were stored immediately and kept for further analyses [24].

Determination of antioxidant enzymes catalase and superoxide dismutase

Hemolysates containing about 50 g Hb/L prepared according to McCord and Fridovich [24] were used to determine the CAT activity which was expressed in U/gHb × 1,000. The CAT activity was determined according to Beutler [25]. Lysates were diluted with distilled water (1:7 v/v) and treated with chloroform-ethanol (0.6:1 v/v) to remove hemoglobin. Then 50 μL of CAT buffer, the 100 μL of sample, and 1 mL of 10 mM H_2O_2 were added to the samples. Detection was performed at 360 nm. Distilled water was used as a blank probe. The SOD activity was determined by the epinephrine method of Misra and Fridovich [26] and it was expressed in U/gHb × 1,000. A hundred μL of lysate and 1 mL carbonate buffer were mixed, and then 100 μL of epinephrine was added. Detection was performed at 470 nm.

Determination of reduced glutathione

A level of GSH was determined spectrophotometrically, and it was based on GSH oxidation via 5,5-dithiobis-6,2-nitrobenzoic acid. The GSH extract was obtained by combining 0.1 mL 0.1 % EDTA, 400 μL haemolysate, and 750 μL precipitation solution (containing 1.67 g metaphosphoric acid, 0.2 g EDTA, 30 g NaCl, and filled with distilled water until 100 mL; the solution was stable for 3 weeks at +4 C°). After mixing in the vortex machine and extraction on cold ice (15 min), it was centrifuged on 4,000 rpm (10 min). Distilled water was used as a blank probe. Measuring was performed at 420 nm. The concentration is expressed as micromoles per mL of RBCs [25, 27].

Statistical analysis

In case of continuous data, the variables were presented as the mean value ± standard deviation (SD). The Kolmogorov-Smirnov test was used for evaluation of distribution of biochemical data. A statistical significance between the groups was tested by the Friedman (repeated measure) test (post hoc Wilcoxon test). All the analyses were estimated at $p < 0.05$ level of statistical significance. A complete statistical analysis of data was done by the statistical software package, SPSS Statistics 18.

Results

A total of 18 women, the average age 52.22 ± 8.82 years, were enrolled in the study. The patients presented SLE with an average time without symptoms of healing of 20.2 ± 5.0 months when they underwent the HBOT.

The values of the serum parameters such as CRP, Hb, creatinine, albumim, C3, ANA, chronic kidney disease estimated (CKD eGFR), were not statistically significantly different when compared the initial values, with the values after 10 days and after a month of the therapy (Table 1).

Levels of superoxide anion radical

We noticed a statistically significant decreased ($p < 0.05$) levels of $O_2^{\bullet-}$ after 30 days of the HBOT beginning compared to the initial values of this parameter, and significantly decreased values after 10 days of the HBOT compared to the initial values (11.92 ± 6.86, 8.26 ± 13.6, 8.39 ± 4.94 nmol/mL, respectively) (Figure 1).

Table 1

Comparison of selected laboratory parameters - the initial value, value after 10 days and after a month of HBOT

Laboratory parameters in serum	Initial (mean ± SD)	10th day (mean ± SD)	30th day (mean ± SD)
CRP (mg/L)	5.16 ± 5.7	4.93 ± 6.06^{ns}	3.26 ± 2.25^{ns}
Hb (g/L)	129.3 ± 10.27	131.00 ± 12.51^{ns}	129.44 ± 14.13^{ns}
Creatinine (mmol/L)	72.00 ± 22.84	78.55 ± 26.09^{ns}	74.55 ± 23.55^{ns}
Albumin (g/L)	42.33 ± 2.95	40.66 ± 2.50^{ns}	42.33 ± 2.95^{ns}
C3 (g/L)	1.05 ± 0.23	1.05 ± 0.29^{ns}	1.03 ± 0.25^{ns}
ANA (IU/mL)	1.11 ± 1.16	1.44 ± 1.33^{ns}	1.00 ± 1.11^{ns}
CKDeGFR (mL/min/1.73 m^2)	89.00 ± 22.46	84.33 ± 19.53^{ns}	87.44 ± 20.91^{ns}

^{ns} non significant differences in comparison to the initial values
HBOT – hyperbaric oxygen therapy; CRP – C-reactive protein; Hb – hemoglobin; C3 – complement 3; ANA – antinuclear antibody; (CKD eGFR) – a chronic kidney disease estimated glomerular filtration rate.

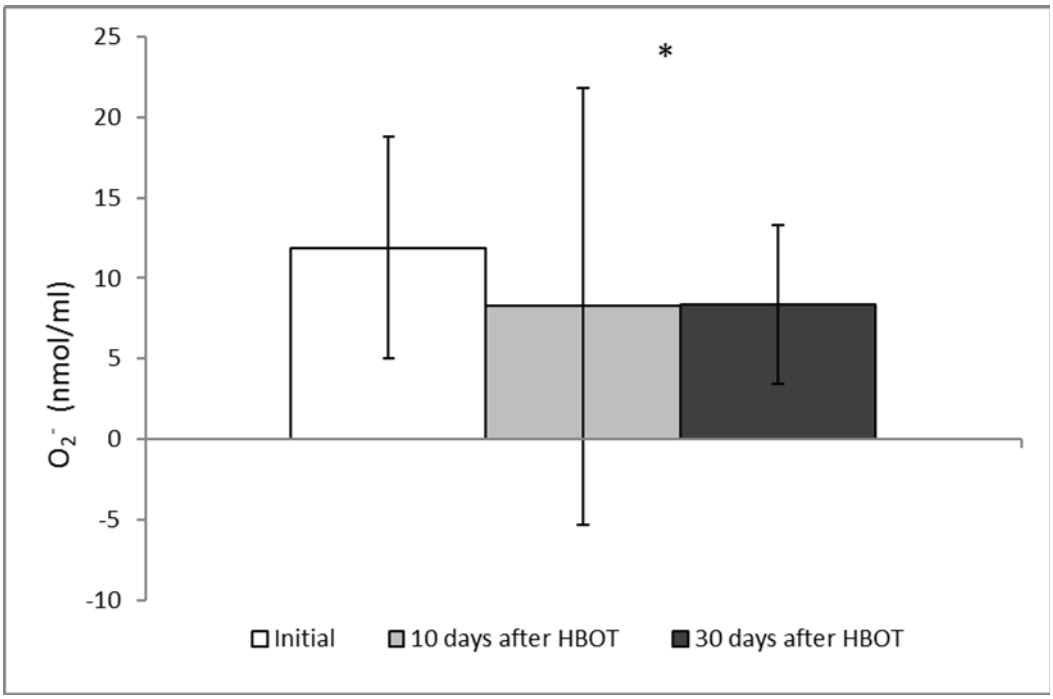

Fig. 1 – Level of superoxide anion radical (O2-) in the urine samples (the values are presented as mean and standard deviation). The statistical significances are presented as a significance between the values after 10 days vs. initial values and after 30 days of hyperbaric oxygen therapy (HBOT) vs. the initial values (*p < 0.05).

Levels of nitrites

The levels of NO_2^- before and after the HBOT were similar in all terms observed (2.72 ± 0.16, 2.77 ± 0.29, and 2.76 ± 0.20 nmol/mL, respectively). We found that this parameter was not significantly affected by the HBOT when comparing the initial values to the values after 10 days of the HBOT and 1 month after the treatment begining (Figure 2).

Levels of hydrogen peroxide

During the observed period of HBOT, there were no statistically significant changes of H_2O_2 levels when compared the initial values, the values after 10 days and after 1 month of the HBOT. This parameter was not changed during the study (1.52 ± 0.08, 1.51 ± 0.07, 1.54 ± 0.17 nmol/mL, respectively) (Figure 3).

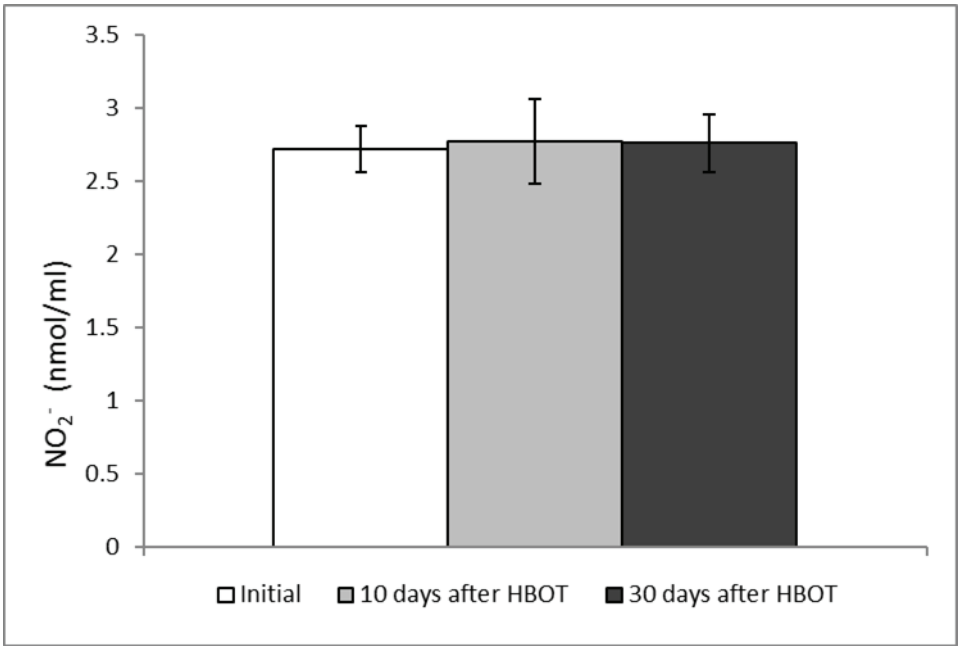

Fig. 2 – Levels of nitrites (NO_2^-) in the urine samples (the values are presented as mean and standard deviation). There was no significant difference before and after the hyperbaric oxygen therapy (HBOT).

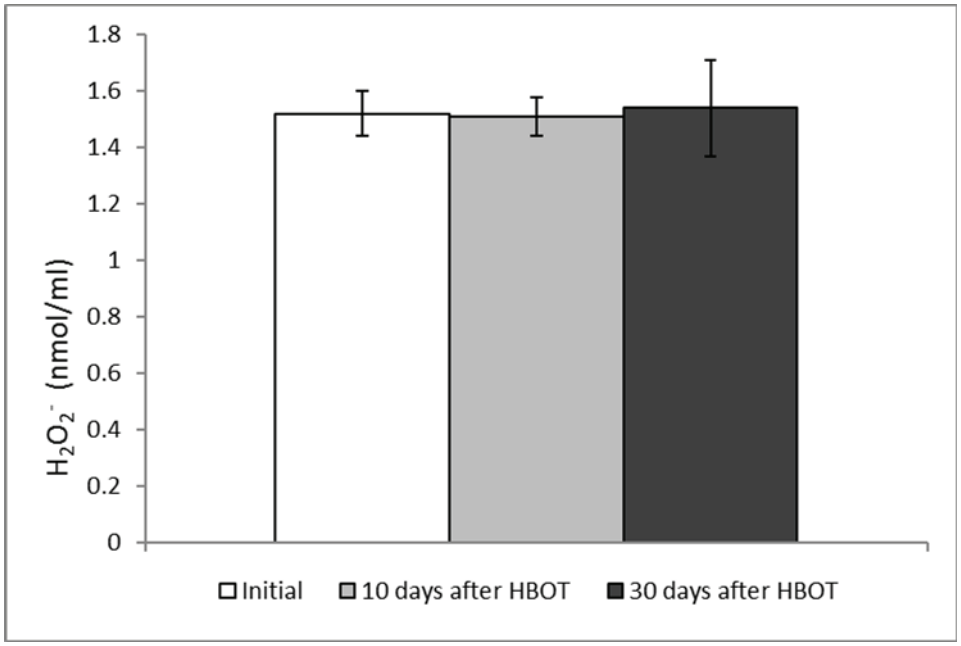

Fig. 3 – Levels of hydrogen peroxide (H_2O_2) in the urine samples (the values are presented as mean and standard deviation). There were no significant differences before and after the hyperbaric oxygen therapy (HBOT).

Concentration of TBARS

Among the examined groups, TBARS concentration was not significantly altered suring the study (1.01 ± 0.11; 1.09 ± 0.11; 1.05 ± 0.96 μmol/mL, respectively) (Figure 4).

Activity of superoxide dismutase (SOD)

In the study group, we noticed a decreased activity of SOD after the HBOT when compared the initial value to the value after 10 days of the therapy (27.58 ± 8.86, and 19.47 ± 10.63 U/gHb×10^3, respectively). However, a month after, the values were similar to those from the beginning of the study protocol and the activity was 27.11 ± 28.26 U/gHb×10^3. Those changes were without a statistical significance (Figure 5).

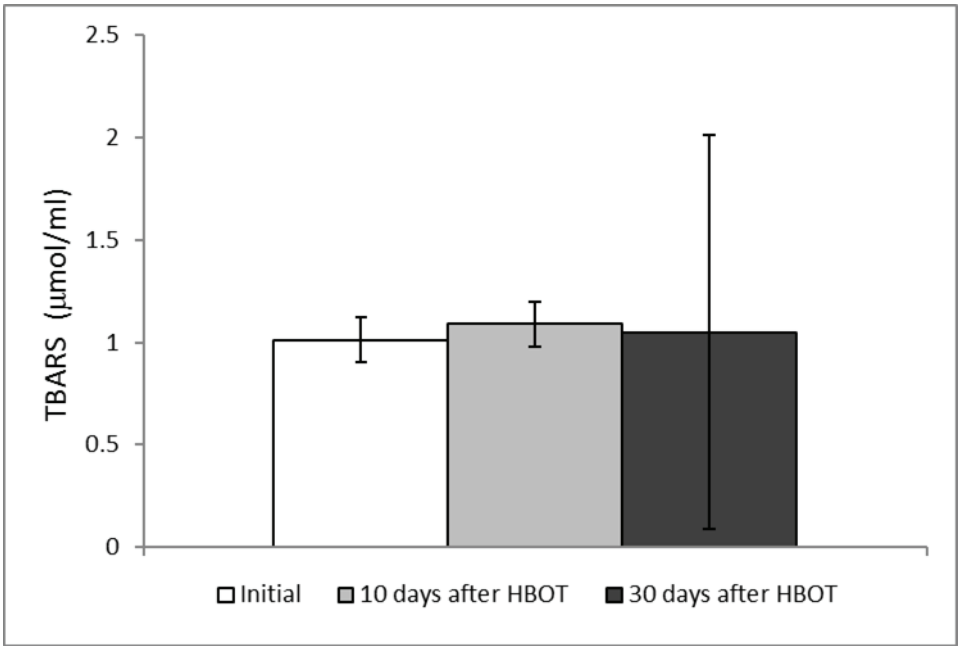

Fig. 4 – Concentration of TBARS in the urine samples (the values are presented as mean and standard deviation). There were no significant differences before and after the hyperbaric oxygen therapy (HBOT).
TBRS – thiobarbituric acid reactive substances.

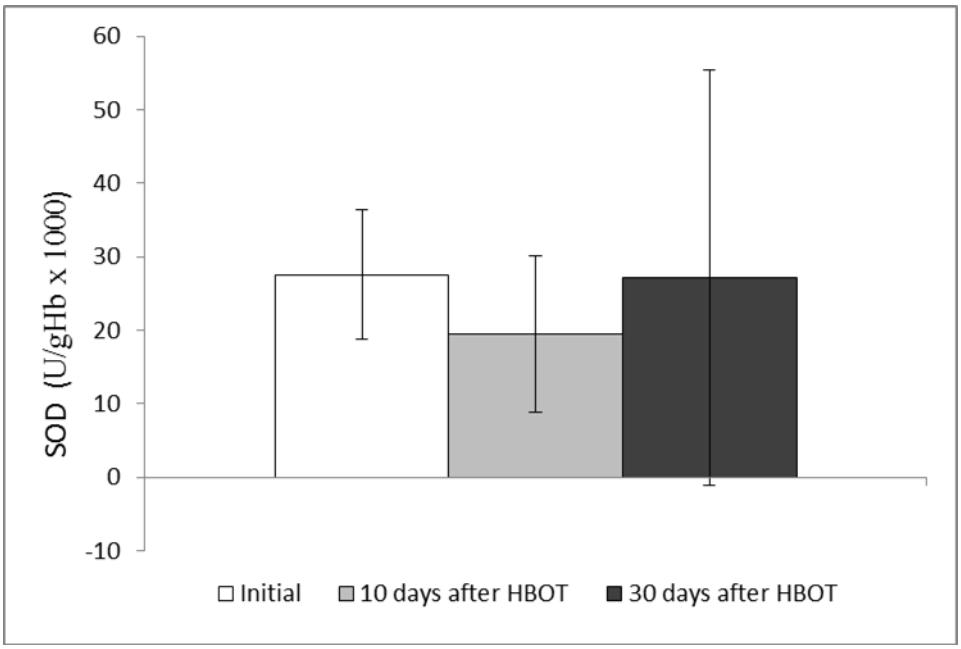

Fig. 5 – Activity of superoxide dismutase (SOD) in the blood samples (the values are presented as mean and standard deviation). Comparing the initial values of SOD and those after the hyperbaric oxygen therapy (HBOT), it was observed decrease in the level, but without a significant difference. After 30 days activity of SOD is similar to the initial values.

Level of reduced glutathione

A level of GSH was not statistically significantly increased in our group (initially 66.34 ± 16.31, 79.43 ± 36.77 μmol/mL RBC after the HBOT). However, a month after values were similar to those from the beginning of the study protocol and the activity was 69.72 ± 22.32 μmol/mL RBC (Figure 6).

Activity of catalase

We observed a decrease in the activity of CAT after 10 days of the HBOT compared to the initial value (4.77 ± 2.93 and 5.44 ± 3.55 U/gHb×10^3, respectively), and it continued to decrease, so after a month it was lower than before the HBOT and after 10 days of the HBOT (3.82 ± 2.49 U/gHb×10^3, respectively). However those differences were not statistically significant (Figure 7).

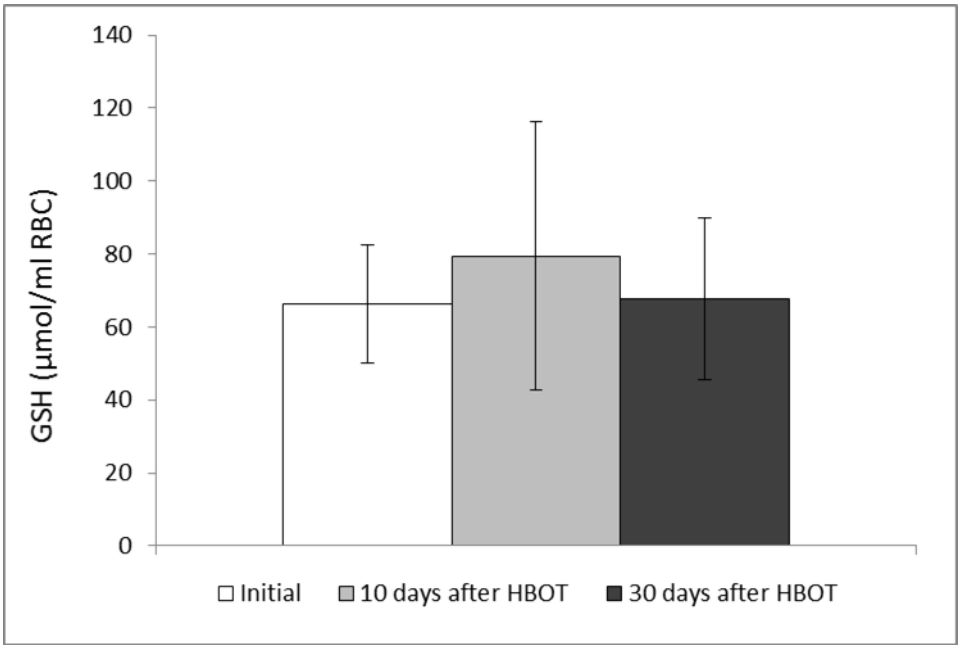

Fig. 6 – Level of reduced glutathione (GSH) in the hemolysate samples (the values are presented as mean and standard deviation). Comparing the values of GSH initially and after the hyperbaric oxygen therapy (HBOT)] there were no statistically significant differences.

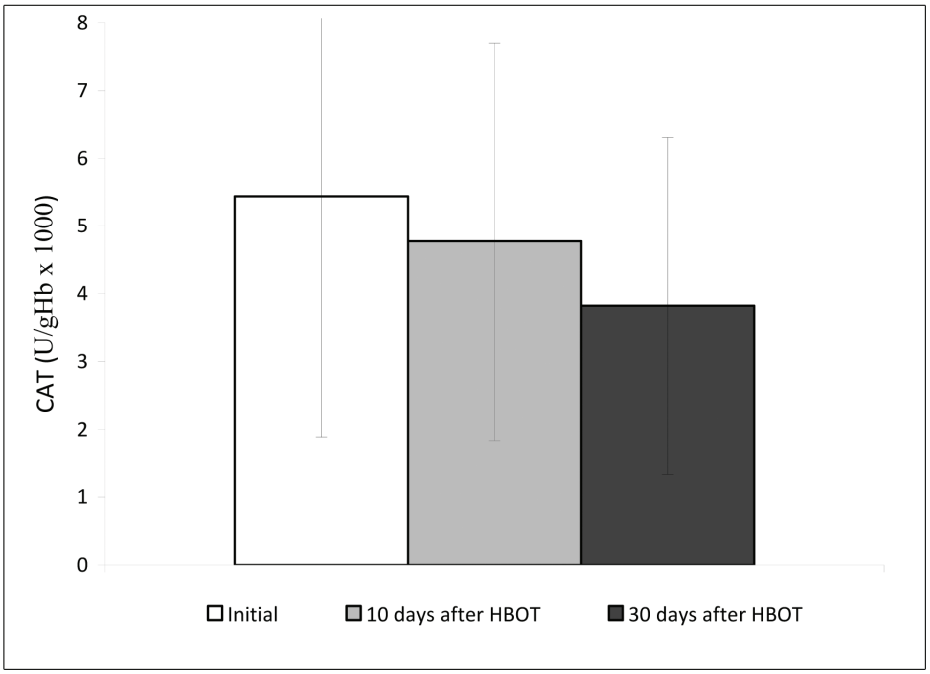

Fig. 7 – Activity of catalase (CAT) in the hemolysate samples (the values are presented as mean and standard deviation). Comparing the initial values and those after 10 days and 30 days, the hyperbaric oxygen therapy (HBOT), there were no statistically significant differences.

Discussion

This study was designed in the field of physiology research of hyperbaric oxygenation with a special emphasis on potential systemic effects of disturbed redox balance, induced by a systemic disease before and after the application of oxygen. Actually, the HBOT leads to an increase in the amount of dissolved oxygen in plasma, creating a diffusion gradient which facilitates the transition of oxygen from the capillaries to the ischemic tissues [17]. Studies reported controversial results regarding the effect of the HBOT on the oxidative stress and enzymes of antioxidative defense in the several pathophysiological models. The role of ROS and RNS in therapeutic responses of the HBOT in the patients with SLE has still not been completely revealed and explained [28–31].

Immune dysfunction, genetic, hormonal and environmental factors are included in an etiology of SLE, however molecular mechanisms underlying this systemic autoimmune response remain largely unknown [31, 32]. It is believed that the oxidative stress has an important role in the pathogenesis of SLE. Excessive production of ROS (including $ONOO^-$) can damage all biomolecules such as lipid, protein and DNA and cause a formation of different products which can be detected in biological fluids [2–5]. In case of the patients with SLE, this fact can be useful since their abundance correlates with the disease activity and organ damage [3]. Our study included the patients with SLE in whom the disease was in remission, which was maintained before and after the HBOT was applied. Comparing the laboratory parameters (CRP, hemoglobin, creatinine, albumin, complement C3, ANA) before and after the performed therapy as well as after a month, we did not notice the statistically significant differences in their values. We examined the effects of 10 session of the HBOT on the parameters of redox balance in the patients with SLE. We found the statistically significant decreased levels of $O_2^{\bullet-}$ after the HBOT, which were held after 30 days. There is a concern that the HBOT might increase the oxidative stress via the production of reactive oxygen species, however, the oxidative stress appears to be less of a concern at the hyperbaric pressures under 2.0 ATA [33]. The patients in our study were exposed to the higher pressure such as 2.2 ATA and we revealed the beneficial effects of hyperbaric oxygen on O_2^- levels. On the other hand, other pro-oxidants, such as NO_2^-, TBARS and H_2O_2 were not affected by the HBOT. In order to validate our results, we excluded all patients with renal disease or urinary infection, because the oxidative stress parameters may have not been removed from plasma because of insufficient excretion and may continue to rediffuse in circulation [30–34]. So, because of this fact, we could not be sure in unchanged levels of the oxidative markers.

Literature data regarding the effects of the HBOT on SLE is limited, and it is hard to compare our results to the others due to the fact that available researches were mostly focused on the effects of the HBOT on ulcers healing. One of a few studies which examined the effects of the HBOT in a SLE patient was a case report conducted by Olivieri et al [17]. They described the SLE patient with a case of refractory vasculitic ulcer responding to the HBOT, which was used in combination with immunosuppressive therapy. Jou et al. [35] reported their experience with the use of the HBOT for the treatment of intractable hemorrhagic cystitis in a SLE patient treated with cyclophosphamide. They concluded that this treatment was very successful, with no recurrent hematuria after the HBOT during 6 months [35]. Efrati et al. [16] reported that the HBOT may serve as an effective safe treatment for the patients with vasculitis having nonhealing skin ulcers, which is in agreement with the results of previously mentioned authors as well as with ours regarding a safety of the HBOT. The increase in tissue oxygenation appeared to be one of the major components responsible for the high cure rates in the patients with ulcers [16, 17].

In order to complete our picture about the influence of HBOT on redox status, we examined the activity of the antioxidant enzyme system. GSH is an important endogenous antioxidant and prime scavenger of free radicals in cells. One of the body's most powerful natural antioxidant enzymes are SOD and CAT. SOD, essential to catalyze the dismutation of superoxide, has been shown to protect cells from oxygen free radicals. Exposure to ROS from a variety of sources led to development of a series of defence mechanisms to neutralize these species and so protect cells against their toxic effects and that protection is achieved mainly by enzymatic antioxidants such as catalase. Some research conclude that hyperbaric oxygen treatment below 2.0 ATA can increase the activity of antioxidant enzymes including SOD, GSH and CAT [36–40].

Regarding a component of antioxidant defense including SOD, GSH, CAT we observed that the levels of GSH were higher (but without statistical significance) after 10 days exposed to hyperbaric oxygen treatment and a month later, too.

We believe that these beneficial results in regard to levels of $O_2^{\bullet-}$ and GSH after the HBOT imply the possibility that the study with a lager number of patients or changes of number of treatments could have results which would be statistically significant for these parameters. That refers to results noticed for SOD and CAT.

Activities of SOD and CAT were affected by the HBOT, but not statistically significantly. We noticed that the activity of SOD decreased after 10 days, but returned to the initial level after 30 days, and level of CAT decreased after a month compared to the initial value and value after 10 days of the HBOT. However, these changes were not statistically significant. Considering that differences in the activity of SOD and CAT between the peroxide initial level and the level after the HBOT were insignificant, we did not observe any significant influence on prooxidants such as hydrogen.

We believe that smaller number of patients in our study influenced our results. The assumption is that the future studies of a different design (larger number of patients, more treatments, additional analysis, etc.) could clarify the fact that we did not get a correlation between the decreased level of O_2^- (statistically significant in our study) and the increased values of the antioxidant protection parameters (GSH was not statistically significantly changed in our study).

We decided to treat the SLE patients with the HBOT not only because it improves the oxygenation of ischemic

tissues and exerts the beneficial effects on vascular inflammatory response by regulating the chemotaxis of leukocytes, but also because it facilitates the healing process of infected wounds promoting the deposition of collagen, angiogenesis, epithelialization and facilitating the oxygen-dependent killing by leukocytes [16–18]. Previous studies suggested that vasculitis skin ulcers in the patient suffering from SLE had been treated successfully with the HBOT [17].

Given the fact that the HBOT can modify oxidation-reduction reactions and because of the mentioned beneficial effects of the HBOT in different tissues in the patients with SLE, this protocol of therapy can be one of the possibility.

Conclusion

Our results highlighted some of the beneficial effects of hyperbaric oxygen treatment on redox balance among the patients suffering from SLE. However, the management of SLE is complex and more research is required to establish the complete mechanism by which the HBOT can modify oxidation-reduction reactions in the patients with SLE, so it can become an additional potential therapeutic strategy in the treatment of SLE.

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In vitro investigation of erosive effect of carbonated beverages on enamel and dentin

In vitro studija o erozivnom uticaju gaziranih napitaka na gleđ i dentin

Zorica Panić*, Ivana Stojšin†, Ognjenka Janković‡, Karolina Vukoje*, Tatjana Brkanić†, Ljiljana Tadić Latinović§

University of Novi Sad, *Faculty of Medicine, Novi Sad, Serbia; †Clinic for Dentistry of Vojvodina, Novi Sad, Serbia; University of Banja Luka, ‡Faculty of Medicine, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; § University Clinical Center of Banja Luka, Banja Luka, Republic of Srpska, Bosnia and Herzegovina

Abstract

Background/Aim. Excessive consumption of acidic dietary substances, such as carbonated beverages, increased the chances of dental erosion. The aim of this study was to determine influence of carbonated beverages on enamel and dentin, during different intervals. **Methods.** Sixty samples were obtained from fifteen impacted third molars. Tooth crown was divided into four parts. One part was a control sample, immersed in destilled water and other three parts were the experimental samples, stored in the following tested beverages: carbonated water, Coca-Cola and Schweppes Bitter Lemon. For each beverage, pH was measured before immersion of the samples. The first group of twenty samples were analysed and photographed, using a Scanning Electron Microscope (SEM), after 60 minutes, the second group after 24 hours and the third group after 7 days of exposure to drinks. The enamel was analysed on the outer surface of the cusps and longitudinal section. Dentin was analysed on longitudinal section. An individually adopted scale was used for determination of the degree of erosive changes of these dental tissues. The data were analysed using the analysis of varance (ANOVA). **Results.** The pH levels of the tested beverages was bellow the critical pH for enamel demineralisation. The SEM images showed different intensity of erosive changes caused by the tested beverages. The analysis by ANOVA revealed a significant difference between all groups of the treated samples, after 60 minutes of exposure to beverages. The highest values of erosive changes showed the samples that were immersed in Schweppes Bitter Lemon. The analysis of the samples after 24 hours and 7 days showed that the samples immersed in Coca-Cola and Schweppes Bitter Lemon can be classified as one group that was statistically significantly different compared with the control samples and samples immersed in carbonated water. **Conclusion.** Prolonged exposure of dental tissue to carbonated beverages cause erosive changes and a loss of surface profile.

Key words:
carbonated beverages; immersion; enamel microabrasion; dentin.

Apstrakt

Uvod/Cilj. Prekomerno konzumiranje kiselih namirnica, uključujući i gazirane napitke, povećava mogućnost za nastanak dentalnih erozija. Cilj ovog rada je da se ispita uticaj gaziranih napitaka na gleđ i dentin tokom različitih vremenskih intervala. **Metode**. Šesdeset uzoraka je dobijeno od 15 impaktiranih trećih molara. Krunica zuba je podeljena na četiri dela. Jedan deo bio je kontrolni uzorak, potopljen u destilovanu vodu, a preostala tri dela su bili eksperimentalni uzorci, koji su potapani u ispitivane napitke: gaziranu vodu, Coca-Cola-u i Schweppes Bitter Lemon. Za svaki napitak izmerena je pH vrednost pre potapanja uzoraka. Prva grupa od 20 uzoraka je analizirana i fotografisana pomoću skenirajućeg elektronskog mikroskopa (SEM) posle 60 minuta, druga grupa posle 24 sata i treća grupa posle sedam dana izloženosti delovanju napitaka. Gleđ je analizirana na spoljašnjoj površini kvržice i na uzdužnom preseku. Dentin je analiziran na uzdužnom preseku. Za određivanje stepena nastalih erozivnih promena pomenutih zubnih tkiva primenjena je individualno prilagođena skala. Za analizu podataka korišćena je analiza varijanse (ANOVA). **Rezultati.** Izmerena pH vrednost ispitivanih napitaka bila je niža od kritične pH vrednosti pri kojoj dolazi do demineralizacije gleđi. Na SEM mikrofotografijama uočen je različit stepen erozivnih promena uzrokovanih delovanjem ispitivanih napitaka. Analizom podataka primenom ANOVA dobijena je statistički značajna razlika između svih grupa tretiranih uzoraka, nakon 60 minuta izloženosti delovanju napitaka. Najveći intenzitet erozivnih promena bio je prisutan na uzorcima koji su po-

Correspondence to: Zorica Panić, Dr Svetislava Kasapinovića 33, 21 000 Novi Sad, Serbia. E-mail: zoricapanic1987@gmail.com

topljeni u Schweppes Bitter Lemon. Analiza uzoraka nakon 24 sata i sedam dana izloženosti delovanju napitaka pokazala je da uzorci potopljeni u Coca-Cola-u i Schweppes Bitter Lemon mogu biti svrstani u jednu grupu koja je statistički značajno različita u odnosu na kontrolne uzorke i uzorke potopljene u gaziranu vodu. **Zaključak.** Povećano izlaganje zubnih tkiva delovanju gaziranih napitaka uzrokuje erozivne promene i gubitak površinske strukture.

Ključne reči:
gazirana pića; imerzija; gleđ, mikroabrazija; dentin

Introduction

The consumption of soft drinks has increased enormously in the last few years [1, 2]. Soft drinks are non-alcoholic, flavored, carbonated beverage, usually commercially prepared and sold in bottles and cans [3]. Although not necessarily common to all types of carbonated beverages, the principal properties of note are carbonation, acidity and high levels of sugar or artificial sweeteners [4]. The effects of these beverages on dental hard tissues were extensively investigated in the last decade. Meta-analysis of the studies assessing tooth erosion and diet showed that high consumption of soft drinks, including carbonated beverages, was associated with an increased chance of tooth erosion. In fact, some studies considered soft drinks as the main cause of the perceived rise in the occurrence of dental erosion in young people [5–7]. The erosive potential of these drinks is thought to involve several factors: the types of acid content, pH value, titratable acidity, and ion concentration [8]. Intraoral pH, after drinking an acidulated drink, decreases to below pH5 within 2 to 3 minutes [9]. After acidic attact, pH takes about 25 minutes to change the acid environment, as further stimulated saliva neutralises any residual acid [9]. A single acidic attack is therefore of a minor importance, but if repeated, the ability of saliva to deal with the acid reduces. Hence, the danger is the frequent use of soft drinks over time [10].

The aim of this study was to investigate, *in vitro*, the influence of different types of carbonated drinks on the dental hard tissues, enamel and dentin during time and to identify which types of these drinks are potentially the most aggressive toward the dental hard tissues.

Methods

Sixty samples for this study were obtained from 15 impacted third molars. After extraction, the soft tissue that remained was removed from the teeth surface, the teeth were washed thoroughly under running water and then immersed in 5.25% solution of sodium hypochlorite for one hour. The teeth were stored in the saline solution at the room temperature for no more than one month until the experiment started. A tooth crown was separated from the root using a water-cooled diamond saw, that was also used for the preparation of cutting lines on occlusal surfaces. Two cutting lines, one with vestibulo-oral and other with mesio-distal direction, were prepared through fissures, around peaks of the cusps. The chisel was placed on an intersection point of cutting lines and hit with a hammer, causing breakage of tooth crown in four parts. One part of every tooth was a control sample, immersed in destilised water, and three were experimental samples, stored in carbonated beverages: carbonated water, Coca-cola and Schweppes Bitter Lemon. The pH level was measured for each beverage, using the digital pH meter (Economic pH009) at room temperature of 23ºC, on opening of the bottle, before immersion of these samples into the adequate beverage. To ensure measurement accuracy the pH meter was calibrated each time before use, at pH 4, 6.9 and 9.2, using standard buffer solutions for all levels. The samples were divided into three groups of twenty samples, obtained from five teeth. The first group was stored in beverages for 60 minutes, the second group for 24 hours and the third group for 7 days. The beverages for the third group were replaced every 24 hours. After taking out from beverages, the samples were washed thoroughly under running water. Each quarter of the teeth was dried in a vacuum apparatus, fixed to the respective carriers and sputter-coated with gold. The prepared samples were examined using the Scanning Electron Microscope (SEM) JEOL, JSM 6460 LV, at a magnification of ×500, ×5000 and ×10000. The enamel was observed on the outer surface of cusps and longitudinal section. Dentin was also observed on the longitudilnal section. The SEM is one of the most frequently used devices for the qualitative assessing ultramicroscopic surface alterations associated with erosion both on enamel and dentine [11, 12]. Grading of the severity of surface alteration could be done on the individually adopted scales [13]. In this study, the individual scale with four values was used to evaluate the degree of dissolution of the dental hard tissues: 0 – unchanged morphology of the tissues with all basic structural units of the observed surface; 1 – morphological units were changed, but still recognized; 2 – loss of obvious lines between morphological units, they merged with each other; 3 – complete loss of structure, the morphological units were not recognisable.

Detemination of morfology changes was done for all images at magnification of ×500, ×5000, ×10 000. The choosen figures of the samples, at magnification of ×500, are presented below. The dissolution values of enamel and dentin were analyzed by the one-way ANOVA and the post hoc Scheffé test, a statistical significance was set at $p \leq 0.05$.

Results

Measuring of pH showed the lowest value for Coca-Cola and Schweppes Bitter Lemon was also highly acidic while carbonated water had the highest pH level.

The SEM images of samples that were stored in beverages for 60 minutes indicate that all experimental beverages caused erosive changes of morfology of enamel and dentin. Outer surface of control enamel was smooth and unchanged, with an intact aprismatic surface layer. The perikymata lines

could be observed (Figure 1a). Enamel surface of sample stored in carbonated water showed partial loss of aprismatic layer and formation of the pores and superficial irregularities (Figure 1b). The alterations the surface morphology after exposure to Coca-Cola and Schweppes Bitter Lemon became much more pronounced. The aprismatic layer was almost completely removed. An increased number of porosities on the enamel surface could be noticed. The localized areas of distinctive honeycomb structure could be observed, which was due to the formation of micropores with preferential dissolution of the center of the enamel prisms and interprismatic enamel became convex and protruded (Figures 1c, and 1d). After 24 hours of exposure to beverages the enamel surface showed a marked visible effect (Figure 2a). The superficial irregularities of samples treated with carbonated water were more expressed (Figure 2b). The samples treated with Coca-Cola and Schweppes Bitter Lemon showed enhanced change of morfology, with marked signs of dental erosion, craters, grooves and cracks (Figure 2c, and 2d). The areas of type II demineralisation, where peripheral zones of enamel prisms were dissolved and cores were protruding, were visible on the sample treated with Schweppes Bitter Lemon (Figure 2d). After seven days, carboneated water caused the enamel surface looked quite rough (Figures 3a and 3b). The surfaces of enamel, after immersion into Coca-Cola and Schweppes Bitter Lemon, became grooved, with deep cracks and craters and complete loss of morfology (Figures 3c and 3d).

The SEM images of longitudinal section of control enamel showed the groups of enamel rods distinctly defined and unchanged, with almost linear orientation (Figure 4a). The localized areas of demineralization could be noticed at the samples stored in carbonated water, where the enamel rods were not obvious as in the control sample (Figure 4b). Coca-Cola caused the alterations that were more significant; increased dissolved regions were present where the morphological units were not clearly visible and the breakage lines became rounded (Figure 4c). The morfology of enamel exposed to Schweppes Bitter Lemon was completly lost, breakage lines were not visible and the orientation of enamel rods could not be defined (Figure 4d). These alterations of surface enamel, caused by the experimental beverages increased and became more pronounced as the exposure time increased (Figures 5, and 6).

The longitudinal section of dentin in the control sample and the sample exposed to carbonated water for 60 minutes showed morfology of sound dentin, with the unchanged morfological units (Figures 7a, and 7b). The tubules were surrounded by sound peritubular dentin that was clearly devided from interubular dentin. The samples stored in Coca-Cola and Schweppes Bitter Lemon represented the tubules with increased lumen, because of dissolution of peritubular dentin. The areas with undefined morfology could be observed (Figures 7c, and 7d). The samples treated with carbonated water for 24 hours and 7 days showed the localised areas of demineralisation, where dental tubules were not visible (Figures 8, 9a and 9b). Samples immersed in Coca-Cola and Schweppes Bitter Lemon showed the recognizable dentinal tubuls, but with lost sharp borders between them. Peritubular and intertubular dentin was not visible (Figures 8, and 9c, and 9d).

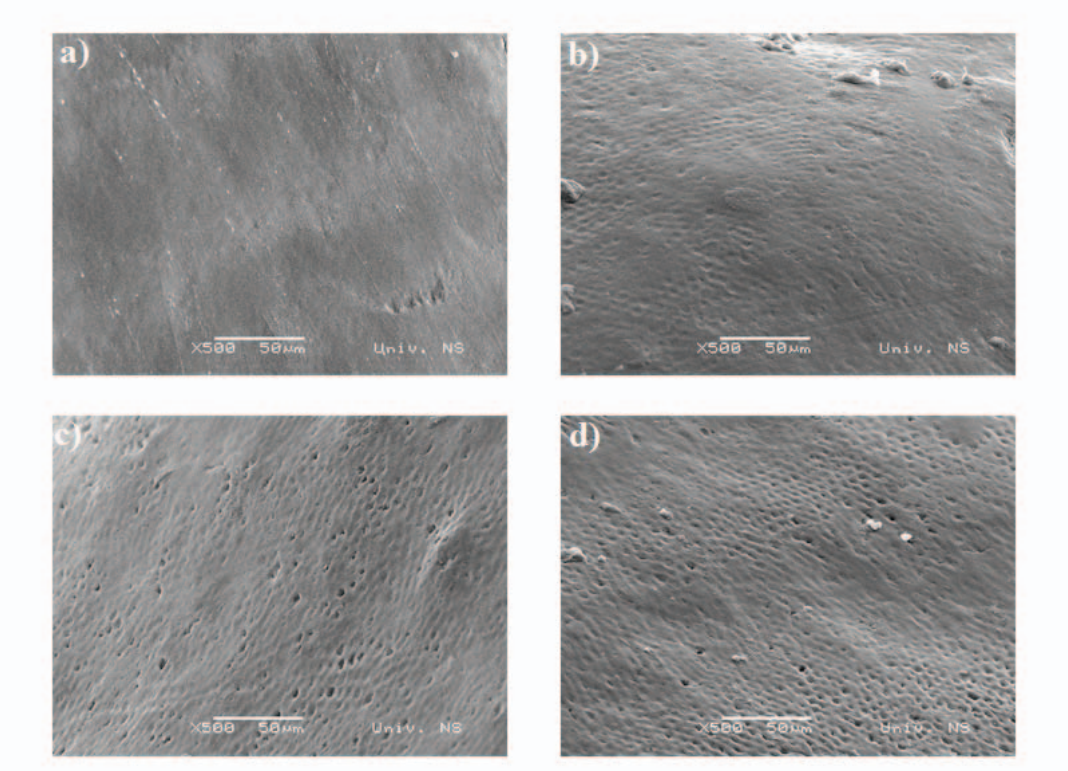

Fig. 1 – Scanning electron microscope (SEM) image of enamel surface after 60 minutes of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

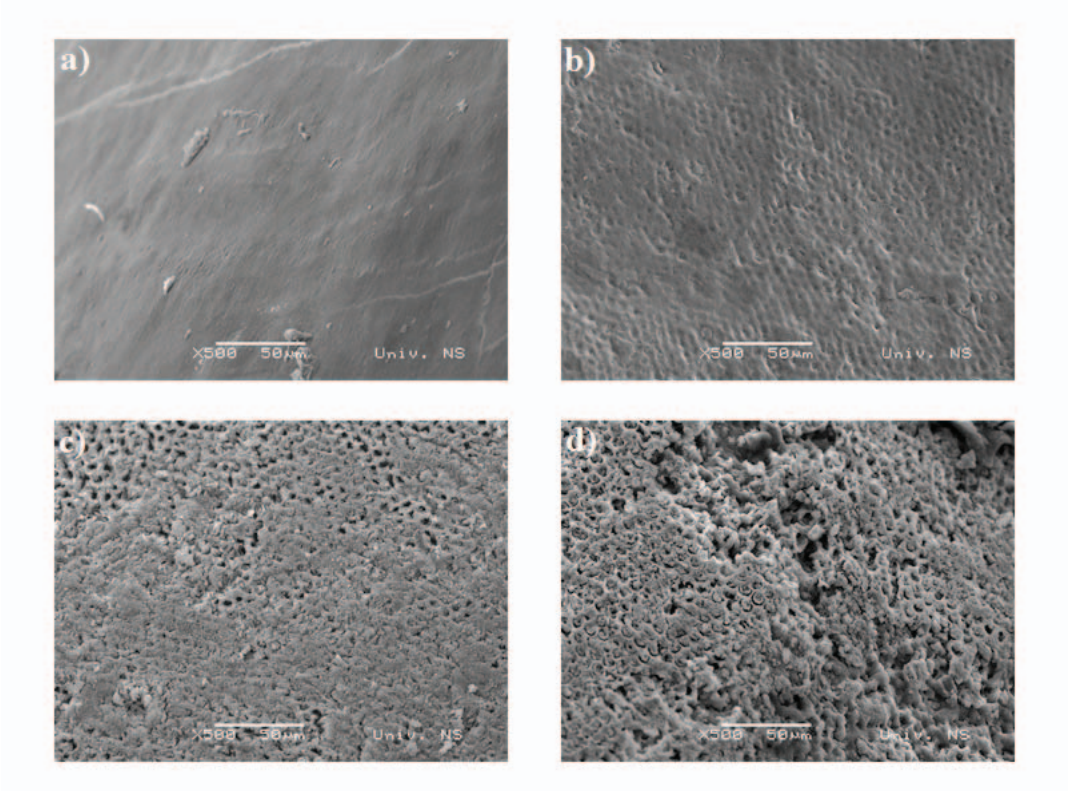

Fig. 2 – Scanning electron microscope (SEM) image of enamel surface after 24 hours of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

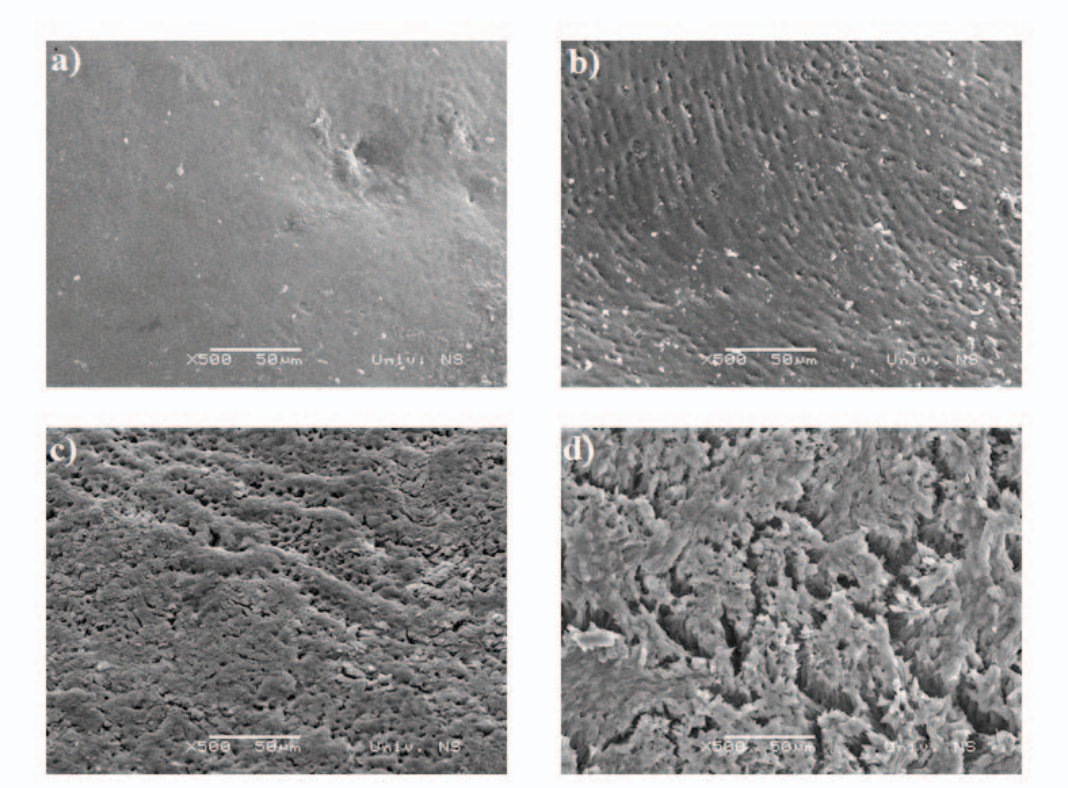

Fig. 3 – Scanning electron microscope (SEM) image of enamel surface after 7 days of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

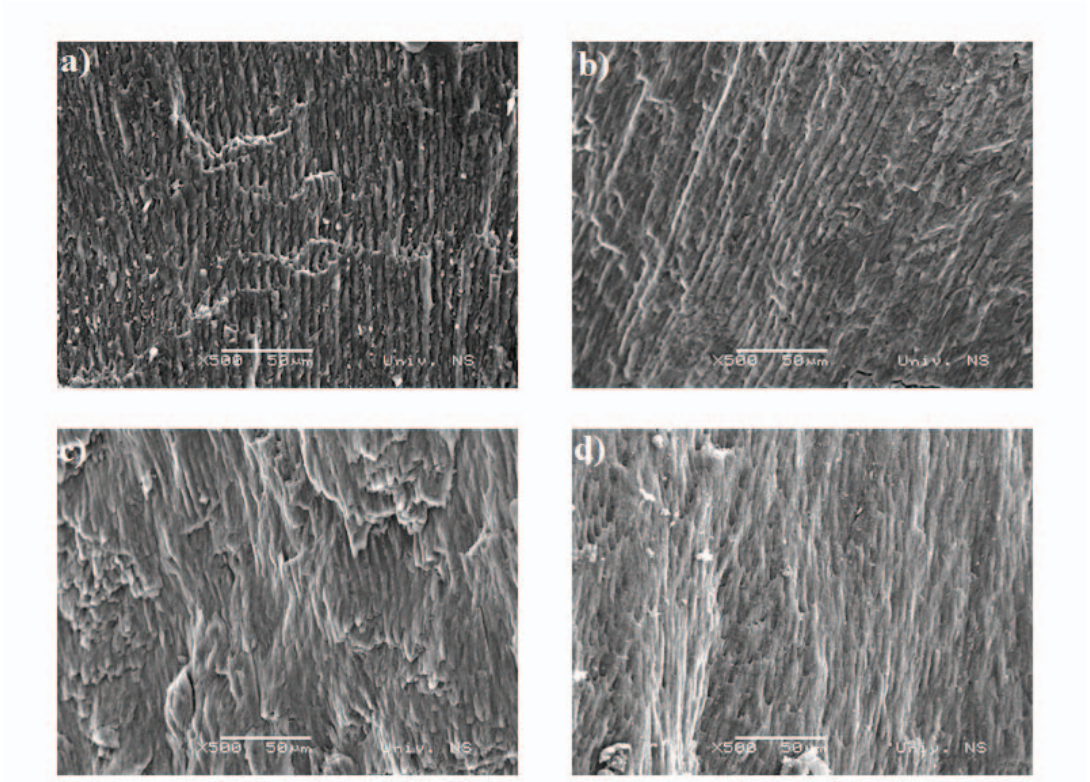

Fig. 4 – Scanning electron microscope (SEM) image of enamel, longitudinal section, after 60 minutes of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

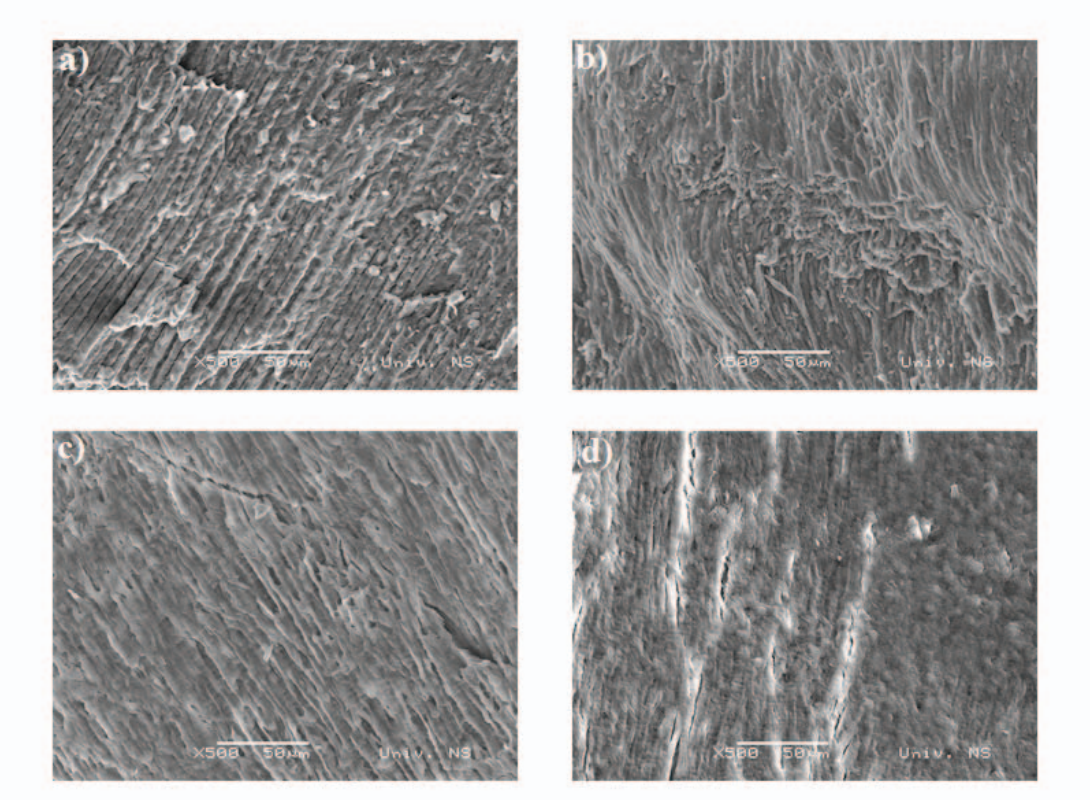

Fig. 5 – Scanning electron microscope (SEM) image of enamel, longitudinal section, after 24 hours of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

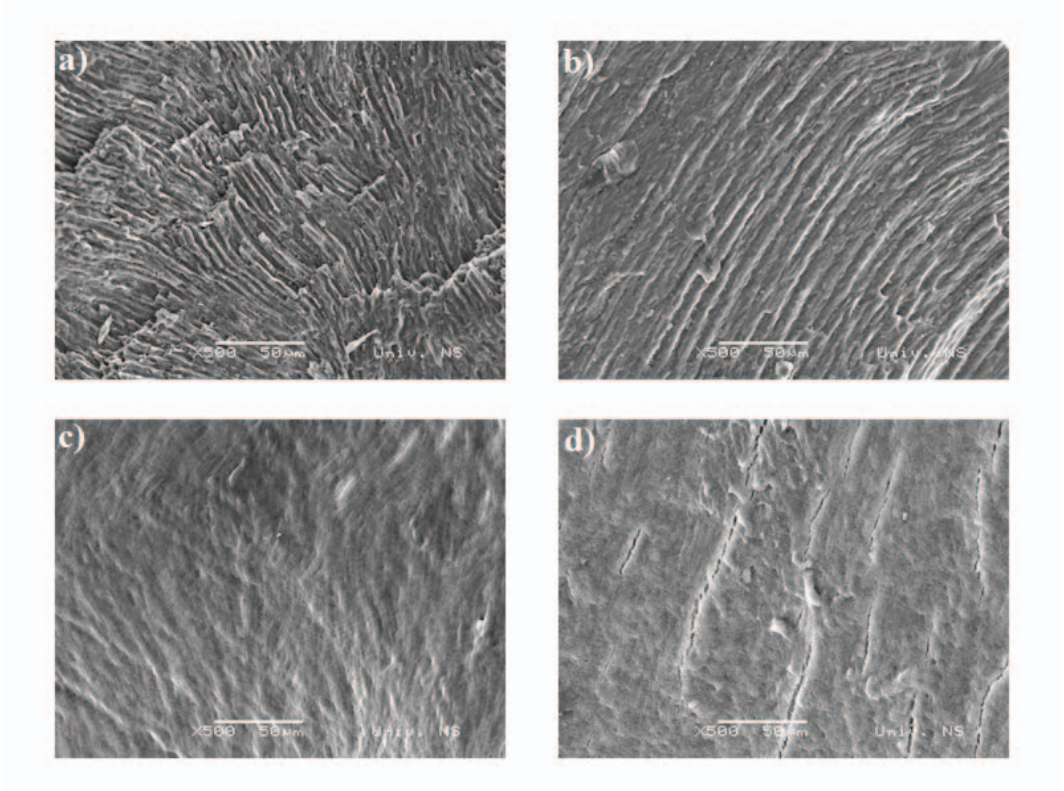

Fig. 6 – Scanning electron microscope (SEM) image of enamel, longitudinal section, after 7 days of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

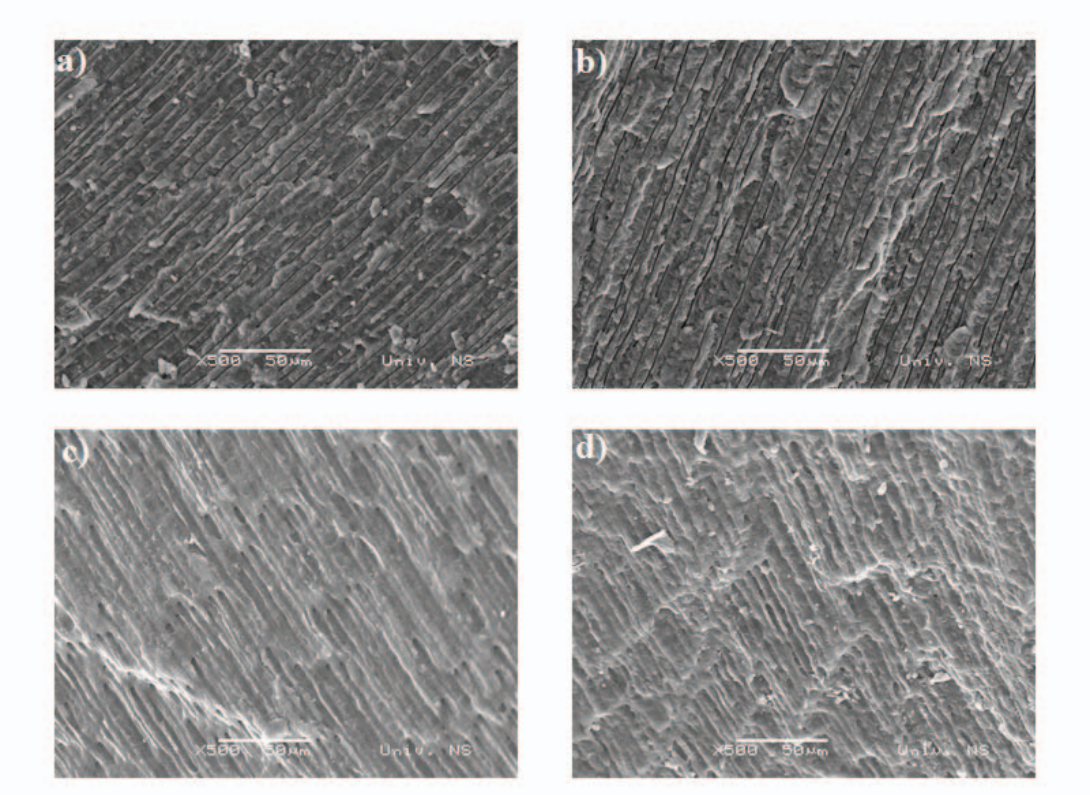

Fig. 7 – Scaning electron microscope (SEM) image of dentin, longitudinal section, after 60 minutes of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

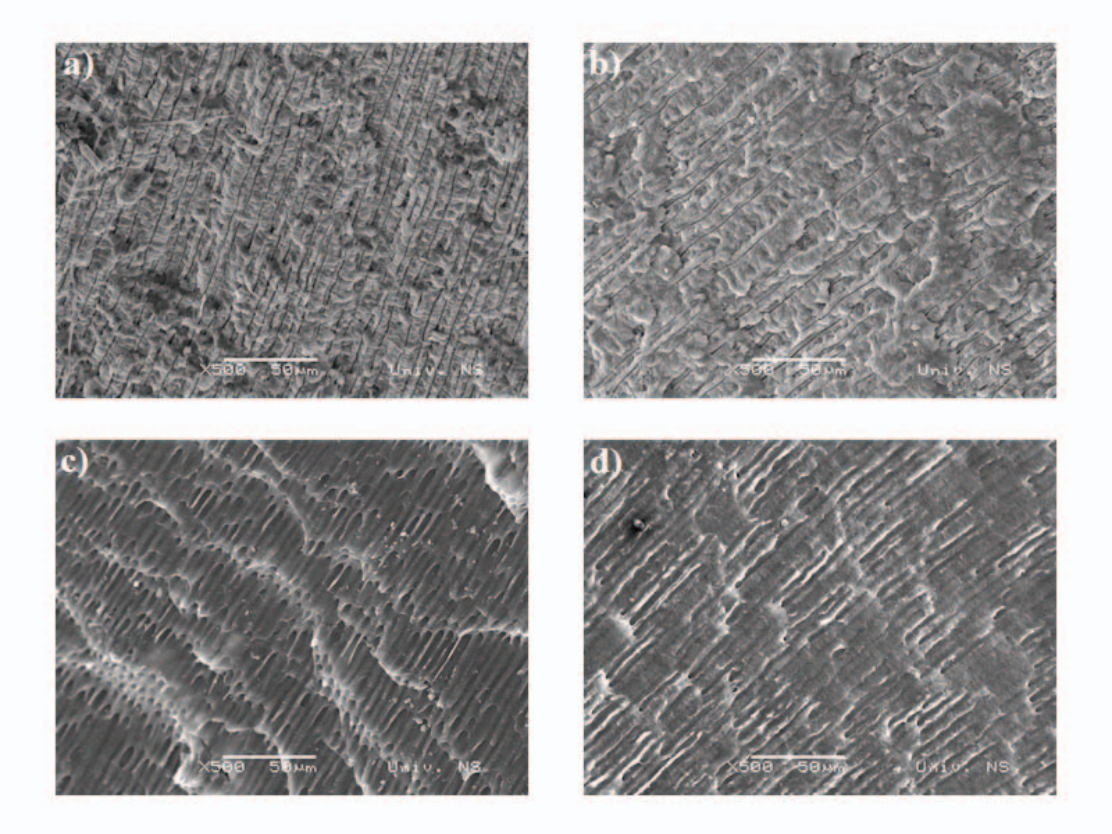

Fig. 8 – Scanning electron microscope (SEM) image of dentin, longitudinal section, after 24 hours of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

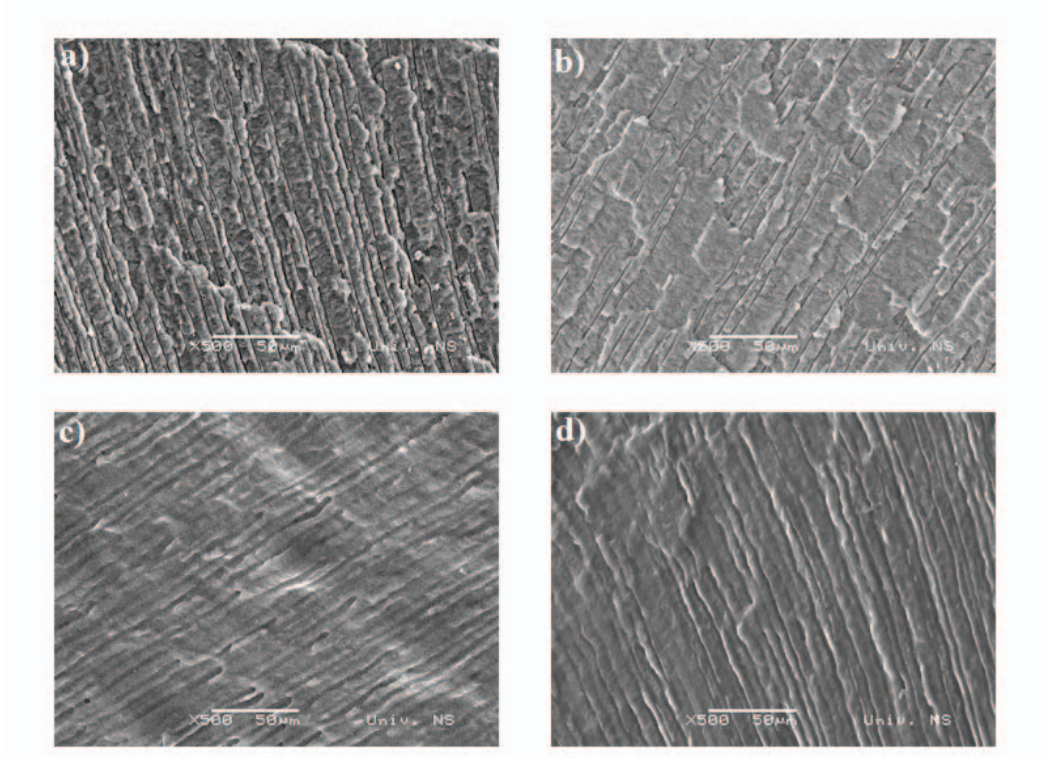

Fig. 9 – Scanning electron microscope (SEM) image of dentin, longitudinal section, after 7 days of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

Table 1

The degree of erosive changes of enamel and dentin during different time intervals of exposure to beverages

Type of beverage	Degree of morphology changes, mean ± SD					
	60 min	F (p)	24 h	F (p)	7 days	F (p)
Distilled water	0.000 ± 0.000		0.000 ± 0.000		0.000 ± 0.000	
Carbonated water	0.733 ± 0.458	44.212	1.133 ± 0.458	44.212	1.467 ± 0.458	44.212
Coca-Cola	1.200 ± 0.561	(0.000)	2.000 ± 0.561	(0.000)	2.467 ± 0.561	(0.000)
Schweppes Bitter Lemon	1.733 ± 0.458		2.467 ± 0.458		2.800 ± 0.458	

Analysis by ANOVA reveals consistent significant difference among the groups of treated samples; SD – standard deviation.

The analysis by ANOVA revealed a consistent significant difference among the groups of treated samples. After 60 minutes of exposure to beverages ($F = 44.212$, $p < 0.01$), the post hoc Scheffé testing revealed a statistically significant difference among all groups of samples. The experimental beverages produced a statistically significant alteration in the dental hard tissue morfology. The highest values of erosive changes showed the samples that were immersed in Schweppes Bitter Lemon (Table 1). The analysis of the samples after 24 hours also showed a significant difference among the groups ($F = 73.373$, $p < 0.01$). The control group was statistically different from the other groups. There was also a statistically significant difference between carbonated water and other groups, while Coca-Cola and Schweppes Bitter Lemon produced the statistically similar changes of structural units of enamel and dentin (Table 1). After 7 days of immersion in the tested beverages, the assessed values of the sample groups were statistically different ($F = 95.734$, $p < 0.01$). The samples that were immersed in Coca-Cola and Schweppes Bitter Lemon presented the similar changes of morfology and they could be classified as one group that was statistically significantly different when compared with the control samples and samples immersed in carbonated water. There was a statistically significant difference between the changes produced by carbonated water and the changes produced by other beverages (Table 1).

Discussion

In vivo influence of soft drinks on dental health is in correlation with the exposure time to acidic challenges, which is determined by the frequency of consumption and drinking habits [12, 14]. Study about the association of frequency of intake of some drinks and dental erosion showed the proportion of students with increased dental erosion as the frequency of drink increased [14]. Moreover, dietary habits also had a significant association with dental erosion; keeping the drinks in mouth for a long time increased the risk of dental erosion by 2.7 times compared with those who swallowed the drinks immediately [14]. *In vitro* studies, including those with the SEM analysis, also showed a progressive destruction of the enamel ultrastructure with the increase of the exposure time [8, 15].

Based on an average daily consumption of 25 ounces of soft drink and a residence time in the mouth of 5 seconds, the total exposure time to beverages would equal 22,750 seconds (380 minutes or 6.3 hours) per year [16]. It is more likely that the exposure time for a beverage on the dentition is closer to 20 seconds before salivary clearance occurs; this would make the annual exposure of dental enamel to soft drinks approximately 90,000 seconds (that is, 1,500 minutes or 25 hours) per year [16]. Test periods of 24 hours and 7 days used in this study can be compared with one and 7 years, if soft drinks are consumed in a way that was indicated before.

The erosive potential of an acidic drink is not exclusively dependent on its pH value, but is also strongly influenced by its mineral content, its titratable acidity ('the buffering capacity') and by the calcium-chelation properties of food and beverages [13, 14]. The formulae and contents of pop-Cola beverages are closely guarded industrial secrets, and sparse data about acidity is provided on their consumer packages [17].

All carbonated beverages contain carbonic acid formed by carbon dioxide in solution [18]. Erosive potential of sparkling mineral water tested in this study is low because there is no added other acids to its content. Like in other studies, less demineralization was observed on the teeth that were exposed to sparkling mineral water compared with other carbonated beverages [19]. Even after 7 days of exposure to carbonated water, the stractural units of dental hard tissues were still recognized. Acidity and erosive potential of other tested drinks is influenced by added acids, such as phosphoric acid that is present in Coca-Cola and citric and ascorbic acids present in Schweppes Bitter Lemon.

From a theoretical point of view, both acids, phosphoric and citric, are very erosive because complete dissociation of one molecule results in the formation of three hydrogen atoms [20]. Most *in vitro* researches has shown that citric acid is more erosive than phosphoric acid [21]. Citric acid has double actions and is very damaging to the tooth surface. This means that citric acid at lower pH, provides hydrogen ions to directly attack the mineral surface and at higher pH, the citrate ion draws the calcium out of the crystal surface. Phosphoric acid provides hydrogen ions at low pH and binds calcium at higher pH. Citrate, however, forms a complex with calcium because of the relative sizes and three-dimensional shapes of the molecules [13]. The pH level is an important variable, and chelation has a striking effect on tooth tissue erosion at high pH [21]. The chelating action of citric acid and adition of ascorbic acid can be associated with a higher potential of Schweppes Bitter Lemon for the dissolution of the hy-

droxyapatite crystals. A different degree of erosive changes between the samples treated with Coca-Cola and Schweppes Bitter Lemon can be observed at samples after 60 minutes exposure. With the prolonged exposure and progression of acid etching, effects of both beverages became similar, resulting in the formation of a completely demineralized layer and a loss of structural units.

The erosion potential of popular beverages is important for clinical guidelines regarding beverage consumption practices and development of potentially "safer" beverages, especially for children [16].

Conclusion

Prolonged and repeated exposure of dental tissue to carbonated beverages caused the erosive changes at a microscopic level that can be associated with serious clinical problems. Due to the fact that beverage intake cannot be limited, it is important to identify the excessive consumption of carbonated beverages as etiological factor for dental erosion. Dietary advice and preventive care are mandatory for the patients at a risk of developing dental erosion, although modification of erosive potential and development of low erosive beverages are also possible.

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Platelet aggregability and anticoagulant proteins activity during dobutamine stress echocardiography in asymptomatic patients four months after percutaneous coronary intervention

Agregabilnost trombocita i aktivnost antikoagulantnih proteina tokom stres ehokardiografije sa dobutaminom kod asimptomatskih bolesnika četiri meseca nakon perkutane koronarne intervencije

Zoran Jović*†, Vesna Subota‡, Boris Džudović†§, Zorica Mladenović*†, Jelena Marić Kocijančić*†, Predrag Djurić*†, Marijan Spasić*†, Nemanja Djenić†§, Radoslav Romanović†§, Vladimir Miloradović‖¶, Radomir Matunović*†, Slobodan Obradović†§

Military Medical Academy, *Clinic of Cardiology, §Clinic of Emergency Internal Medicine, ‡Institute of Clinical Biochemistry, Belgrade, Serbia; University of Defence, †Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; Clinical Center, ‖Department of Cardiology, Kragujevac, Serbia; University of Kragujevac, ¶Faculty of Medical Sciences, Kragujevac, Serbia

Abstract

Background/Aim. Platelets aggregability (PA) and the activation of hemostasis during myocardial ischemia within physical or mental stress, can be one of many factors that influence the process of stent thrombosis after the percutaneous coronary intervention (PCI). The aim of the study is to investigate the relationship between the PA and activity of anticoagulant proteins with myocardial ischemia during the dobutamine stress echocardiography (DSE) in the asymptomatic patients 4 months after the PCI. **Methods.** The study population included 74 asymptomatic patients who had a successful PCI 4 months before a high-dose DSE. PA on epinephrine (EPI) and adenosine diphosphate (ADP) were determined by the Light Transmission Aggregometry (LTA), together with plasma activity of protein C and antithrombin before the DSE and at the peak stage of the stress test. The patients were divided into several groups on the basis of whether they have baseline or induced disturbance of segmental myocardial kinetics or not. All patients were on the clopidogrel and aspirin therapy at the time of DSE. **Results.** There were no statistically significant difference in the PA ADP (47.50% *vs* 50.20%; $p = 0.970$) as well as on EPI (59.30% *vs* 60.30%, $p = 0.600$) before and at the peak of DSE. A statistically significant difference was found in the anticoagulant activity of the antithrombin (84.85% *vs* 74.75%, $p = 0.001$) and protein C (77.75% *vs* 67.60%, $p < 0.001$). A significance of differences in antithrombin and the protein C, refered to the result before and at the peak levels of the test. There was no significant difference in the PA and plasma activity of anticoagulant proteins in the patients with or without induced myocardial ischemia at the peak of DSE. The patients who had an increased wall motion score index at the peak of DSE, had a higher EPI induced PA than the patients with normal myocardial contractility (68.60% *vs* 54.70%, respectively; $p = 0.017$). **Conclusion.** There are no changes in the PA before and after DSE, however, plasma activity of anticoagulant proteins decreased at the peak level of the test. The PA on EPI significantly increases at the peak of DSE in the patients with segmental myocardial hypocontractility.

Key words:
antithrombins; echocardiography, stress; percutaneous coronary intervention; platelet aggregation.

Apstrakt

Uvod/Cilj. Agregabilnost trombocita i proces aktivacije hemostaze tokom ishemije miokarda u sklopu fizičkog ili mentalnog stresa mogu biti jedan od brojnih faktora koji utiču na proces tromboze stenta nakon perkutane koronarne intervencije (PKI). Cilj rada bio je da se ispita povezanost agregabilnosti trombocita i aktivnosti antikoagulantnih proteina sa miokardnom ishemijom tokom dobutamin stres ehokardiografije (DSE) kod asimptomatskih bolesnika, četi-

Correspondence to: Zoran Jović, Military Medical Academy, Clinic of Cardiology, Crnotravska 17, 11 000 Belgrade, Serbia.
E-mail: zockydr@gmail.com; zockydr@sezampro.rs

ri meseca nakon PKI. **Metode.** Studijsku populaciju činila su 74 asimptomatska bolesnika koja su imala uspešnu PKI četiri meseca pre visokodozne DSE. Agregabilnost trombocita na epinefrin (EPI) i adenozin difosfat (ADP) određena je metodom optičke agregometrije – *light transmission aggregometry* (LTA) zajedno sa aktivnošću proteina C i antitrombina u plazmi pre i u piku opterećenja tokom stres testa. Bolesnici su bili podeljeni u nekoliko grupa shodno tome da li su imali poremećaje kontraktilnosti određenih segmenata miokarda u miru ili u naporu. Svi bolesnici su u vreme DSE bili na terapiji klopidogrelom i aspirinom. **Rezultati.** Nije bilo statistički značajne razlike u agregabilnosti trombocita na ADP (47,50 *vs* 50,20; $p = 0,970$) kao ni na EPI (59,30% *vs* 60,30%; $p = 0,600$) pre i u piku DSE. Statistički značajna razlika je utvrđena u aktivnosti antikoagulantnih proteina, antitrombina (84,85% *vs* 74,75%; $p = 0,001$) i proteina C (77,75% *vs* 67,60%; $p < 0,001$). Značajnost razlike u aktivnosti antitrombina i proteina C odnosi se na rezultat pre i u piku testa. Nije utvrđena značajna razlika u agregabilnosti trombocita i aktivnosti antikoagulantnih proteina u plazmi kod bolesnika sa ili bez indukovane ishemije u piku DSE. Bolesnici koji su imali povišen indeks pokretljivosti zida leve komore (*wall motion score index*) u piku DSE su imali veću agregabilnost trombocita na EPI nego bolesnici sa normalnom kontraktilnošću miokarda (68,60% *vs* 54,70% $p = 0,017$). **Zaključak.** Nema promena vrednosti u agregabilnosti trombocita pre i posle DSE, međutim, dolazi do smanjenja aktivnosti antikoagulantnih proteina u plazmi u piku testa. Agregabilnost trombocita na EPI značajno raste u piku DSE kod bolesnika sa segmentnom hipokontraktilnošću miokarda.

Ključne reči:
antitrombini; ehokardiografija, stres; perkutana koronarna inervencija; trombociti, agregacija.

Introduction

During the last decades, ischemic heart disease is the most common cause of morbidity and mortality in the developed world. Although the mortality rate from IHD declined over last four decades all over the world, it is still responsible for a third of all deaths in the patients older than 35 years [1, 2]. An important factor in the disease incidence and complications during the percutaneous coronary interventions (PCI) is a platelet aggregability, changes in these parameters and resistance to antiplatelet therapy [3–7]. The aggregation of platelets and activation process of hemostasis during myocardial ischemia within the physical or mental stress can be an important factor in stent thrombosis after the PCI [4].

Platelets are oval or round plates with a usual diameter of about 2 microns resulting from fragmentation of megakaryocytes in the bone marrow, liver, spleen and lungs, from where they are released into the bloodstream. The most important physiological functions of platelets are: active participating in all phases of hemostasis, both physical and chemical processes, as well as the release and activity of specific platelet factors. In addition, they have a role in the process and maintaining the integrity of the endothelium, phagocytosis, body detoxification and transport of goods [5–8]. Platelets play a key role in the pathophysiology of thrombosis after the plaque rupture. The plaque rupture occurs spontaneously in the patients with an acute coronary syndrome, or may be iatrogenic induced in the patients undergoing the PCI. Among the multiple mediators of platelet activation, adenosine diphosphate (ADP) plays a key role. Thienopyridines are irreversible inhibitors of the P2Y12 ADP receptor. Clopidogrel is a second generation thienopyridine that is, in combination with aspirin, proved to be superior to oral anticoagulants in the prevention of thrombotic complication after stenting of the coronary arteries [3, 8]. Protein C and antithrombin are natural plasma proteins that play an important role in the process of anticoagulation. Their deficiency leads to the development of procoagulant conditions, and one of the complications can be artery thrombosis [9–13]. The association of stress caused by ischemia, quantified by dobutamine stress echocardiography (DSE), and changes in platelet function and other parameters of hemostasis in patients after the PCI, have not been tested so far.

The aim of the study was to investigate the relationship between the platelets aggregability and activity of anticoagulant proteins with stress-induced myocardial ischemia during the dobutamine stress echocardiography in the patients after the PCI with stent implantation.

Methods

Subjects

The prospective study population included 74 patients with ischemic heart disease. At least 5 days before the PCI, the patients were on the dual antiplatelet therapy (aspirin 100 mg + clopidogrel 75 mg). All patients had completed the PCI 4 months before admission to the study and did not have angina. There was no difference in the dual antiplatelet therapy in the patients with the acute coronary syndrome and stable angina pectoris, both groups received the same dose of aspirin and clopidogrel. The study population was on average 58 years old and 35% of them were women. The blood samples had been taken from all patients before the test and at the maximum load of the SE [14, 15]. The platelets aggregability was determined by the method of Light Transmission Aggregometry (LTA) [3, 16] before and at the maximum load of the DSE. The LTA is used for the ADP and EPI tests. We also determined the concentration of protein C, and antithrombin in all plasma samples. The main characteristics of patients are shown in Table 1.

Dobutamine stress echocardiography test

Four months after the PCI, all patients underwent the high-dose DSE. Dobutamine infusion was initiated with 10 μg/kg/min, then increased by 10 μg/kg/min every 3 minutes until a dose of 40 μg/kg/min was reached. Each stage was regularly monitored for the blood pressure and ECG. Also,

the side effects during the dobutamine test were followed in all patients. The regional wall abnormalities of the left ventricle were analyzed in accordance with the adopted the 16 divisions segmented model of the left ventricule by echocardiography recommended by American Cardiology Society. The regional wall-motion abnormality of each segment was evaluated by use of the 4-point scoring system: 1 = normal wall thickening; 2 = hypokinesia; 3 = akinesia; 4 = dyskinesia. Each segment of the left ventricle was individually assessed as viable if its regional contractility from the initial akinesia, hypokinesia or dyskinesia to hypokinesia or normokinesia were significantly improved at low doses of dobutamine while altering from the dyskinesia to akinesia was not considered as a marker of viability. If we had a worsening of motion from the baseline maintained motility after the dobutamine administration, these segments were evaluated as ischemic responses. Global mobility of the left ventricle in basal conditions and after the administration of low concentrations of dobutamine was determined based on the obtained values. The global mobility was presented like as Index of mobility – Wall Motion Score Index (WMSI), which is a measure of the average deviation from the ideal mobility segment that is preserved mobility of all 16 surveyed segments.

$$\frac{\text{WMSI} = \text{No. normokinesia} \times 1 + \text{No. hypokinesia} \times 2 + \text{No. akinesia} \times 3 + \text{No. diskinesia} \times 4}{16}$$

In physiological conditions, WMSI is 1, the WMSI value of 1.1 or higher, indicates a higher degree of the left ventricular dysfunction. WMSI was calculated in basal conditions and at the high dobutamine doses of 40 mg/kg. The findings of the DSE were considered positive when the regional wall motion of a normal or hyperkinetic segment was deteriorated. The interpretation of the test was done by an expert, echocardiographer.

Hemostatic parameters

At the same day when we performed the PCI, blood was sampled from the brachial veins in order to determine the platelets aggregability with the LOTA method. After the PCI, 4 months later, 2 samples of blood were taken from the brachial vein into the tubes containing sodium citrate, 3.8% below the minimum path, 30 min after standstill. The DSE was performed 2 to 3 hours after a blood cannula had been placed into the brachial vein. At the maximum load, blood was sampled from the venous cannula previously placed, into the two tubes containing 3.8% sodium citrate. Platelet rich plasma (PRP) was obtained by centrifugation at 150 x g for 10 min at room temperature. The platelet aggregation response to ADP (20 mmol/L) was recorded 5 min after addition of the agonists using the agregometar BCT-system (Dade-Behring, Germany). For determining the activity of protein C and antithrombin, platelet poor plasma (PPP) was taken, citrated and centrifuged at 2,000 x g for 15 min at room temperature and frozen at -80 °C to final weighing [4]. The activity of the protein C and antithrombin was determined by a colorimetric assay (Berichrom, Dade-Behring, Germany). All procedures were carried out according to the manufacturer's instructions.

Statistics

The Wilcoxon's test was used to compare two related samples as well those of the population without a normal distribution. The Mann-Whitney U test was used for comparison of two independent samples of the population who did not have a normal distribution. For the processing of the data, we used the SPSS (Statistical Package for the Social Sciences 20.0 for PC, SPSS Inc., Chicago, IL, USA). The values of $p < 0.05$ were considered significant, while those of $p < 0.01$ were considered to be statistically highly significant.

Results

The main characteristics of patients are shown in Table 1. There was no significant difference in the platelet aggregability both in the LTA tests on ADP ($p = 0.970$) and epinephrine (EPI) ($p = 0.600$), before and at the peak of the test (Table 2).

Table 1

Basic characteristics of patients included in the study

Basics parameters	Patients (n =74)
Age (years), mean ± SD	58 ± 9
Male, n (%)	48 (64.9)
Diabetics, n (%)	16 (21.6)
Smokers, n (%)	53 (71.6)
Treated hypertension, n (%)	71 (95.9)
Hypercholesterolemia, n (%)	32 (47.1)
BMI (kg/m^2), mean ± SD	27.05 ± 3.92
Body surface (m^2), mean ± SD	1.97 ± 0.22
STEMI patients, n (%)	11 (14.9)
NTEMI patients, n (%)	13 (17.6)
UAP patients, n (%)	18 (24.3)
SAP patients, n (%)	32 (43.2)

n – number of patients; SD – standard deviaton; BMI – body mass index; STEMI – ST-elevation myocardial infarction; NTEMI – non ST-elevation myocardial infarction; UAP – unstable angina pectoris; SAP – stable angina pectoris.

Table 2

Differences in the platelet aggregation and anticoagulation protein activity before and at the end of the stress echo test

Hemostatic parameters	Stress test, median (IQR)		*p* value
	before	at the end	
Platelet aggregability on ADP (%)	47.50 (31.65–74.40)	50.20 (31.90–71.30)	0.970
Platelet aggregability on EPI (%)	59.30 (44.60–74.90)	60.30 (44.50–74.50)	0.600
Antithrombin activity (IU/L)	84.85 (63.10–98.40)	74.75 (45.43–92.25)	0.001
Protein C activity (IU/L)	77.75 (39.88–105.00)	67.60 (32.30–97.25)	< 0.001

IQR – interquartile range; ADP – adenosine diphosphate; EPI – epinephrine.

A statistically significant difference was found in the activity of the anticoagulant proteins, antithrombin (p = 0.001) and protein C (p < 0.001) (Table 2). Significant ischemia was found in 16 of 74 patients. At the peak of the test, we registered very significant drop in the concentration of antithrombin and protein C levels (Table 2).

There was no significant difference in the platelet aggregability on ADP in the groups of patients with the positive and negative stress echo test (p = 0.829). The group with the positive stress echo test had the median of platelet aggregability on ADP of 38.10% with the inter-quartile range (IQR) from 30.05% to 72.80%, while that with the negative stress echo test on ADP had the median of platelet aggregability on ADP of 52.00% with IQR from 32.65% to 71.35% (Figure 1).

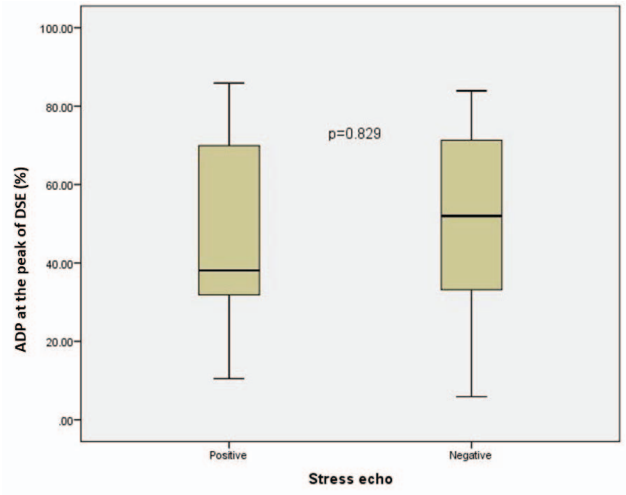

Fig. 1 – Platelet aggregability on adenosine diphosphate (ADP) in the groups of patients with positive and negative DSE test (stress echo). DSE – dobutamine stress echocardiography.

There was no significant difference in the platelet aggregability on EPI between groups (p = 0.465). The group with the positive stress echo test had median of platelet agregregability on EPI of 62.00% with IQR from 51.30% to 80.70%, while the group with negative stress echo test had the median of platelet aggregability on EPI of 59.00% with IQR from 43.83% to 73.60% (Figure 2). There was no statistically significant difference between groups regarding antithrombin activity (p = 0.081). The group with the positive stress echo test had the median of antithrombin activity of 86.30 IU/L with IQR from 58.00 IU/L to 101.25 IU/L, and the group with negative stress echo test had the median of antithrombin activity of 70.00 IU/L, with IQR from 36.35 IU/L to 87.50 IU/L (Figure 3).

There was no statistically significant difference between groups regrading activity of protein C (p = 0.240). The stress echo test positive group had the median of protein C activity of 64.50 IU/L with IQR from 11.20 IU/L to 94.43 IU/L, while the negative echotest group had the median of protein C activity of 67.70 IU/L with IQR from 35.10 IU/L to 99.00 IU/L (Figure 4).

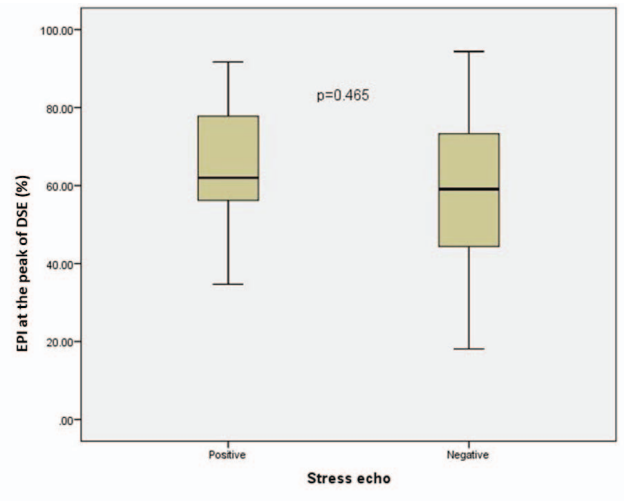

Fig. 2 – Platelet aggregability on epinephrine (EPI) in the groups of patient with positive and negative DSE test (stress echo). DSE – dobutamine stress echocardiography.

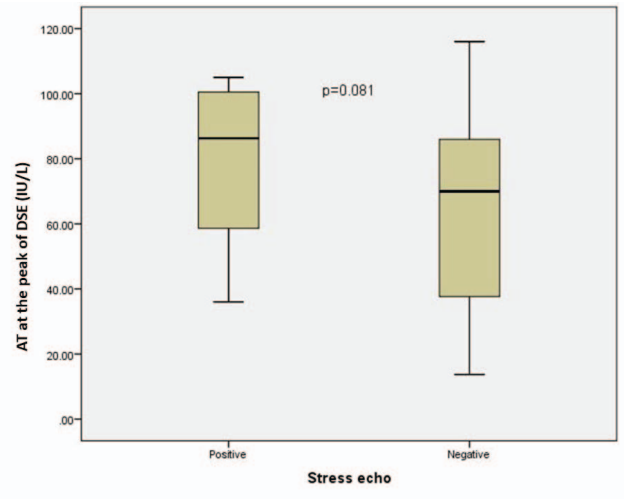

Fig. 3 – Antithrombin (AT) activity in the groups of patients with positive and negative DSE test (stress echo).

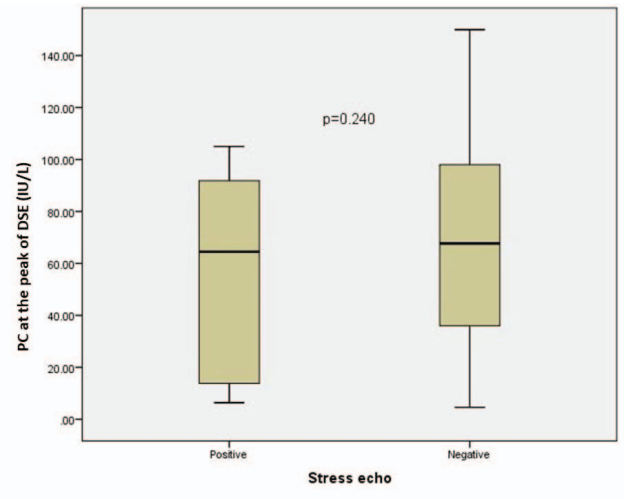

Fig. 4 – Protein C (PC) activity in groups of patients with positive and negative dobutamine stress echocardiography (DSE) test (stress echo).

Table 3

Differences in the platelet aggregability and anticoagulation protein activity compared with the Wall Motion Score Index (WMSI) at the peak of stress echo test

Hemostatic parameters	WMSI = 1 (n = 39) median (IQR)	WMSI > 1 (n = 35) median (IQR)	*p* value
Platelet aggregability on ADP (%)	49.70 (30.30–66.43)	65.20 (32.75–77.40)	0.275
Platelet aggregability on EPI (%)	54.70 (41.05–67.48)	68.60 (52.15–85.25)	0.017
Antithrombin activity (IU/L)	65.80 (33.50–82.15)	75.30 (56.15–98.65)	0.115
Protein C activity (IU/L)	63.00 (26.35–99.00)	84.00 (33.40–91.75)	0.921

IQR – interquartile range; n – number; ADP – adenosine diphosphate; EPI – epinephrine.

In the groups of patients with WMSI equal to 1.0 or greater than 1.0, it was found that there was a statistically significant difference between the groups in determining platelet aggregability on EPI, while concerning other parameters, platelet aggregability on ADP, antithrombin and protein C activities, there was no significant difference between the groups at the peak of the DSE test (Table 3).

Discussion

The most important finding in our investigation was that no one in the group of asymptomatic coronary patients, 4 months after the PCI, had an increase in the EPI and ADP dependent patelets aggregability at the peak of DSE; furthermore they had decreasing activity of both protein C and antithrombin. In the subgroup of patients with the detected regional motion abnormality at the peak of DSE, we measured the higher EPI induced platelets aggregability than in the subgroup of patients with a completely normal ventricular contractile function. These findings implicate a potential hypercoagulable state, especially during the inotropic and chronotropic myocardial stress in the coronary patients after the PCI, particularly in the presence of wall motion disturbances.

The treadmill test, which was used in most of the studies of hemostasis during physical exercise, had pretty or rather low sensitivity in the diagnosis of myocardial ischemia in comparison to the DSE [17–21] , which limits its capability to induce changes in hemostasis with appearance of ischemia. The DSE was used in our study, because of its better sensitivity to detect ischemia, so we could expect that the changes in hemostasis can be firmly associated with myocardial ischemia. The DSE lasts 12 min and it is not realistic to expect that in certain patients, due to the positive inotropic effects, dobutamine may cause adverse events. In our study, there were no such events on the effect of dobutamine during the test. Also, we found that the platelets aggregability on EPI was significantly higher in the patients with the regional left ventricle wall motion disturbances. They associate chronotropic and inotropic stress of myocardium and insufficient coronary flow with catecholamines induced enhanced platelets aggregability which can cause arterial thrombosis, especially at the spot of coronary stents.

In our previous study, which included 37 asymptomatic coronary patients, 4 to 8 months after the PCI, with even more sophisticated single photon emission computed tomography (SPECT) adenosine-exercise stress testing, we showed a significant decrease of antithrombin, but not the protein C activity without changes in the platelets aggregability at the peak of stress test [4]. In the adenosine-exercise stress SPECT test, redistribution of the blood flow and tachycardia induced ischemia, while in the DSE tachycardia and inotropic myocardial stimulation were the main factors causing ischemia. These differences, in the way of inducing ischemia, may be the reason for the EPI induced platelets aggregability did not change in the adenosine-exercise SPECT test and it was increased at the peak of DSE in the patients with the regional wall motion dysfunction.

The limitation of this study is a relatively small number of patients.

Conclusion

There are no changes in the platelets aggregability before and after the DSE, however, plasma activity of anticoagulant proteins decreased at the peak level of the test. The platelet aggregability on EPI significantly increases at the peak of DSE in the patients with a pronounced segmental myocardium hypocontractility (elevated WMSI). There is a need for more clinical trials, with a larger number of patients, to optimize the antiplatelet therapy in the patients with the coronary artery disease who had the PCI. Also, it is essential to determine additional hemostatic parameters which can interplay with the stress, myocardial ischemia and antiplatelet agents.

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Evaluation of emotional distress in people with diabetes mellitus

Procena emocionalnog distresa kod osoba obolelih od šećerne bolesti

Verica Petrović *†, Vesna Vidaković ‡, Ornela Jelisavac †

***Health Center Banja Luka, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; University of Banja Luka, †Faculty of Medicine, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; ‡Health Center Prijedor, Prijedor, Republic of Srpska, Bosnia and Herzegovina**

Abstract

Background/Aim. Despite the modern ways of treating diabetes mellitus (DM), a half of the patients do not achieve the optimal metabolic control, which increases a risk of complications and occurrence of emotional "burnout" of patients. The goal of the research was to determine the level of emotional distress of patients with diabetes, and the association of the level of emotional distress with sociodemographic traits and the disease characteristics. **Methods.** The research was conducted from 01.01.2016. to 04.30.2016. in the Comunity Health Center in Banja Luka. The research was conducted in a group of 113 randomly selected patients (63 men, 50 women) with diabetes, with an average age of 63.93 years. A majority of respondents have type 2 DM (91.12%). The Problem Areas in Diabetes Questionniare (PAID) was used in the research. Another questionnaire used contained questions related to sociodemographic traits (sex, age, education), and disease traits (type of DM, duration of the illness, types of therapy that the patient uses in the treatment of DM, the value of hemoglobin A1c (HbA1c) over the last three months, the presence of microvascular complications). **Results.** According to the total score of the PAID questionnaire, 64 (56.6%) respondents had a moderate level of emotional distress. The level of emotional distress is higher in the group with a higher HbA1c values, but without a statistically significant difference compared to the group with the achieved goal HbA1c values. Two-thirds of respondents with microvascular complications manifest a moderate level of distress. The association between occurrence of distress with the occurrence of microvascular complications was confirmed. **Conclusion.** More than a half of patients with DM have an elevated level of distress of a moderate degree, and two-thirds of patients with DM with the microvascular complications have an elevated level of distress of a moderate degree, which indicates the importance of interdisciplinary cooperation of endocrinologists, vascular surgeons and psychiatrists.

Key words:
diabetes mellitus; stress, psychological; glycated hemoglobin a; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Uprkos savremenom načinu lečenja dijabetes melitusa (DM), polovina bolesnika ne postiže optimalnu metaboličku kontrolu, što povećava rizik od komplikacija i pojave emocionalnog "sagorevanja" obolelih. Cilj ovog istraživanja bio je da se utvrdi nivo emocionalnog distresa obolelih od dijabetesa i povezanost nivoa emocionalnog distresa sa sociodemografskim karakteristikama i karakteristikama bolesti. **Metode.** Istraživanje je sprovedeno od 01.01.2016. do 30.04.2016. u Domu zdravlja u Banja Luci. Istraživanje je rađeno na grupi od 113 slučajno odabranih bolesnika (63 muškarca, 50 žena) obolelih od dijabetesa, prosečne starosti 63,93 godine. Dominirali su ispitanici sa DM tip 2 (91,12%). U istraživanju je korišćen *Problem Areas In Diabetes Questionare* (PAID) upitnik o problematičnim područjima u šećernoj bolesti. Korišćen je i Upitnik koji sadrži pitanja koja se odnose na sociodemografske karakteristike [(pol, dob, stepen obrazovanja) i karakteristike bolesti (tip DM, dužina trajanja bolesti, vrsta terapije koju bolesnik koristi u liječenju DM, vrijednosti hemoglobina A1c (HbA1c) rađenog unazad tri mjeseca, prisustvo mikrovaskularnih komplikacija)]. **Rezultati.** Prema ukupnom skoru PAID upitnika 64 (56,6%) ispitanika imalo je umeren nivo emocionalnog distresa. Nivo emocionalnog distresa bio je veći u grupi sa većim vrednostima HbA1c, ali bez statistički značajne razlike u odnosu na grupu sa postignutim ciljnim vrednostima HbA1c. Dve trećine ispitanika sa mikrovaskularnim komplikacijima ispoljavalo je umeren nivo distresa. Potvrđena je povezanost pojave distresa sa pojavom mikrovaskularnih komplikacija. **Zaključak.** Više od polovine bolesnika sa DM imalo je povišen nivo distresa umerenog stepena, a 2/3 osoba sa DM sa mikrovaskularnim komplikacijama imalo je povišen nivo distresa umerenog stepena, što

Correspondence to: Verica Petrović, Bana Lazarevića 9, 78 000 Banja Luka, Republika Srpska, Bosnia and Herzegovina.
E-mail: drverica@hotmail.com

ukazuje na značaj interdisciplinarnarne saradnje endokrinologa, vaskularnih hirurga i psihijatara.

Ključne reči:
dijabetes melitus; stres, psihički; hemoglobin a, glukozilovan; ankete i upitnici.

Introduction

Diabetes mellitus (DM) represents a group of metabolic diseases, which manifest itself with a systematic disorder of metabolism of carbohydrates, fats, and proteins, due to an absolute or relative lack of biologically active insulin [1, 2].

Knowledge of the long-term, lifelong disease affects people suddenly, usually in full health, and requires the mobilization of all adaptive psychological mechanisms to help them to overcome the disease and integrate it into their daily life. Diabetes requires daily responsibility and patients' self-care by using insulin, pills, diet, exercise, and checking blood glucose levels every day for the rest of their lives. Each type of emotional response serves a purpose, but can be harmful if it is too intense or too long. The most common types of response during the adjustment to diabetes are: denial and disbelief, fear and helplessness, anger, guilt, sadness and depression. Due to the chronic nature of this disease, it is important to constantly monitor changes in physical health, and also the psychological changes [1–3].

Research indicates that the distress shortly after the diagnosis of diabetes is usually low, especially in asymptomatic individuals that are not in the intensive treatment regimen. Their distress increases after 2–3 years, which is likely correlated with the progression of the illness. People with type 2 diabetes who are treated with insulin, which implies a greater disease progression and a more demanding treatment regimen, have a higher distress level caused by diabetes than those treated with lifestyle change and/or oral medication. Significant predictors of distress increase from non-clinical to a clinical level during one year are female sex, presence of major depressive disorder in the medical record, a large number of acute stressful events in the given time period, a large number of complications of diabetes and poor self-treatment with diet and physical activity [3].

The guidelines of the American Diabetes Association (ADA) state specific directions for psychosocial assessment and monitoring of patients with diabetes. The general principle of this approach is that the assessment of the psychosocial needs of patients should be a part of the regular medical monitoring of patients. The assessment of psychological illnesses is particularly important for the patients with unsatisfactory treatment results. These guidelines emphasize that psychosocial testing should be performed at the time of diagnosis, during routine controls, and especially in the cases when complications arise or if a more intensive treatment regimen is required [3].

There are many instruments used for measuring the psychosocial aspects in diabetes treatment. The Problem Areas in Diabetes Questionnaire – PAID, is one of the recent questionnaires, which was developed in 1995 by G. Welch, as a measure of emotional adaptation to life with diabetes, consists of 20 questions. Each question is connected with the five-point Likert scale, which reflects a degree which a certain statement is perceived as a current problem [4, 5].

The aim of this study was to determine the level of emotional distress in the patients with diabetes and its association with sociodemographic characteristics (gender, age, education) and the characteristics of the disease [type of diabetes, duration of diabetes, treatment regimen and hemoglobin (Hb)A1c level].

Methods

This is a prospective study, conducted in the period from 01.01.2016 to 04.30.2016 in the Educational Centre of Family Medicine in Banja Luka.

All patients with diabetes registered in two randomly selected family medicine teams were the target group. Of the total of 120 registered patients, 7 patients did not participate in the survey, while 113 questionnaires were correctly completed and used for further statistical analysis.

The survey instrument was a custom-made questionnaire containing two parts. The first part of this questionnaire contained questions related to gender, age, education level, type of DM, disease duration, therapy regimen used in the treatment of DM, HbA1c-made in the past three months and the presence of microvascular complications of DM. The second part was related to the PAID. With 20 items. Each item had five possible answers, with the values from 0 to 4, where 0 represented the answer "not a problem" and 4 indicated "a serious problem". The values were added together and multiplied by 1.25, so the total score possible was from 0–100. The respondents with a score of 40 or more had a high level of emotional distress and require special attention. The PAID scores in these patients might be reduced by 10 to 15 points, if they underwent the medical interventions and educational programs [4]. Extremely low scores (0–10) combined with a poor glycemic control may be indicative for denial [5].

The study included 113 subjects (the patients with diabetes type 1 (DT1) and type 2 DT2), 63 (55.8%) men and 50 (44.2%) women, divided into three age categories. There were 5 (4.4%) patients at the age of 40 years, 56 (49.6%) from 41–65 years, and 52 (46%) over 65 years. As to the qualifications 21 (18.6%) respondents had primary school education, 73 (64.6%) had secondary school education and 19 (16.8%) had a university degree. According to the type of diabetes, there were 10 (8.8%) patients with DT1, and 103 (91.2%) with DT2. According to the therapy used for diabetes treatment, 69 (61.1%) patients were treated with oral antidiabetic drugs (OAD), 17 (15%) used the combination therapy (OAD + insulin), and 27 (23.9%) used insulin only. In our study, 34 (30.1%) patients had a good glycemic control (HbA1c $\leq$ 6.5/7.0%), while the number of patients with a

poor glycemic control (HbA1c ≥ 6.5/7.0%) was larger and counted 79 (69.9%). According to the duration of disease, 49 (43.4%) respondents had DM up to 5 years, 38 (33.6%) had it in a range of 6–10 years and 26 (23%) the disease lasted 11 years and over. Of all respondent 39 (34.5%) had microvascular complications.

To determine the distribution of respondents by the categories of socio-demographic variables and the examined characteristics of the disease, the descriptive statistics was applied, i.e., frequencies and percentages. To determine the relationship of socio-demographic variables and characteristics of the disease to the level of emotional distress, the χ^2 test was applied. In cases where the frequency response in the individual categories was less than 5, the χ^2 test was not precise, and instead, the Fisher's exact index of probability was applied.

Results

According to the presence of emotional distress, a majority of respondents, 64 or 56.6% had a moderate level of distress. A high level of emotional distress was present in 24 (21.3%) patients, while 25 (22.1%) patients had no emotional distress (Table 1).

A moderate level of distress was the most common in both sexes. A high level of distress was equally present both in men (22.6%) and women (22.0%). A slightly higher proportion of women (26%) expressed no distress compared to men (19.1%). These differences were not statistically significant ($\chi^2 = 974$, df = 2, $p = 0.614$).

Several sub-groups according to the distress level and three different age groups had fewer than 5 respondents, so the χ^2 square test was not reliable ($\chi^2 = 3.015$, df = 4, $p = 0.555$), and the Fisher's Exact Test = 2.658, $p = 622$, was applied. There were no statistically significant differences in the level of distress among the different age categories.

Comparing the level of emotional distress among respondents with different levels of education, over a half (63%) of the respondents with secondary education had a moderate level of distress. No statistically significant differences in the level of distress was found among the patients with different levels of education (Fisher's Exact Test = 4.237, $p = 0.375$).

Table 1

Presence of emotional distress in the patients with diabetes

Patients		Not expressed distress (0–9.99)	Moderate level of distress (10–39.99)	Pronounced level of distress (≥ 40)	*p*
Total number, n (%)	113 (100)	25 (22.1)	64 (56.6)	24 (21.3)	
Gender, n (%)					
men	63 (55.8)	12 (19.1)	38 (60.3)	13 (20.6)	$\chi^2 = 0.974$, df = 2, $p = 0.614$
females	50 (44.2)	13 (26.0)	26 (52.0)	11 (22.0)	
Age (years), n (%)					
< 40	5 (4.4)	0 (0.0)	4 (80.0)	1 (20)	Fisher's Exact Test – 2.658, $p = 0.622$
40–65	56 (49.6)	12 (21.4)	34 (60.7)	10 (17.9)	
≥ 66	52 (46)	13 (25.0)	26 (50.0)	13 (25.0)	
Education, n (%)					
primary school	21 (18,6)	7 (33.3)	9 (42.9)	5 (23.8)	Fisher's Exact Test = 4.237, $p = 0.375$
secondary school	73 (64.6)	13 (17.8)	46 (63.0)	14 (19.2)	
higher and university	19 (16.8)	5 (26.3)	9 (47.4)	5 (26.3)	
Type of diabetes, n (%)					
DT1	10 (8.8)	0 (0)	9 (90)	1 (10)	Fisher's Exact Test = 4.616, $p = 0.069$
DT2	103 (91.2)	25 (24.3)	55 (53.4)	23 (22.3)	
Therapy, n (%)					
OAD*	69 (61.1)	19 (27.5)	38 (55.1)	12 (17.4)	Fisher's Exact Test = 5.679, $p = 0.216$
OAD + insulin	17 (15)	4 (23.5)	9 (53)	4 (23.5)	
insulin	27 (23.9)	3 (7.4)	17 (63.0)	7 (29.6)	
Level of HbA1c, n (%)					
good-regulated (≤ 6.5/7.0%)	34 (30.1)	7 (20.6)	21 (61.8)	6 (17.6)	$\chi^2 = 0.573$, df = 2, $p = 0.751$
poorly regulated (> 6.5/7.0%)	79 (69.9)	18 (22.8)	43 (54.4)	18 (22.8)	
Duration of diabetes (years), n (%)					
≤ 5	49 (43.4)	16 (32.6)	24 (49.0)	9 (18.4)	$\chi^2 = 6.980$, df = 4, $p = 0.137$
6–10	38 (33.6)	6 (15.8)	25 (65.8)	7 (18.4)	
≥ 11	26 (23)	4 (11.5)	15 (57.7)	8 (30.8)	
Microvascular complications, n (%)					
no	74 (65.5)	23 (31.1)	36 (48.6)	15 (20.3)	Fisher's Exact Test - 11.215, $p = 0.012$
yes	39 (34.5)	2 (5.1)	28 (71.8)	9 (23.1)	

***OAD – oral antidiabetic drugs; DT1 – diabetes mellitus type 1; DT2 – diabetes mellitus type 2; HbA1c – hemoglobin A1c.**

There was no statistically significant difference in the level of emotional distress among the respondents with type 1 diabetes and type 2 (Fisher's Exact Test = 4.616, p = 0.069). Given the small number of cases of type 1 diabetes, these two categories of diabetes could not be compared in percentage.

There was no significant difference in the level of emotional distress among the respondents who used different types of therapy (Fisher's Exact Test = 5.679, p = 0.216).

In most cases, the respondents with both good and poorly controlled HbA1c levels had expressed a moderate level of distress. A slightly higher proportion of subjects with poorly controlled HbA1c (22.8%) had a pronounced level of distress as compared to the subjects with well controlled HbA1c (17.6%). These differences were not statistically significant (χ^2 = 573, df = 2, p = 0.751).

About a third of respondents who suffered from the disease up to 5 years (32.6%) did not have an expressed distress. The number of respondents that did not have expressed distress reduced with the duration of the disease (32.6%; 15.8%; 11.5%). Comparing the level of emotional distress depending on the duration of the disease, a statistically significant difference was not determined (χ^2 = 6.980, df = 4, p = 0.137).

There was a statistically significant difference in the prevalence of the emotional distress between the patients who did not and patients who did have microvascular complications (Fisher's Exact Test – 11.215, p = 0.012). It was found that one third of respondents who did not have microvascular complications did not have pronounced distress (31.3%), while the respondents with present microvascular complications, in most cases, had moderate levels of distress (71.8%). The aforementioned differences were statistically significant.

Discussion

The PAID questionnaire showed that most of our respondents had moderate levels of emotional distress. In the Republic of Srpska (RS) in 2015, the presence of psychological distress was assessed in the adults with diabetes using the vitality scale of the questionnaire short form (SF) 36, recommended by the European Health Interview Survey (EUROHIS) and the European Community Health Indicators Monitoring (ECHIM) project. The study showed that the highest percentage of respondents (74.4%) in the month preceding the survey had a negative emotional state, such as sadness, depression, etc., (score ≤ 58), a quarter of respondents (24.9%) had a score of between 58 and 78, while a very small number of respondents (0.6%) had a more positive mental health (score ≥ 78) [2]. Although we used a different questionnaire (PAID), it can be stated that the results of our research are a little better when it comes to the mental health of our patients. Around one-quarter of our respondents (22.1%) had no distress which was significantly higher than the mentioned study in the RS. Unlike the mentioned study where a majority of respondents had a negative emotional state (74.4%), in our study 21.3% of respondents had a high level of emotional distress. The largest percentage of our respondents had a moderate distress (56.6%). The results of a research in France, from the second multinational DAWN study by Reach et al. [6], conducted on 500 diabetic patients (420 with type 2 and 80 with type 1 DM), showed that about a half of participants (44%) had a high level of distress in the PAID 5 scale. The Diabetes Attitudes, Wishes and Needs (DAWN2) multinational study included 8596 patients with diabetes in 17 countries. The study results showed that 44% of participants had a high level diabetes-related distress on the PAID scale, the lowest number of participants with a high level of distress (20%) were in the Netherlands, and the highest (about 60%) in Algeria [7].

In our research, an association between the level of emotional distress with sociodemographic variables (gender, age, education) was not confirmed, however, some studies had confirmed this relationship. The study conducted by Lee et al. [8] in the Republic of Korea on 440 patients with DT2, with equal representation by gender (51.4 : 41.6%), found, like in our study, that the total score of the PAID 5 scale was significantly higher for females than for males. When it comes to age, in contrast to our study, the survey by Reddy et al. [9], on 184 participants with DT1 and DT2 showed that the DM-related distress negatively correlates with age. A study about diabetes-related distress and depression, conducted on 700 patients with DT2 in Malaysia, showed that younger people had a higher level of distress [10].

In our research, we did not find a statistically significant correlation between the level of emotional distress with the type of diabetes, duration of disease, nor the type of therapy used for diabetes treatment. Due to the small number of patients with DT1 it was not possible to compare the percentage of emotional distress by type of diabetes. Most of the study did not confirm a difference of emotional distress among the respondents with DT1 and DT2 [7].

The level of emotional distress was higher in the group with the higher HbA1c levels, but without statistically significant differences compared to the group with the target levels for HbA1c.

About a third of respondents (32.7%) who had the disease up to 5 years did not have a pronounced distress. The number of respondents did not have the distress reduction due to the duration of the disease. A high level of stress was present in the patients who had the disease up to 10 years (18.4%); this percentage rises to 30.8% with the patients who had the disease for 11 years and more, but there was not a statistically significant difference. As in our study, Stoop et al. [11], in their study which included 526 patients with type 2 diabetes, showed no significant association between diabetes duration and the total level of emotional distress.

With the patients using insulin, moderate distress level was higher (63%) than in other two groups, but with no statistically significant difference. In a Dutch Diacource study conducted on 590 people with type 2 diabetes, Kasteleyn et al. [12] found that the participants with insulin treatment had a higher score on the PAID scale than participants on the OAD therapy.

An association between the occurrence of distress and occurrence of microvascular complications was confirmed. There was a statistically significant difference in the prevalence of emotional distress among respondents who had and those who did not have some microvascular complications. Approximately a half of our respondents without microvascular complications had a moderate level of distress (48.6 %), which was a statistically significantly less compared to those with microvascular complications (71.8%).

As in our research, Kasteleyn et al. [12], established a correlation between the total score on the PAID scale and present microvascular complications.

Conclusion

More than a half of patients with DM have an elevated level of distress of a moderate degree, and two-thirds of patients with DM with the microvascular complications have an elevated level of distress of a moderate degree. The results indicate the need to assess the emotional distress of all patients with diabetes in the family health centers, as well as the importance of an interdisciplinary approach to the treatment of patients with diabetes. In addition to the endocrinologists and vascular surgeons, involving the psychiatrists would be significant.

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CASE REPORTS

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Stress fracture of the femoral neck after the Pipkin type IV hip injury

Stres prelom vrata butne kosti nakon zadnjeg iščašenja kuka – tip IV po Pipkinu

Nemanja Gvozdenović*†, Srdjan Ninković*†, Mladen Jovanović*‡, Dušica Marić*

University of Novi Sad, *Faculty of Medicine, Novi Sad, Serbia; Clinical Center of Vojvodina, †Department for Orthopaedic Surgery and Traumatology, ‡Department for Plastic and Reconstructive Surgery, Novi Sad, Serbia

Abstract

Introduction. Hip fractures/dislocations of Pipkin type IV are rare, often accompanied by complications and poor outcome. We describe a complication in the form of a stress fracture of the femoral neck (SFOFN) after the Pipkin type IV fracture – dislocation of the hip. **Case report.** A healthy male, TAXI driver, aged 60, was injured in a traffic accident and admitted as a polytraumatised patient with the Pipkin type IV hip injury. Open reduction and internal fixation had been done. Completely recovered, 9 months after the injury during a walk he felt pain in the operated hip and was unable to bear weight. We noted a dislocated subcapital SFOFN which did not form on the site of the previous osteosynthesis. **Conclusion.** Pipkin type IV hip injury as a result of polytrauma, unstable joint and osteosynthesis, inadequate weight bearning and disposal of physical therapy, increases the risk of complications such as avascular necrosis, or as in our case, a stress fracture (caused by weight overload).

Key words:
hip dislocation; hip fracture; multiple trauma; fractures, stress; orthopedics; reoperation.

Apstrakt

Uvod. Povrede kuka (fraktura-luksacija) Pipkin tipa IV su retke, često praćene komplikacijama i lošim krajnjim rezultatom. Opisujemo slučaj komplikacije lečenja frakture-luksacije kuka tipa Pikin IV u vidu stres preloma vrata butne kosti (SFOFN). **Prikaz bolesnika.** Taksista star 60 godina, inicijalno zdrav, povređen je u saobraćajnom udesu kao politraumatizovan, sa zadnjim iščašenjem kuka i prelomom zadnjeg zida acetabuluma i glave butne kosti, tipa Pipkin IV. Odmah je operativno zbrinut – načinjena je otvorena repozicija kuka i osteositeza ulomaka solitarnim zavrtnjevima. Poptuno oporavljen, devet meseci nakon inicijalne povrede u toku hoda osetio je bol u predelu operisanog kuka i nemogućnost oslonca. Konstatovana je dislokacija i supkapitalna SFOFN koji nije nastao na mestu prethodne osteosinteze. **Zaključak.** Pipkin tip IV povreda kuka nastala u politraumi, nestabilan kuk i osteosinteza, kasno ili prerano davanje oslonca i odlaganje fizikalne terapije povećavaju mogućnost nastanka komplikacija, u vidu avaskularne nekroze ili kao u našem slučaju, stres preloma (preloma preopterećenja).

Ključne reči:
kuk, iščašenje; kuk, prelom; povrede, multiple; prelomi usled zamora; ortopedske procedure; reoperacija.

Introduction

Traumatic hip dislocations are usually caused by high energy force and occur often in polytrauma patients [1]. There are several classifications of hip dislocation set by different authors and all of them are based on the direction of hip dislocation [2]. Posterior hip dislocations constitute about 90% of all traumatic dislocations and usually occur in traffic accidents [2]. Pipkin classification is generally accepted and it is the most commonly used classification of hip dislocation associated with fractures of the upper part of the femur [2–4]. According to Pipkin, type I represents a dislocation associated with the fracture of the femoral head under the *fovea centralis capitis*; type II represents a hip dislocation associated with the fracture of the head above the *fovea centralis*; type III represents type I and type II associated with femoral neck fracture; type IV represents type I and type II associated with the fracture of acetabulum. The higher Pipkin classification grade is, the final treatment results become worse as well as the complication rate [4].

Frequency of complications and final functional outcome are affected by the associated bone lesions and the time passed from the dislocation to the reposition of the dislocated hip joint, and therefore, the reposition represents an urgent orthopedic procedure [3,4].

Correspondence to: Nemanja Gvozdenović, University of Novi Sad, Faculty of Medicine, Clinical Center of Vojvodina, Department for Orthopaedic Surgery and Traumatology, Hajduk Veljkova 1-7, 21 000 Novi Sad, Serbia. E-mail: nemanja.gvozdenovic@mf.uns.ac.rs

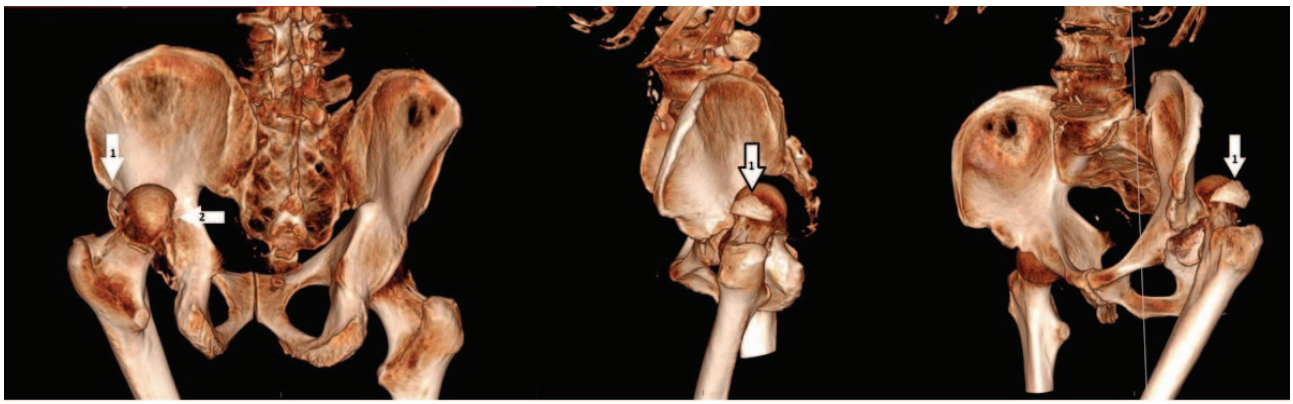

Fig. 1a – Computed tomography 3D reconstruction of the pelvis with both hips – the left hip without initial fracture of the femoral neck.
Arrow 1: Fracture of the posterior acetabular wall; Arrow 2: Fracture of the femoral head.

Complications of the hip fracture/dislocations can be: early – interruption of the vascularization of the femoral head due to the damage of the blood vessels of the femoral neck, injury of the sciatic nerve, infection, inability to perform reposition; late – avascular necrosis (AVN), post-traumatic osteoarthritis and heterotopic ossification [4].

The main goal of this case report was to show SFOFN as complication after the Pipkin type IV hip injury without any previous clinical or radiological signs.

Case report

A 60-year-old male TAXI driver without previous comorbidities was injured in a traffic accident as a driver and was taken immediately to the Emergency Center of the Clinical Centre of Vojvodina (ECCCV) for treatment.

At the reception, the patient was unstable and advanced trauma life support measures were applied immediately. Initial diagnosis was polytrauma with the Injury Severity Score of 22. The patient had injuries of the face, tounge and chest (pneumothorax with rib fractures, II-V right and II-VII left) as well as the posterior dislocation of the left hip associated with the fracture of the posterior acetabular wall and fracture of the femoral head (Figures 1, a and b).

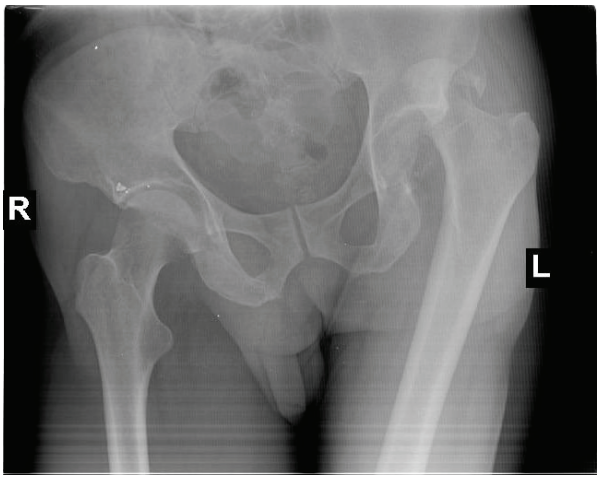

Fig. 1b – Antero-posterior pelvis radiography film: left hip fracture/dislocation of Pipkin type IV.

Pneumothorax and face injuries were resolved immediately by the maxillofacial and general surgeons. Closed reposition of the hip was done under general anesthesia. The position and the stability of the hip was maintained by the transtibial traction with the load of 6 kg. On the third day, after the patient's general condition stabilized, using posterior (Koher-Langebeck) approach, osteosynthesis of the posterior acetabular wall and the femoral head were done with two screws each (Figure 2).

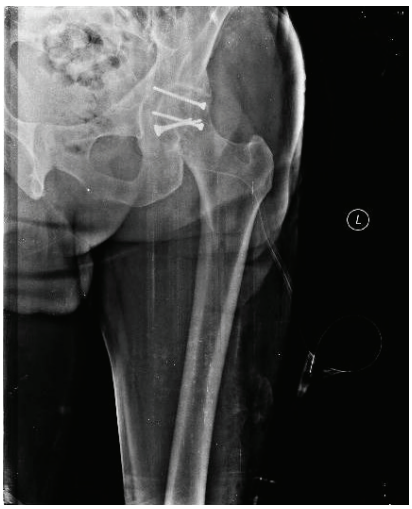

Fig. 2 – Antero-posterior left hip joint radiography film after the open reduction and internal fixation with solitary screws.

Verticalization and physical therapy began immediately, on the first day after the surgery. Full weight bearing on the leg was accomplished 3 months later with monthly controls and radiographic verifications of the progress of the fracture sanation (Figure 3). After the end of the physical therapy the patient returned to his daily activities and job fully functionally recovered. Six months later, respectively 9 months after the injury, the patient felt intense hip pain in the previously operated leg while walking and was unable to bear weight. He was taken to the ECCCV and dislocated subcapital stress fracture of the left femoral neck was diagnosed (Figures 4, a and b).

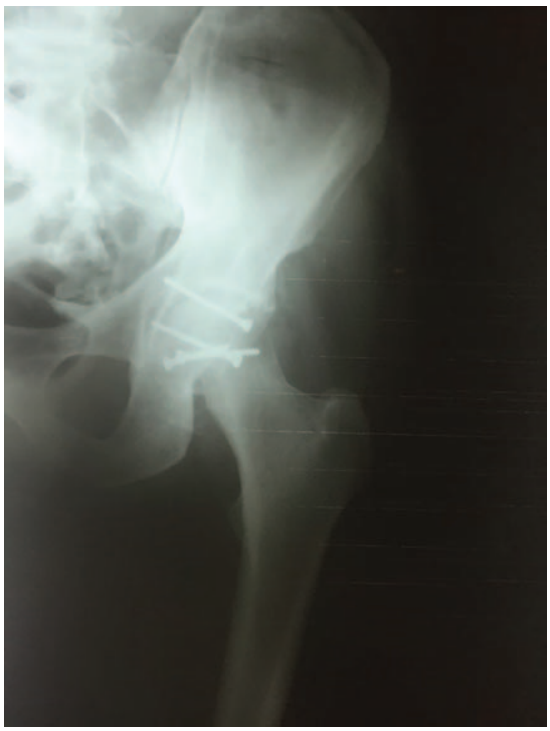

Fig. 3 – Radiography 3 months after the injury – full sanation of fractures without avascular necrosis.

After preoperative preparations, a total hip replacement with cement fixation was done (Figure 5). Intraoperatively, a complete recovery of acetabular and femoral head fractures with no signs of strong arthrosis were diagnosed but with softening of the bone tissue in the femoral neck which, presumably had enabled the occurrence of SFOFN as a complication of the primary treatment of the dislocation.

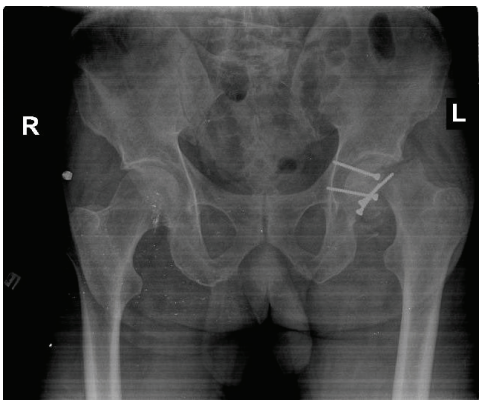

Fig. 4a – Antero-posterior pelvis radiography film made 9 months after the injury after sudden pain in the hip: subcapital stress fracture of the neck of the left femur at the place with no previous fracture.

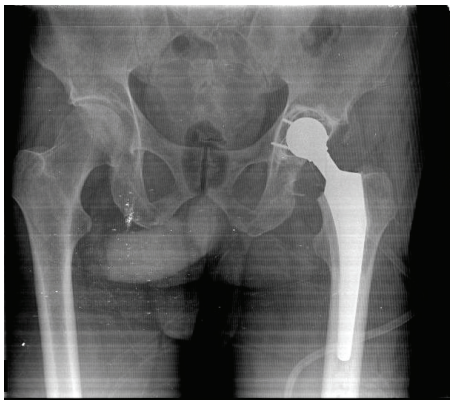

Fig. 5 – Final result – antero-posterior pelvis radiography: total hip replacement with cement fixation on the left hip after a stress fracture of the femoral neck.

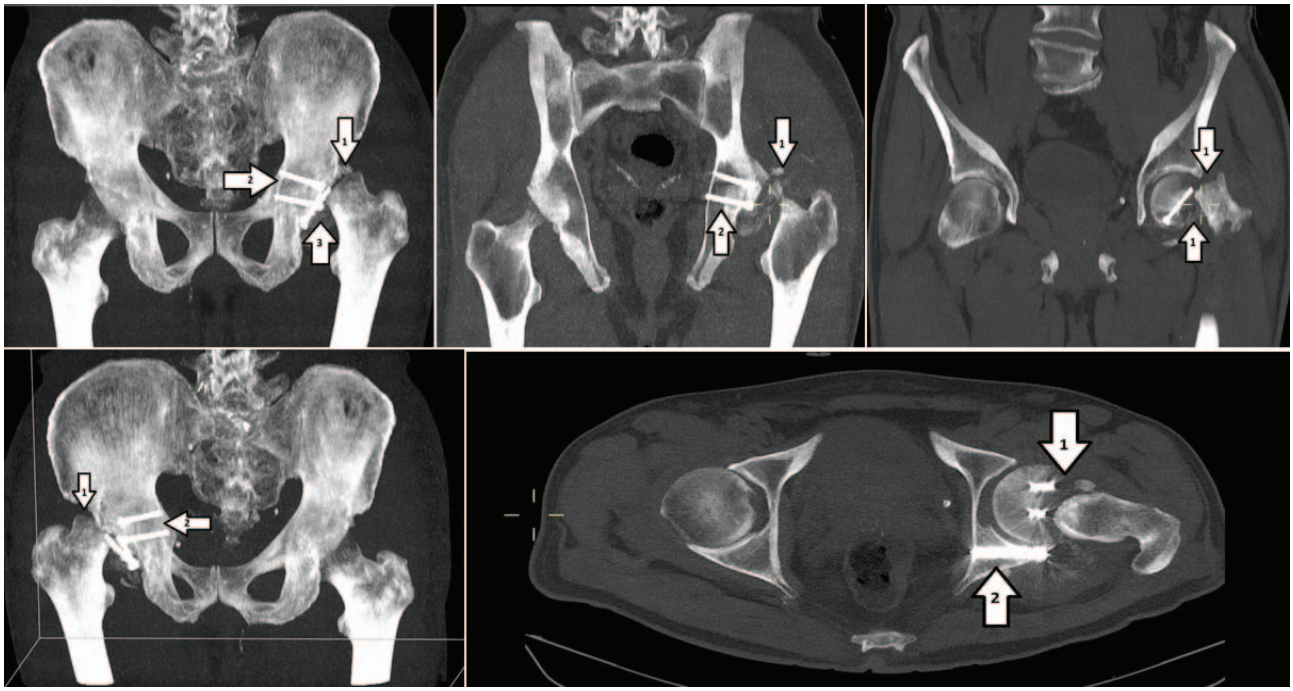

Fig. 4b – Computed tomography 3D reconstruction of the pelvis with both hips: stress fracture of the left femoral neck. Arrow 1: Stress fracture of the femoral neck; Arrow 2: Healed acetabular fracture – posterior wall; Arrow 3: Healed fracture of the femoral head.

Discussion

Traumatic hip dislocation usually occurs with high intensity force in traffic accidents in younger peope [1, 2]. Giannoudis et al. [4] noted that almost 50% of the patients with the Pipkin type IV fracture dislocations had bad final outcomes developing some of the complications.

Although we applied all recommended methods for a hip fractures/dislocations treatment, the patient developed a complication in the form of SFOFN.

Stress fractures are definied as the spontaneously occurred fractures caused by the weight overload. They usually occur in the lower leg and the foot in middle-aged women. They may also be caused by the repetitive mechanical stress, amenorrhoea, nutritional disorders, osteoporosis, rheumatoid arthritis, the Paget disease, hyperparathyroidism, steroid therapy and pregnancy [5, 6]. According to Devas 5, there are two types of SFOFN: type I – transversal fracture (occurs in younger people, usually caused by the repetitive mechanical stress) and tends to be complicated by dislocation and type II – compressive fracture (usually occurs in elderly people with systemic diseases with no tendency for dislocation) [5]. Our patient was not a diabetic and without any other disease or pathological condition as a predisposition for this complication. We assume that in our case SFOFN occurred due to the local softening of the bone tissue (intraoperative findings) as a consequence of an early stage of AVN, or local osteopenia caused by insufficient activity and weight bearning of the injured extremity. Lee and Suh [6] published the case of Type I SFOFN that occurred in a heavy alcohol drinking woman after the development of the aseptic necrosis of the femoral head. Glimcher and Kenzora [7] explained the occurrence of SFOFN together with AVN of the femoral head as a result of the difference between the elastic moduli and the compliance of the two bone types. According to these authors, the stress fracture occurs in subhondral necroticly altered bone or at the junction between necrotic and emerging reparative bone. Our patient also had dislocated SFOFN type I. Vinod et al. [8] published a case report with similar type of subcapital neck fracture (radiological characteristics) calling it „unclassified type of neck fracture“, but their patient had a sure sign of AVN. Even though the occurrence of AVN is characteristic after the type IV fracture of the acetabulum, in our case, there were no previous radiological indicators, nor did the patient had any symptoms that would indicate the development of AVN of the femoral head.

AVN of the femoral head occurs in about 5%–53% of cases of posterior dislocations of the hip as a late complication. Its occurrence is affected by the time of reposition, associated injuries, injuries caused by the influence of the "high energy force" and injuries of the blood vessels responsible for feeding the hip joint [2]. The initial, so called "asymptomatic stage" of the occurrence of AVN was characterized by an absence of pain and normal bone architecture on radiography [7]. A definite diagnosis of AVN (based on radiography film) of the femoral head usually occurs 2 years after the initial injury when the hip joint becomes painful and also when the radiological changes of the femoral head developed and are clearly visible [7, 9, 10]. In our case, the patient did not have any symptoms, he had full recovery with no signs of development of AVN of the femoral head and neck on the control radiography (which did not exclude its presence, because it had been 9 months since the initial injury).

Borschmann et al. [9] noticed in their research an increased incidence of SFOFN based on the osteopenic bone in the patients who were in bed for a long time and who did not immediately begin physical therapy. The higher incidence of stress fractures based on osteopenic bone was also confirmed by Myburgh et al. [11] in their research conducted on the athletes. Our patient did not bear weight on the injured leg for 3 months because of the fracture sanation, but the physical therapy began immediately and was perfomed for 6 months till full recovery. The relevant literature data indicate that there must be a minimum loss of 30%–50% of the bone before it can be detected by radiography [9, 11]. In our case, during the control radiological examinations, any radiological presence of osteopenia was not observed, which was later confirmed intraoperatively. We assume that osteopenia in the femoral head and neck has facilitated the occurrence of the SFOFN. Treatment of dislocated SFOFN is always surgycal, usually by a total hip replacement which we did in our case [5, 6].

Limitations of this case report are that we did not make the postoperative magnetic resonance imaging (MRI) of the hip and the dual-energy radiography absorptiometry (DEXA) examination. The MRI was not done because of earlier implantation of iron screws and complete recovery of the patient (no doubt about the development of complications). The DEXA examination for the detection of osteoporosis was not done considering the injured patient was a middle-aged male previously healthy with no comorbidities.

Conclusion

The Pipkin type IV hip fracture/dislocations as a part of polytrauma, late reduction of the hip, unstable osteosynthesis, too late or too early weight bearing and disposal of physical therapy increase the possibility of occurrence of complications, like avascular necrosis and stress fracture of femoral neck caused by the local osteopenia, and that is how we would explain this case.

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CASE REPORT

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Tracheal localization of inflammatory myofibroblastic tumor in adults: A case report

Trahealna lokalizacija inflamatornog miofibroblastnog tumora kod odraslih

Branislav Oluić*, Radomir Vešović[†], Zlatibor Lončar*[‡], Jelena Stojšić[§], Nataša Mujović[‡‖], Dejan Nikolić[‡¶]

Clinical Center of Serbia, *Emergency Center, [†]Clinic for Thoracic Surgery, [§]Service of Histopathology, [‖]Clinic for Physical Medicine and Rehabilitation, Belgrade, Serbia; University of Belgrade, [‡]Faculty of Medicine, Belgrade, Serbia; University Children's Hospital, [¶]Physical Medicine and Rehabilitation Department, Belgrade, Serbia;

Abstract

Introduction. Inflammatory myofibroblastic tumor (IMT) is a rare neoplasm. This disease, of unknown etiology, runs an unpredictable course. Its definitive diagnosis calls for a detailed histopathological analysis including immunohistochemistry. Microscopically, IMT is composed of myofibroblastic spindle and inflammatory cells in different proportions. It presents infrequently in adults with nonspecific symptomatology. The presence of IMT is described in every anatomical region but the tracheal one is especially uncommon. **Case report.** A 41-year-old female patient checked into our institution due to exacerbation of asthma-like symptoms such as shortness of breath, cough and exertion intolerance. She was originally treated as the asthmatic patient with the bronchodilator therapy with no success. Chest x-ray done during one of the outpatient follow-up appointments pointed to a suspected change in the tracheal distal part. After her admission to our institution, the following diagnostic procedures were performed: spirometry, chest computed tomography (CT) scan, chest magnetic resonance imaging (MRI) and bronchoscopy and the change in tracheal distal third was confirmed. Right-sided thoracotomy with mobilization of lung, tracheal resection and termino-terminal (T-T) anastomosis was undertaken. Subsequent histopathological analysis of surgically removed afflicted tracheal part of them trachea including immunohistochemistry enabled us to definitively of diagnose IMT. Four years after surgical resection, the patient showed no recidivism of illness. **Conclusion.** Definitive IMT diagnosis requires the detailed diagnostic tests, most importantly, an adequate histopathological analysis including immunohistochemistry**.** Complete surgical resection is the treatment of choice in case of IMT. Further monitoring of patients is necessary due to a risk of recurrence.

Key words:
diagnosis; immunohistochemistry; inflammation; surgical procedures, operative; thoracotomy; tracheal neoplasms.

Apstrakt

Uvod. Inflamatorni miofibroblastni tumor (IMT) je retka neoplazma. Bolest je nepredvidivog toka i nejasne etiologije i za njenu definitivnu dijagnozu je potrebna detaljna patohistološka analiza uz primenu imunohistohemije. Mikroskopski, IMT čine miofibroblastne vretenaste i inflamatorne ćelije u različitom odnosu. Ređe se viđa kod starijih osoba i nespecifične je simptomatologije. Opisano je prisustvo IMT-a na svim anatomskim lokalizacijama, a trahealna lokalizacija je veoma retka. **Prikaz bolesnika.** Bolesnica stara 41. godinu javila se u našu ustanovu zbog progresije simptoma sličnih astmi u vidu otežanog disanja, kašlja i brzog zamaranja. Ranije je lečena bronhodilatatornom terapijom bez uspeha. Na kontrolnim ambulantnim pregledima radiografijom grudnog koša uočena je suspektna promena u distalnom delu traheje. Nakon prijema u našu ustanovu učinjene su dijagnostičke metode – spirometrija, kompjuterizovana tomografija (CT) grudnog koša, nuklearna magnetna rezonanca (NMR) i i bronhoskopija. Navedene dijagnostičke pretrage su potvrdile postojanje promene u distalnoj trećini traheje. Učinjena je desna torakotomija sa mobilizacijom pluća i resekcijom traheje i termino-terminalnom (T-T) anastomozom. Patohistološkom analizom operativnog materijala, uz primenu imunohistohemije, postavljena je dijagnoza IMT-a. Bolesnica je četiri godine nakon operacije bile bez recidiva bolesti. **Zaključak.** Za postavljanje dijagnoze IMT-a potrebne su detaljne dijagnostičke pretrage, posebno adekvatna patohistološkom analiza sa imunohistohemijom. Metod izbora u lečenju IMT-a je kompletna hirurška resekcija. U cilju detekcije mogućih recidiva neophodne su dalje kontrole.

Ključne reči:
dijagnoza; imunohistohemija; zapaljenje; hirurgija, operativne procedure; torakotomija; traheja, neoplazme.

Correspondence to: Radomir Vešović, Clinical Centre of Serbia, Clinic for Thoracic Surgery, Koste Todorovića 26, 11 000 Belgrade, Serbia. E-mail: rvesovic@gmail.com

Introduction

Inflammatory myofibroblastic tumor (IMT) is an infrequent mesenchymal tumor of unclear etiology and more frequent occurrence among children and young adults in the first two decades of life [1, 2]. The most common incidence is in the lungs as peripheral nodes, while its manifestation in the trachea is extremely rare. According to the World Health Organization (WHO), IMT is a lesion composed of myofibroblastic spindle cell population accompanied by an inflammatory infiltrate of plasma cells, lymphocytes, and eosinophils [2] and its diagnosis depends on relevant histopathological analysis. Before its definitive diagnosis, a detailed histopathological analysis, which includes an immunohistochemistry, is necessary [3, 4]. Even though it was previously considered a benign ailment, the latest research indicates its recurrence potential or malignant nature, depending on the proliferative index (Ki-67) [5]. The surgical resection is the method of choice in its treatment with the excellent chance of patient survival [1]. We reported a 41-year-old female patient with IMT of the trachea.

Case report

A 41-year-old female patient was admitted to our institution due to the exacerbation of asthma-like symptoms. In the months prior to admission, she experienced difficulties of breathing, cough without sputum and exertion intolerance. The worsening of those symptoms over time and their appearance even at rest were noted. She was originally treated with bronchodilator therapy with no effects. During one of the outpatient follow-up visits, the chest x-ray showed a change in the distal left wall of the trachea (Figure 1) and she was referred to our hospital.

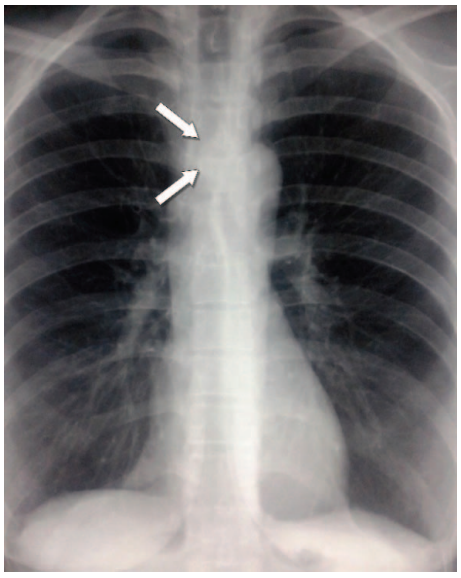

Fig. 1 – Chest X-ray showing a lesion on the left distal wall of the trachea (arrows).

Upon admission, the pulmonary function test parameters informed of the severe obstructive ventilatory defect of the upper respiratory tract forced expiratory volume (FEV1) – 32%, forced vital capacity (FVC) – 84%, Tiffenean (Tiff) – 29%.

The laboratory analysis values fell within their respective reference intervals. The computed tomography (CT) of the thorax exposed semicircular thickening of the size 32 x 15 mm at the level of the distal third of trachea, above bifurcation, that significantly reduced the tracheal lumen (Figure 2).

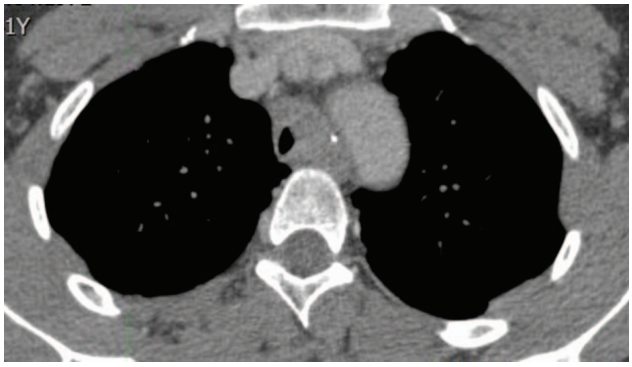

Fig. 2 – Computed tomography showing a mass on the left tracheal wall with intraluminal propagation.

Subsequently, the chest magnetic resonance imaging (MRI) revealed that on a distance of approximately 16 mm from the tracheal carina, laid a tumor that filled the lumen almost in its entirety (free lumen is approximately 8 x 4 mm in magnitude) and its dimensions were 11 x 19 x 33 mm (Figure 3).

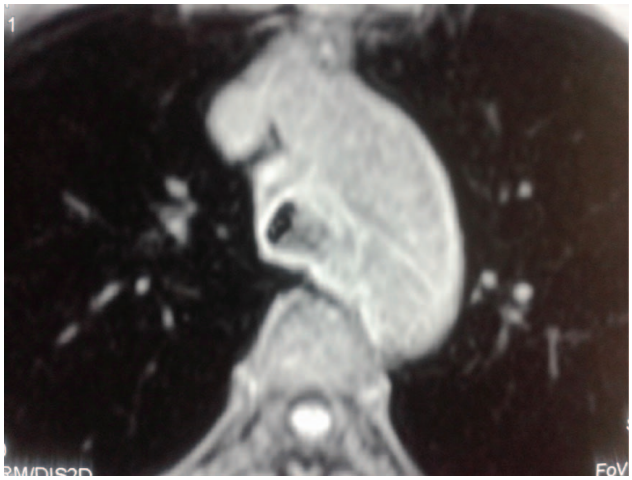

Fig. 3 – Magnetic resonance imaging scan demonstrating a tumor of the left lateral tracheal wall, with lumen reduction.

Repeated bronchoscopy disclosed the tumorous lesion on the XIII tracheal ring that blocked two thirds of tracheal lumen (Figure 4). The histopathological findings revealed chronic inflammation and squamous metaplasia of respiratory epithelia of trachea only.

After completed the preoperative procedure, right-sided thoracotomy was performed with mobilization of lung and resection of trachea in the length of approximately 3 cm and termino-terminal (T-T) anastomosis.

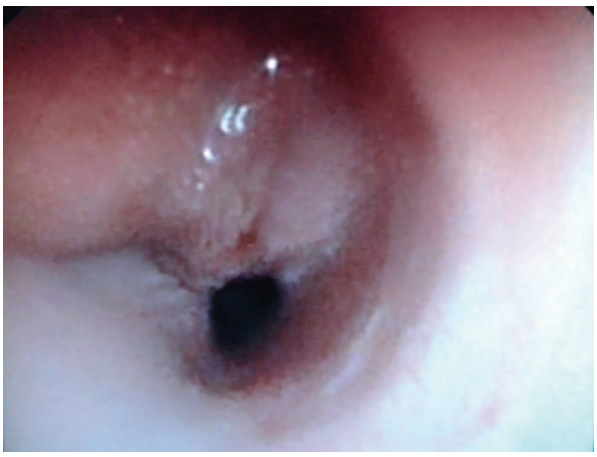

Fig. 4 – Bronchoscopy showing a stenosis of the trachea as a result of a tumor mass at the level of the XIII tracheal ring.

Microscopically, the nonencapsulated inflammatory myofibroblastic tumor contained a mixture of spindle cells arrayed in the fascicles or arranged in a storiform pattern.

These cells with the oval nuclei and inconspicuous nucleoli had abundant lightly eosinophilic cytoplasm. Mitoses and cytologic atypia were not found. Admixed with the spindle proliferation, there was an inflammatory infiltrate containing a numerous lymphocytes and a prominent number of plasma cells associated with lymphoid follicles. Histiocytes were also obtained including some Touton type giant cells (Figures 5a and 5b). Immunohistochemically, all of tumor cells expressed vimentin (Figure 5c) and some of them smooth-muscle-actin (SMA) (Figure 5d). The anaplastic lymphoma kinase (ALK) expression was detected in some tumor cells (Figure 5e). Cytokeratin-AE1/AE3 was not expressed, excluding the spindle cell type of lung carcinoma, and, in like manner, S-100 protein, excluding neuroectodermal tumor. Ki67 was not found in any of the examined tumor cells.

The postoperative course proceeded with no complications. The patient was released from the hospital on the 10th postoperative day. From there on, she has been undergoing the regular follow-up visits and four years after the procedure, the patient shows no recidivism of the illness.

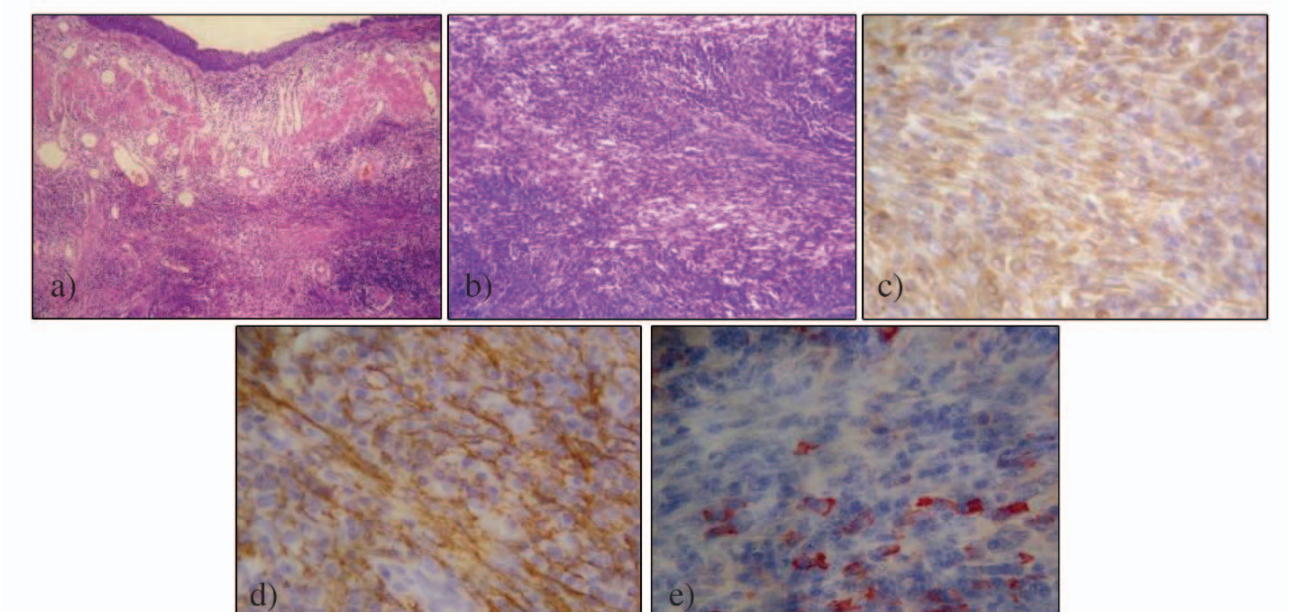

Fig. 5 – a) Nonencapsulated proliferative cells are present in deeper layers of the tracheal wall. Respiratory epithelia is with squamous metaplasia [hematoxylin and eosin (HE) stain (×10)]; b) Mixture of small spindle cells with prominent, hyperchromatic nuclei and histiocytes with clear nuclei intermingled with lymphocytes and plasma cells (HE, ×20); c) Small spindle cells express, Vimentin (×40); d) A majority of cells express smooth-muscle-actin (SMA, ×40); e) The anaplastic lymphoma kinase (ALK-P) expression in some cell is specific for the myofibroblastic lung tumor (ALK-P, ×40).

Discussion

IMT is a rare mesenchymal tumor most commonly located in the lungs making only 0.04% of all lung tumors [1], while its presence in the trachea is exceptionally uncommon [6, 7].

Etiology of this tumor is uncertain. Explanations provided for the IMT development are the following: an inflammatory reaction as a result of a trauma, an autoimmune reaction or an infection. However, in the majority of reported IMT cases, no trauma or infection was detected prior to the diagnosis [8, 9]. As of late, IMT is widely recognized as a mesenchymal tumor with a low malignant potential in view of its observed characteristics: local recidivism and invasion, metastasis, malignant transformation and certain genetic transformation such as chromosome translocation ALK genes in reported cases [5, 10].

IMT of respiratory organs is the most frequently related to an ambiguous symptomatology involving difficulties with breathing, cough and fatigue [11]. Dyspnea as the most com-

mon tracheal IMT clinical symptom is manifested early in the course of illness because of the tracheal obstruction [10].

However, forming the correct diagnosis is usually delayed when symptoms are as vague and common and having in mind the low illness incidence [3].

IMT of trachea is generally located in the distal third of trachea [12].

Identifying an IMT preoperatively is hindered because of its cellular pleomorphism feature even after an adequate bronchoscopy [11, 13]. Its diagnosis is ordinarily being confirmed after the surgical resection [13]. The above argument also demand an immunohistochemistry analysis [3, 4].

Upon the morphological pattern and immunohistochemistry results, we diagnosed IMT. Until the WHO 2004 lung tumor classification, inflammatory myofibroblastic tumor of the lung was categorized as an "inflammatory pseudo tumor" [14]. Characteristic morphology of nonencapsulated tumor is a mixture of spindle cells in fascicular and storiform pattern and collagen, accompanied with lymphocytes, plasma cells and histiocytes. Using the vimentin expression, we confirmed mesenchimal proliferation and using the focal SMA expression we confirmed the myofibroblastic origin of tumor. Absence of S-100 proliferation excluded the neuroectodermal origin of tumor and the absence of Cytokeratin-AE1/AE3 expression allowed exclusion of spindle cell carcinoma, although according to the literature their presence could be observed in some IMTs. Its focal reactivity is potentially explained by the alveolar entrapment. We did not find the Ki67 expression and that could be a good prognostic sign that we were not supposed to expect the local tumor recurrence after the tumor was removed completely. We were guided by the IMT diagnosis recommendation both of the 2004 and the more recent 2015 WHO classification of lung tumors [14, 15]. In the described tumor, ALK was expressed in some cells. Cessna et al. [16] in 2002 found that 40% of IMTs expressed ALK in some of tumor cells.

Since IMT could immitate other lesions such as lymphroproliterative diseases, certain expert opinions call for a histopathological confirmation before the surgical resection that could shed light on whether less invasive procedure like the endoscopic removal can be implemented. Nonetheless, it is widely accepted that in the case of tracheal IMT with the transmural propagation or recidivism, the complete surgical resection of the afflicted part of the trachea is recommended [3, 4, 7, 11, 12].

Due to the small number of reported cases of tracheal IMT, till 2013 (there were only 11 recorded cases of tracheal IMT in adults), we cannot say anything definitive about its recidivism rate [8]. This is the first reported case of tracheal IMT in adults in our hospital.

Radiotherapy is being used in the patients when surgery is not applicable, or they are inoperable with limited results [3, 4, 12].

In the course of the IMT treatment, beside the surgical resection as the method of choice, corticosteroids are prescribed as well. The results of their use vary, from the ineffective to complete illness remission [2, 17, 18]. The adoption of chemotherapy in the IMT treatment did not produce satisfactory results [19].

After the complete IMT removal, the prognosis is usually excellent, and with several recidivism cases being reported, subsequent regular follow-ups of patients are recommended [7, 12].

Conclusion

Inflammatory myofibroblastic tumor is rarely thought of, considering its vague clinical course and nonspecific radiographic findings. It cannot be ignored especially in the pediatric population. Surgical resection is the method of choice in the treatment of inflammatory myofibroblastic tumor. When tumor is resected in its entirety, this method yields an excellent rate of patient survival. The histopathological examination and diagnosis supported by immunohistochemistry are necessary to determine the histological type of tumor and its biological behavior. Other treatment techniques (chemotherapy, radiotherapy, corticosteroids) are less frequently used and with the lower success rates. Further monitoring of patients is necessary in order to detect recidivism.

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CASE REPORT

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Clinical characteristics of hereditary hemorrhagic telangiectasia – case series and review of the literature

Kliničke karakteristike nasledne hemoragijske telangiektazije – prikaz serije bolesnika i pregled literature

Dragan Popović*†, Aleksandra Sokić-Milutinović*†, Srđan Djuranović*†, Tamara Alempijević*†, Sanja Zgradić†, Vera Matović‡, Ljubiša Tončev†, Snežana Lukić*†

University of Belgrade, *Faculty of Medicine, Belgrade, Serbia; Clinical Centre of Serbia, †Clinic for Gastroenterology and Hepatology, ‡Emergency Center, Belgrade, Serbia

Abstract

Introduction. Hereditary hemorrhagic telangiectasia (HHT) is a rare autosomal dominant disorder with estimated prevalence of one in 5,000 to 10,000. The disease has age-related penetrance and the HHT signs and symptoms occur and worsen with age. A diagnosis of HHT is based on the Curacao`s criteria. **Case report.** We report a case series of 6 patients diagnosed with HHT, 5 with definite and one with probable diagnosis according to the Curacao criteria. In 5 patients, the recurrent epistaxis occurred in adolescence as the first presentation while one patient presented with melena. The diagnosis was delayed in 5 patients and the presence of HHT was diagnosed during or after the fifth decade. In 4 patients, the overt gastrointestinal bleeding occurred in the later course of the disease. The asymptomatic pulmonary circulation arteriovenous malformations were detected in 2 patients. The cerebral arteriovenous malformations were not detected. **Conclusion.** Hereditary hemorrhagic telangiectasia is a rare disorder affecting multiple organs. It should be considered in the adolescents with recurrent epistaxis and in the differential diagnosis of anemia with signs of the gastrointestinal bleeding in order to shorten the delay in the diagnosis and subsequently improve the outcome of the disease.

Key words:
telangiectasia, herediatary hemorrhagic; diagnosis, differential; hemoptysis; digestive system; hemorrhage; arteriovenous malformation.

Apstrakt

Uvod. Nasledna hemoragijska teleangiektazija (HHT) je redak autosomno-dominantni poremećaj sa prevalencijom javljanja 1 na 5,000 do 10,000. Bolest je uzrasno zavisna i HHT simptomi i znaci se rano javljaju i pogoršavaju sa godinama. Dijagnoza se postavlja na osnovu Curaco kriterijuma. **Prikaz bolesnika.** Ovo je serijski prikaz šest bolesnika sa HHT, pet sa definitivnom i jednog sa verovatnom dijagnozom HHT na osnovu Curaco kriterijuma. Kod pet bolesnika ponavljane epistakse su se javile u adolescentnom dobu kao prva manifestacija bolesti, dok je prvi znak bolesti kod jednog bolesnika bila melena. Kod pet bolesnika dijagnoza HHT postavljena je tek u toku i nakon pete decenije života. Kod četiri bolesnika manifestno gastrointestinalno krvarenje se javilo u daljem toku bolesti. Asimptomatske arteriovenske malformacije plućne cirkulacije uočene su kod dva bolesnika. Cerebralne arteriovenske malformacije nisu uočene ni kod jednog bolesnika. **Zaključak.** Nasledna hemoragijska teleangiektazija je retka bolest koja zahvata više organa. Na ovu bolest bi trebalo misliti kada adolescent imaju ponavljane epistakse i diferencijalno dijagnostički kod anemija sa znacima gastrointestinalnog krvarenja, a u cilju pravovremenog postavljanja dijagnoze bolesti i poboljšanja ishoda iste.

Ključne reči:
teleangiektazija, nasledna, hemoragijska; dijagnoza, diferencijalna; hemoptizije; gastrointestinalni sistem; krvarenje; arteriovenske malformacije.

Introduction

Hereditary hemorrhagic telangiectasia (HHT), also known as the Rendu-Osler-Weber disease is a rare autosomal dominant disorder with estimated prevalence of one in 5,000 to 10,000 [1–3]. A diagnosis of HHT is based on the Curacao`s criteria published in 2000 [4] that include: epistaxis (spontaneous recurrent nosebleed); multiple telangiectasias at the

Correspondence to: Snežana Lukić, Clinical Center of Serbia, Clinic for Gastroenterology and Hepatology, Koste Todorovića 6, 11 000 Belgrade, Serbia. E-mail: lukic.snezana@gmail.com

characteristic sites (lips, oral cavity, fingers, nose); presence of visceral lesions (gastrointestinal telangiectasia, pulmonary, hepatic, cerebral, spinal arteriovenous malformations – AVM); 1st degree relative with HHT.

The diagnosis of HHT is definite if 3 criteria are fulfilled, possible or suspected in the presence of 2 criteria and unlikely if less than 2 criteria are seen in the patient [4].

Case report

We report a case series of 6 patients, 5 with definitive and one with suspected HHT.

The majority of the patients (5 out of 6) had the recurrent epistaxis in their early childhood or adolescence as the first presentation, while one patient initially presented with melena. In 5 patients, the diagnosis was delayed and the presence of HHT was diagnosed in the adulthood (during or after the fifth decade), although the signs were present from the adolescence. In 4 patients, the overt gastrointestinal bleeding occurred in the later course of the disease. Three patients had melena and one patient hematemesis. Clinical findings are presented in Table 1.

Upon admission, physical examination confirmed the presence of mucocutaneous telangiectasia in all patients.

The lowest hemoglobin level at admission was 14 g/L and the highest one was 95 g/L, while the mean value was 67.5 g/L. The lowest value of mean corpuscular volume (MCV) in the time of admission was 52 fL, the highest 82 fL, and the median 71 fL. The serum iron was in the range of 1.9 μmol/L to 15.7 μmol/L, with a mean value of 5.2 μmol/L.

All patients were treated with intravenous iron supplementation while blood transfusion was indicated in 5 patients. Three patients received multiple transfusions and were classified as transfusion dependent. One patient had one blood transfusion weekly until hemoglobin reached 90.6 g/L.

Three patients underwent cauterization of nasal septum varices with acetic acid due to the repeated episodes of epistaxis.

We performed the head and paranasal sinuses computed tomography (CT) in 2 patients and the cerebral arteriovenous malformations (AVM) were not diagnosed, but nasal mucosa polyposis in the right nostril was diagnosed in 2 and bilateral edema of lower turbinate (concha) in one patient. In both patients, arteriography with embolization of nasal blood vessels was suggested aiming to decrease frequency and intensity of epistaxis. In one patient, embolization was successfully performed twice, leading to the reduced number of epistaxis episodes (Figure 1). The other patient did not accept the suggested procedure.

Table 1

Clinical findings in hereditary hemorrhagic telangiectasia (HHT) patients

Patient No	Sex F/M (age)	Epistaxis	Gastrointestinal bleeding	MCT	Hgb (g/L)	Diagnosis at admission	Curacao criteria (n)	Treatment	Improvement
1	F (54)	+	+	+	87	HHT	3	BT, APC, embolization	Yes
2	F (50)	+	-	+	14	Anemia	3	BT, APC cauterization	Yes
3	F (62)	-	+	+	95	HHT	2	BT, APC	Yes
4	M (70)	+	-	+	48	HHT	3	BT, APC	Yes
5	M (57)	+	+	+	70	Anemia	3	BT	Yes
6	M (62)	+	+	+	91	HHT	3	BT, APC	Yes

F – female; M – male; MCT – mucocutaneous telangiectasia; Hgb – hemoglobin at admission; BT – blood transfusion; APC – argon plasma coagulation.

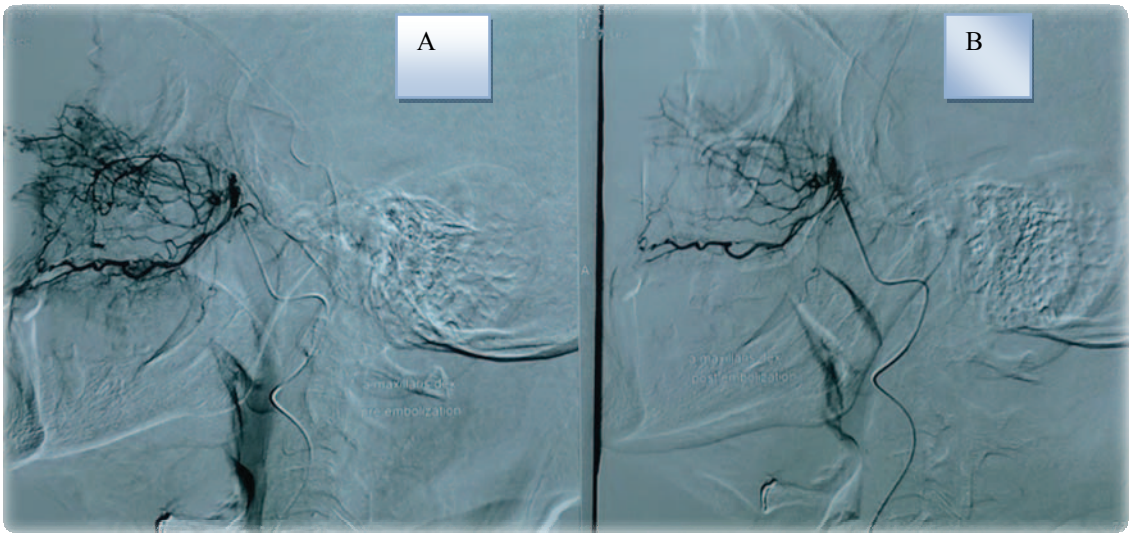

Fig. 1 – (A) Before and (B) after embolisation of right maxillary artery.

Esophagogastroduodenoscopy revealed the presence of esophageal in one and gastric telangiectasia in all patients, while 5 patients had duodenal telangiectasia. In 4 patients, a colonoscopy revealed the presence of telangiectasia in the cecum and on the ileocolonic valve (Figure 2). In 2 patients double balloon endoscopy was performed and jejunal telangiectasia was diagnosed. Five patients underwent endoscopic argon plasma coagulation (APC), and in one patient the procedure was repeated, but a significant reduction in the transfusion frequency was not achieved since the lesions were diffuse. In this patient with a gastrointestinal (GI) bleeding as the first presentation of HHT and repeated unsuccessful APC, a treatment with thalidomide was suggested, but informed consent was not obtained.

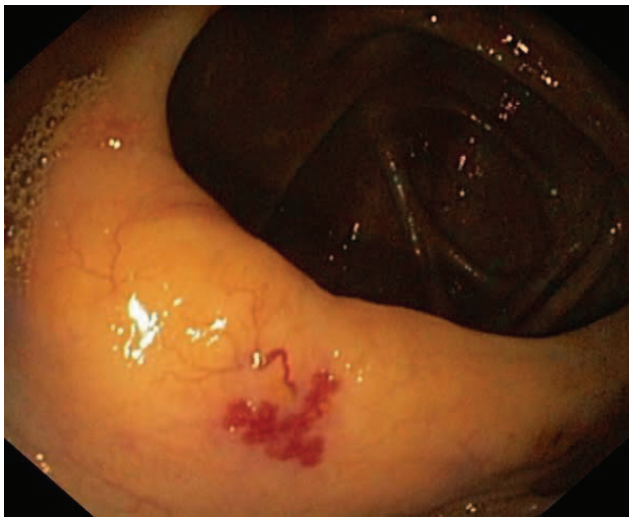

Fig. 2 – Ileocecal valve angiodysplasia.

In 2 patients, the pulmonary AVMs were detected using the chest CT with pulmonary angiography.

Discussion

Our case series included 6 patients, 5 with definite (3 positive Curacao criteria) and one with probable (2 positive Curacao criteria) diagnosis of HHT.

In our study, the most common sign was epistaxis that was present in 5 out of 6 patients. According to different authors, epistaxis is a common sign present in 82%–93% of all HHT patients [1, 5, 6]. Recurrent epistaxis occurs during childhood in more than 50% of the HHT patients with an average of 18 nose bleeds a month [1], while by the age of 30 years, over 90% of patients experienced the recurrent epistaxis [2, 7]. Although epistaxis is an early marker of the disease, diagnosis is usually delayed. According to Pierucci et al. [2], a diagnostic delay is 25.7 years, which is consistent with our results where only one patient was diagnosed in the adolescence.

Mucocutaneous telangiectasia in the HHT patients are commonly seen on the face, lips, tongue, oral mucosa, gums, conjunctiva and skin of the trunk, hands, and fingers.

They usually occur in childhood and become numerous and pronounced over time. Bleeding from cutaneous telangiectasia is usually mild to moderate, but in rare cases it is severe and a laser coagulation is indicated [3]. In our study, 5 out of 6 patients had cutaneous telangiectasia. This result is consistent with the results of other studies. In the study of the Irish National Center, cutaneous telangiectasia existed in 57% of patients with the suspected and 80% of patients with the certain HHT diagnosis [5].

Telangiectasia can be localized throughout the GI tract. The stomach and duodenum are the most common sites where GI telangiectasia are diagnosed [1, 8] which is consistent with our results. Telangiectasias were identified in the stomach of all patients, while 5 patients had telangiectasia in the duodenum. The colonic localization was diagnosed in 4 patients, while esophagus telangiectasia was diagnosed in one patient. These results are consistent with the results of other studies [8]. We diagnosed jejunal lesions in 2 patients, as opposed to previously published data where over 50% of patients had jejunal lesions, but this could be explained by a small sample size in our study [8]. Blood loss from the gastrointestinal tract is presented in 15% –30% of patients more often after the age of 30 years and can be acute or chronic [1, 8, 9]. In our study, 5 patients had the overt gastrointestinal bleeding and 3 were transfusion dependent. The number of telangiectasia in the gastrointestinal tract is correlating with the average hemoglobin level as previously reported by Longacre et al. [8] Namely, in the patients with more than 20 lesions, mean hemoglobin was 79 g/L while in the patients with less than 20 telangiectasias, mean hemoglobin was 94 g/L.

The pulmonary AVMs were identified in 40%–60% of the HHT patients [1, 10]. Apart from the hemoptysis and hemothorax, neurological disturbances such as a migraine, transitory ischemic attack (TIA), stroke or brain abscess may occur in the patients as a result of communication between the arterial and venous blood flow in the pulmonary circulation and subsequent embolization of cerebral blood vessels [1, 5]. Therefore, it is essential to conduct screening to detect the AVM in the patients with HHT. In our case series, the asymptomatic pulmonary circulation AVMs were detected in 2 patients. The small pulmonary AVMs were not detectable by CT and in the patients with an initial negative finding, CT should be repeated within 3 to 5 years. If AVM is detected in the arterial branch of more than 3 mm in diameter, it is necessary to perform embolization, with a control thoracic CT scan in 6 months [11]. Antibiotic prophylaxis is mandatory in the patients with the pulmonary AVM before the invasive diagnostic procedures, in order to prevent complications [12].

The cerebral AVMs were not detected in any of the patients in our case series that can be explained both by a small number of patients and low incidence of the cerebral AVM in the HHT patients. Namely, according to previously published studies, incidence of the cerebral AVM varies between 2.3% and 7.7% [5, 13]. Also, recent data from an AVM database demonstrated that out of 531 patients, 12 (2.3%) had the cerebral AVM due to underlying HHT [14].

Conclusion

Hereditary hemorrhagic telangiectasia is a rare genetic disorder that should be considered in the cases of unexplained recurrent epistaxis in the young adults in order to shorten the delay in the diagnosis and subsequently improve the outcome of the disease. HHT should be suspected in the cases of an unexplained occult and the overt gastrointestinal bleeding. In the HHT patients, adequate diagnosis and optimal treatment require a multidisciplinary approach.

Acknowledgments

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Conflict of interest

Authors declare that they have no conflict of interest.

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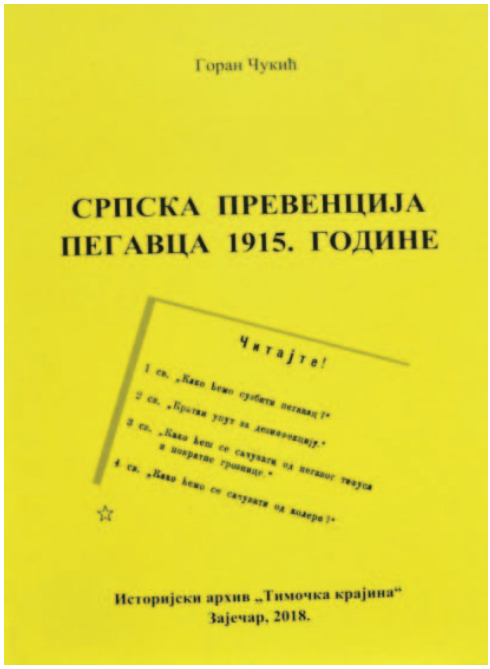
BOOK REVIEW/PRIKAZ KNJIGE

Srpska prevencija pegavca 1915. godine

Autor: Goran Čukić
Izdavač: Istorijski arhiv „Timočka krajina“, Zaječar.
Godina izdavanja: 2018.
Štampa: Radunić doo, Beograd
Tiraž: 250 primeraka
ISBN: 978-86-84483-43-2



Epidemija tifusa u Srbiji, od pozne jeseni 1914. do pred kraj proleća 1915. godine, bila je u tom vremenskom periodu veća opasnost po ostanak srpskog naroda, države i vojske i od samog rata i zato ne prestaje da intrigira stručno-medicinsku i istorijsku javnost ni danas, više od stotinu godina od njene sanacije. Razlozi za to su brojni, između ostalog, što još nisu razjašnjene neke bitne činjenice i događaji, a o nekima postoje nesuglasice ili kontroverze. To su motivi da se autor ove knjige, primarijus dr Goran Čukić, epidemiolog, bavi ovim problemom celih 35 godina, objavivši još dve monografije i 67 stručnih i naučnih radova o pegavom tifusu.

Knjiga ima tri dela, a u okviru njih više poglavlja napisanih na 270 stranica, sa faksimilom 38 originalnih arhivskih dokumenata, 15 fotografija (od kojih su mnoge iz porodičnih albuma i sada objavljene prvi put) i jednom tabelom. Citirano je 146 literaturnih podataka i 181 originalni izvorni dokumenat.

Autor problem nastanka, toka i sanacije tifusa u Srbiji 1914–1915. godine razmatra sa više aspekata, navodi dosta novih i do sada nepoznatih činjenica, predlaže neke zaključke koji su drugačiji od do sada važećih i hrabro, na osnovu argumenata, diskutuje o medicinskim ali i vojnim i sociološkim aspektima ove strašne epidemije. I pored toga, autor je svestan da problem još nije do kraja rešen, da proučavanje treba da se nastavi, da se istražuju inostrani arhivi, da se eventualno dođe do novih činjenica i da se o tome organizuje međunarodna stručna konferencija. Dalja istraživanja bi još bolje osvetlila ulogu srpskog saniteta, međunarodnih sanitetskih misija i stranih eksperata u sanaciji epidemije tifusa, ali i državnog i vojnog rukovodstva Srbije.

Evidentno je zalaganje autora da se što je moguće više prikaže uloga srpskog saniteta u sanaciji epidemije tifusa, da je to do sada nedovoljno istaknuto, smatrajući da je taj doprinos veoma veliki, uprkos velikom broju obolelih i umrlih od tifusa. Posebno naglašava odličnu realizaciju protivepidemijskih mera od strane srpskog saniteta kada su one prihvaćene na medicinskom nivou i državnom i vojnom vrhu.

Kroz knjigu provejava stav autora da je pravilna i pravovremena procena situacije, donošenje realnog zaključka i pokretanje organizovane akcije za sanaciju od presudnog značaja za rešavanje ovog ili sličnog problema. Na taj način se stvaraju uslovi za definisanje doktrinarnih stavova koji važe za sve, a to je garancija da se slična elementarna, opštenarodna nesreća ne ponovi, ili, ukoliko se desi, da će se na nju odgovoriti odranije definisanim rešenjima.

Posebna vrednost monografije je što je u njoj detaljno obrađena aktivnost Državnog sanitetskog odbora za suzbijanje zaraze i Komisije pri Vrhovnoj komandi za suzbijanje zaraznih bolesti. Date su biografije osnivača i članova Komisije: dr Lazara Genčića, dr Čedomira Đurđevića, dr Đorđa Protića, dr Dušana Kopše i magistra farmacije Danila Atanackovića. U posebnom dodatku je dat fototipski prikaz brošura iz serije „Borba protiv zaraze“, u kojima su jednostavnim jezikom date preporuke i uputstva kako suzbiti pegavac i povratnu groznicu, kako vršiti dezinfekciju i kako se sačuvati od kolere. Navedene brošure su veoma doprinele zdravstvenom prosvećivanju običnog naroda, posebno o značaju higijenskih mera u prevenciji zaraznih bolesti.

Knjiga predstavlja vredno štivo iz kojeg mogu lekari, posebno epidemiolozi i infektolozi, ali i istoričari medicine i sanitetski oficiri, da upoznaju deo istorije i tradicije svoje profesije. Poznavanjem tih činjenica bolje ćemo razumeti teškoće, probleme i izazove u vremenu sadašnjem.

Brigadni general u penziji
dr sc. med. Veljko Todorović

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Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost nadležnog etičkog komiteta.

Rezultate prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa *Interneta* citiraju se uz navođenje datuma pristupa tim podacima.

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Tabele

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

Ilustracije

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

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

Skraćenice i akronimi

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

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

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