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IN MEMORIAM

World Cancer Day marks on February 4 each year to raise awareness of cancer and to direct efforts to its prevention, detection, and treatment. This Day was founded by the Union for International Cancer Control (UICC) to support the goals of the World Cancer Declaration, written in 2008. The primary goal of the World Cancer Day is to significantly reduce incidence of illness and death caused by cancer by 2020.

In 2018, World Cancer Day is taking place under the tagline `We can. I can.` and explores how everyone - as a collective or as individuals - can do their part to reduce the global burden of cancer.

Svetski dan borbe protiv raka obeležava se svake godine 4. februara sa ciljem da podigne svest o ovoj bolesti i usmeri napore ka njenoj prevenciji, otkrivanju i lečenju. Ovaj dan je ustanovila Unija za internacionalnu kontrolu raka da bi se poduprli ciljevi Svetske deklaracije o kanceru iz 2008. godine. Glavni cilj Svetskog dana borbe protiv raka jeste da se značajno snizi učestalost kancera, bolesti i smrti prouzrokovane njime do 2020.

U 2018. godini, Svetski dan borbe protv raka održaće se pod sloganom `Mi možemo. Ti možeš`, što znači da svako, bilo kao član kolektiva ili pojedinac, može da dade doprinos u smanjenju globalnog opterećenja ovom bolesti.

ORIGINAL ARTICLES

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The relationship between tacrolimus concentration-dose ratio and genetic polymorphism in patients subjected to renal transplantation

Povezanost odnosa koncentracija-doza takrolimusa i genetskog polimorfizma kod bolesnika sa transplantiranim bubregom

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Abstract

Background/Aim. Tacrolimus concentration-dose ratio as a potential therapeutic drug monitoring strategy was suggested to be used for the patients subjected to renal transplantation. The aim of this study was examining the relationship between tacrolimus concentration-dose ratio, suggested to be used as a therapeutic drug monitoring strategy and the polymorphisms of genes encoding the most important enzymes, such as CYP3A5 and CYP3A4, as well as the transporter P-glycoprotein, for its metabolism and elimination. **Methods.** The study was designed as a prospective case series study, in which the unit of monitoring was the outpatient examination of 54 patients subjected to renal transplantation. Genotyping was performed by 7500 Real-Time PCR System by assessing allelic discrimination based on TaqMan® methodology. **Results.** Patients (n = 13) who were treated with less than 2 mg of tacrolimus/day (0.024 ± 0.006 mg/kg/day) had the tacrolimus concentration-dose ratio larger than 150 ng/mL/mg/kg. In this group, 84.62% patients had CYP3A5 *3*3 allele. All of these patients had CYP3A4 *1*1/*1*1B allele. Regarding ABCB1 C3435T gene, 30.77% of patients had the TT gene variant, while 69.23% of our patients had CC and CT gene variants. **Conclusion.** Tacrolimus concentration-dose ratio greater than 150 ng/mL/mg/kg is cut-off value in patients subjected to renal transplantation which might point to patients who are poor CYP3A5 metabolizers and/or with dysfunctional P-glycoprotein.

Key words:
kidney transplantation; tacrolimus; dose-response relationship, drug; polymorphism, genetic.

Apstrakt

Uvod/Cilj. Odnos koncentracija-doza takrolimusa, kao potencijalna strategija terapijskog monitoringa lekova, upućuje na to da se može koristiti kod bolesnika sa transplantiranim bubregom. Cilj ove studije je bio da ispita vezu između odnosa koncentracija-doza takrolimusa koji je sugerisan kao strategija terapijskog monitoring lekova i genskog polimorfizma gena koji kodiraju najznačajnije enzime, CYP3A5 i CYP3A4, kao i transporter P-glikoprotein, za metabolizam i eliminaciju takrolimusa. **Metode.** Studija je osmišljena kao prospektivna serija slučajeva, u kojoj je jedinica monitoringa bio ambulantni pregled 54 bolesnika sa transplantiranim bubregom. Genotipizacija je urađena na aparatu 7500 Real-Time PCR System za procenu za diskriminacije alela koja se bazira na TaqMan® metodologiji. **Rezultati.** Bolesnici (n = 13) koji su lečeni sa manje od 2 mg takrolimusa na dan (0,024 ± 0,006 mg/kg/dan) imali su odnos koncentracija-doza takrolimusa veći od 150 ng/mL/mg/kg. U ovoj grupi, 84,62% bolesnika je imalo CYP3A5 *3*3 alele. Svi ovi bolesnici su imali CYP3A4 *1*1/*1*1B alele. Što se tiče ABCB1 C3435T gena, 30,77% bolesnika je imalo TT gensku varijantu, dok je 69,23% njih imalo CC i CT gensku varijantu. **Zaključak.** Odnos koncentracija-doza takrolimusa veći od 150 ng/mL/mg/kg je granična vrednost kod bolesnika sa transplantiranim bubregom koji može da ukaže na one bolesnike koji su spori CYP3A5 metabolizeri i/ili su sa disfunkcionalnim P-glikoproteinom.

Ključne reči:
transplantacija bubrega; takrolimus; lekovi, odnos doza-reakcija; polimorfizam, genetički.

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Introduction

Tacrolimus is one of the most important immunosuppressive drugs used for renal transplantation [1]. It is a "critical dose" drug because of its narrow therapeutic range. Underexposure to tacrolimus may result in an acute rejection and graft dysfunction, while overexposure might be followed by serious adverse effects [2]. The clinical usage of tacrolimus can be complicated due to significant inter-individual and intra-individual variability of this drug, as well as significant differences in bioavailability [3]. It is well known from clinical practice that patients who are treated with the equal doses of this drug could have high variability of tacrolimus blood concentrations.

Numerous factors have been identified as contributors to the high tacrolimus pharmacokinetic variability: age, gender, body mass index, albumin concentration, liver dysfunction, hematocrit, time elapsed after transplantation, hepatitis C status, diabetes status, diarrhoea, corticosteroid dosage, drug-drug interactions and food administration [3–6]. As a result, therapeutic drug monitoring (TDM) is particularly important. Recently, tacrolimus concentration-dose ratio (C/D ratio), as a potential TDM and target concentration intervention (TCI) strategy, has been suggested to be used for the patients subjected to renal transplantation [5, 6–8]. The tacrolimus C/D ratio is the ratio between tacrolimus trough concentrations (TTC) (ng/mL) and 24h dose normalized by patient's weight (mg/kg/day) [5]. Tacrolimus C/D ratio, together with TTC, would provide a better estimation of the influence of additional factors, like gender and comedication on tacrolimus exposure in these patients.

Genetic polymorphism is also considered to be one of the most significant causes of tacrolimus pharmacokinetic variability [5, 9–11]. Since polymorphic cytochrome P450 isoenzyme family (CYP) is the most important system involved in tacrolimus biotransformation and elimination, genotyping CYP polymorphisms provides important information that can predict tacrolimus exposure in patients subjected to renal transplantation [9]. Tacrolimus is metabolized mainly by CYP3A4 and CYP3A5 isoenzymes [9]. It is also a substrate of P-glycoprotein efflux pump. P-glycoprotein lowers the blood concentration of tacrolimus by pumping the absorbed tacrolimus back into the intestinal lumen [12]. Polymorphisms of genes which encode these isoenzymes and efflux pump can have a significant influence on tacrolimus blood concentrations in these patients [9, 12].

The aim of this study was to examine the relationship between tacrolimus C/D ratio and polymorphisms of genes encoding the most important enzymes, CYP3A5 and CYP3A4 and transporter, P-glycoprotein, for its metabolism and elimination in order to estimate the influence of genetic polymorphisms on tacrolimus exposure in patients subjected to renal transplantation.

Methods

Study design

The study was designed as a prospective case series study. The unit of monitoring was outpatient examination of 54 patients subjected to renal transplantation in the Centre for Solid Organ Transplantation in the tertiary health care university hospital, the Military Medical Academy (MMA), Belgrade, Serbia. They were all monitored during 4 years, from September 2010 to January 2015, starting one month after renal transplantation.

Patients and therapeutic protocol

All patients were treated in accordance with the established therapeutic protocol in the Centre, as described in the earlier studies [7, 13]. After kidney transplantation, they were subjected to the triple-drug-therapy, including corticosteroids (methylprednisolone or prednisone), myco-phenolate mofetil and tacrolimus (Prograf®, Astellas, Japan), with or without the addition of an induction agent (anti-T lymphocyte globulin) in the early phase after the transplantation. The other drugs were administered according to comorbidity.

On the day of transplantation, tacrolimus was introduced in an initial oral dose of 0.1–0.3 mg/kg/day, divided into 12-hour intervals. The patients were given a dose of 500 mg of methylprednisolone, intravenously, on the day of the surgical intervention, before the transplantation itself; the next 2 days the dose was 250 mg/day, and then, it was reduced to 125 mg/day in the following 2 days, followed by 3 days with a dose of 1.5 mg/kg/day. During the second week after transplantation, a dose of 0.3 mg/kg/day of prednisone was administered orally; the same dosage was used until the end of the first month. The prednisone dose of 10 mg/day was prescribed until the end of the first year after transplantation, while 10 mg dose was recommended every other day, during the second year of treatment and later on. Mycophenolate mofetil was given orally, 1 g, twice a day, starting 2 days before the kidney transplantation. Three months after transplantation, mycophenolate mofetil dose was reduced to 500 mg, twice a day. After this dose reduction, mycophenolate mofetil was taken permanently. Anti-thymocyte globulin was administered intravenously (as a slow intravenous infusion) as a series of divided doses during the first post-transplant week (in a dose of 2–4 mg/kg/day).

The other drugs were administered according to comorbidity. In order to control hypertension, calcium channel blockers (nifedipine, amlodipine), β adrenergic antagonists (propranolol, carvedilol, bisoprolol, atenolol, metoprolol, nebivolol) and/or diuretics (furosemide) were given. As a prophylaxis for peptic ulcers and surgical stress-related bleeding, H_2-antagonists (ranitidine) or proton pump inhibitors (pantoprazole, esomeprazole) were administered. The doses of all concomitant drugs were always within recommended therapeutic range. All the patients were also treated with cotrimoxazole (for *Pneumocystis Jirovecii* prophylaxis) for 6 post-transplant months.

Therapeutic drug monitoring

Tacrolimus TDM was needed to optimize the dosage regime in patients after renal transplantations. Tacrolimus trough concentrations (TTC) were measured by chemiluminescence microparticles immunoassay (CMIA), ARCHITECT i1000SR

Abbott Laboratories; Abbott Park, Illinois, USA) in the Institute for Medical Research, Department for Clinical and Experimental Immunology, the MMA, Belgrade, Serbia. The whole blood samples were taken 12 h after the evening dose, i.e. 10 min before the morning dose, starting a month after renal transplantation. The recommended target concentration range for TTC has been from 6 to 10 ng/mL. Tacrolimus trough concentrations were measured every other day during 2 weeks after renal transplantation and later, on each control examination. The control examinations were conducted twice a week during the first 3 months after transplantation, once a week for the next 3 months, twice a month from the sixth to the ninth month after transplantation, once a month until the end of the second year, and later on, once in every 3 months.

Genotyping for CYP3A5, CYP3A4 and ABCB1

One blood sample from antecubital vein in a vacutainer with anticoagulant EDTA was taken from each patient. DNA was extracted and isoforms of the enzymes CYP3A5 and CYP3A4 as well as of the transporter ABCB1, were genotyped. The 13 adult patients were genotyped for single nucleotide polymorphism (SNP) of CYP3A5 at position 6986A > G (the *3 or *1, rs776746), CYP3A4 at position -392A > G (the *1 or *1B, rs2740574) and ABCB1 at exon 26 (3435C>T, rs1045642). The genotyping was detected by TaqMan® SNP genotyping assays (Life Technologies, USA) on a 7500 Real-Time polymerase chain reaction (PCR) System (Applied Biosystems, USA).

For CYP3A4, ABCB1 and CYP3A5, the observed genotype (allele) frequencies were in Hardy-Weinberg equilibrium ($p > 0.05$).

Statistical analysis

The complete statistical analysis of data was done using the statistical software package, PASW Statistics 18® [SPSS (Hong Kong) Ltd., Hong Kong]. All variables were presented as frequency of certain categories. Continuous variables were presented as means and standard deviations. Continuous variables were compared by using Mann-Whitney U test. The normality of the data was assessed by using Kolmogorov-Smirnov test. Ratios between tacrolimus daily dose per body weight, TTC and tacrolimus C/D ratio were tested by Spearman's coefficient correlation. All the analyses were estimated at $p < 0.05$ level of the statistical significance.

Ethical approval

The principles of ICH Good Clinical Practice were strictly followed and ethical approval N° 01/31-01-13 from the Ethics Committee of the MMA was obtained for the study protocol N° 910-1.

Results

The most important demographic characteristics and biochemical analyses of renal transplant patients are presented in Table 1. The total of 54 patients was subjected to kidney transplantation (34 males or 63% and 20 females or 37%); the average age was 40.46 ± 11.38. The average body mass index was 21.49 ± 3.18 kg/m^2. The total number of 1,872 outpatient examinations were performed during this follow-up (34.67 ± 10.96 outpatient examinations per patient).

A weak correlation between tacrolimus daily dose per body weight and TTC was shown ($r = 0.233$, $p < 0.001$), while the correlation between tacrolimus daily dose per body weight and its C/D ratio was very strong ($r = -0.859$; $p < 0.001$), (Figures 1 and 2). It was observed that the patients who had tacrolimus C/D ratio larger than 150 ng/mL/mg/kg were treated with less than 2 mg of tacrolimus/day and vice versa (Figure 2).

Calculations were made according to tacrolimus daily dose and it could be concluded that in the patients who were treated with less than 2 mg of tacrolimus/day (0.024 ± 0.006 mg/kg/day) the average TTC was significantly lower (5.82 ± 1.92 ng/mL), and tacrolimus C/D ratio was significantly higher (252.82 ± 101.19 ng/mL/mg/kg) in comparison to those treated with more than 2 mg of tacrolimus/day (0.089 ± 0.041 mg/kg/day), (Table 2). In the patients whose tacrolimus C/D ratio was larger than 150 ng/mL/mg/kg, genotyping of genes encoding the most important enzymes, such as CYP3A5 A6986G and CYP3A4 -A392G, and transporter, ABCB1 C3435T, for tacrolimus metabolism and elimination

Table 1

The most important demographic characteristics and biochemical analyses of renal transplant patients

Demographic characteristics	Values
Total number of patients, n	54
Gender: male/female, n	34/20
Age (years), $\bar{x}$ ± SD	40.46 ± 11.38
Height (m), $\bar{x}$ ± SD	1.74 ± 0.09
Weight (kg), $\bar{x}$ ± SD	67.96 ± 13.47
Body mass index (kg/m^2), $\bar{x}$ ± SD	21.49 ± 3.18
Biochemical analyses	
Haematocrit (vol %), $\bar{x}$ ± SD	0.39 ± 0.05
Blood urea nitrogen (mmol/L), $\bar{x}$ ± SD	10.03 ± 15.29
Creatinine (μmol/L), $\bar{x}$ ± SD	133.64 ± 54.49
Proteinuria (g/24 h), $\bar{x}$ ± SD	0.30 ± 0.29

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

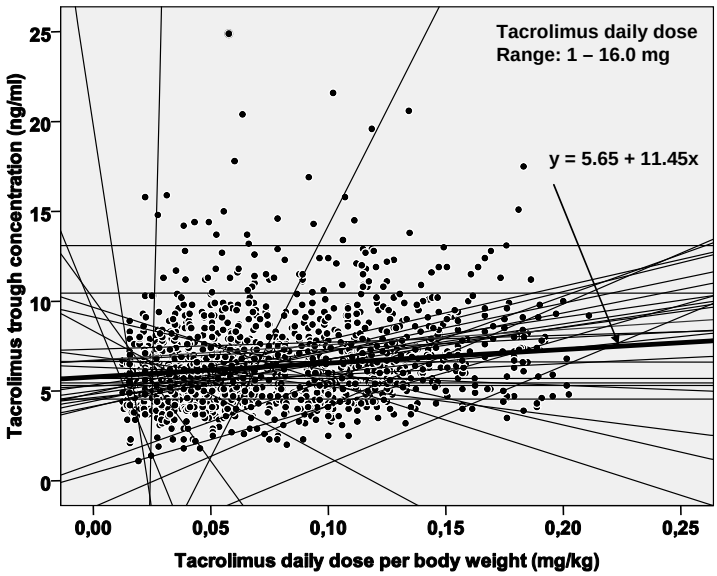

Fig. 1 – Relationship between tacrolimus daily dose and tacrolimus through concentration in patients subjected to renal transplantation

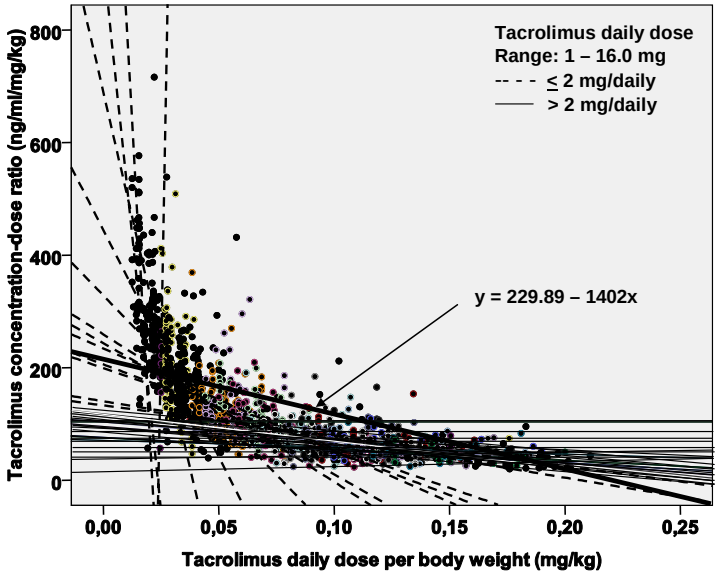

Fig. 2 – Relationship between tacrolimus daily dose and tacrolimus concentration-dose ratio in patients subjected to renal transplantation.

Table 2

Parameters of therapeutic drug monitoring depending on tacrolimus daily dose in patients subjected to renal transplantation

Parameters	Mean ± standard deviation		*p* value
	≤ 2 mg	> 2 mg	(Mann-Whitney test)
Tacrolimus daily dose (mg)	1.60 ± 0.33	5.94 ± 2.91	< 0.001
Tacrolimus daily dose per body weight (mg/kg)	0.024 ± 0.006	0.089 ± 0.041	< 0.001
Body weight (kg)	66.73 ± 8.31	68.16 ± 13.44	0.416
Tacrolimus through concentrations (ng/mL)	5.82 ± 1.92	6.70 ± 2.44	< 0.001
Tacrolimus concentration-dose ratio (ng/mL/mg/kg)	252.82 ± 101.19	92.56 ± 55.01	< 0.001

in renal transplant recipients was performed. The total number of these patients was 13 (Table 3).

In the group of 13 renal transplant recipients most of them (84.62%) had CYP3A5 *3*3 allele (Table 3). On the other hand, 15.38% of patients are homo or heterozygous for CYP3A5 *1 (total of 7.69% *1*1 and 7.69% *1*3). All these patients had CYP3A4 *1*1/*1*1B allele. Regarding ABCB1 C3435T gene, 30.77% of patients had TT gene variant, while CC and CT gene variants were present in 69.23% of them.

Table 3

Genetic polymorphism for CYP3A5, CYP3A4 and ABCB1 C3435T in patients with tacrolimus concentration-dose ratio larger than 150 ng/mL/mg/kg

Genetic polymorphism	% (number of patients)		
CYP3A5 A6986G	AA 7.69 (1)	AG 7.69 (1)	GG 84.62 (11)
CYP3A4 -A392G	AA 92.31 (12)	AG 7.69 (1)	GG -
ABCB1 C3435T	CC 38.46 (5)	CT 30.77 (4)	TT 30.77 (4)

Discussion

A very strong correlation between tacrolimus daily dose expressed per body weight and tacrolimus C/D ratio was found. It was also found a weak correlation between tacrolimus daily dose per body weight and TTC. Tacrolimus trough concentrations, most often used for TDM, are widely accepted as a guide for TCI and individualizing tacrolimus dose requirements in patients subjected to kidney transplantation [14, 15]. On the other hand, although full dose interval area under the concentration-time curve is generally considered as the best marker for tacrolimus exposure, due to its complexity it has not been widely used as a routine method in clinical settings [16, 17]. Quite recently, however, tacrolimus C/D ratio has been suggested as a potentially useful TDM strategy [7]. It, concomitantly with TTC, enabled better estimation of the influence of gender and comedication on tacrolimus exposure in patients subjected to renal transplantation.

It is well known that tacrolimus is primarily metabolised in the intestine and liver by the CYP3A family, especially its CYP3A4 and CYP3A5 members, and is a substrate of P-glycoprotein efflux pump [1, 3]. CYP3A is responsible for > 90% of tacrolimus metabolic elimination [18]. CYP3A4 accounts for 30% of the total cytochrome P450 activity in liver and 70% in small intestines [19]. It was reported that CYP3A5 was expressed at higher levels than CYP3A4 in extra hepatic tissue, such as in the small intestine, colon, lung, oesophagus, kidney, adrenal gland, anterior pituitary, breast, prostate and polymorphonuclear leukocytes [20]. The majority of compounds (tacrolimus, cyclosporine) that are substrates for CYP3A4, are also metabolized by CYP3A5, usually with a higher catalytic efficiency. Therefore, CYP3A5 is the predominant enzyme for metabolism of tacrolimus, with CYP3A4 contributing [21].

The efflux transporter P-glycoprotein also plays a major role in the pharmacokinetics of tacrolimus [21]. P-glycoprotein was found in enterocytes where it decreases intracellular concentrations of tacrolimus, by pumping them back into the lumen of the small intestine. P-glycoprotein also transports calcineurine inhibitors across membranes of hepatocytes and kidney cells, as well as lymphocytes. About 75% of interpatient variability in cyclosporine clearance could be explained by variation of both CYP3A4 activity in the liver, and expression of P-glycoprotein in enterocytes [22].

Polymorphisms of genes which encode previously mentioned most important enzymes and transporter for tacrolimus metabolism and elimination can have significant influence on oral bioavailability of this drug and its blood concentrations [10]. Therefore, larger doses of the drug (2–16 mg) were needed for the patients in order to get significantly higher tacrolimus trough concentrations. On the other hand, our results showed that patients who were treated with less than 2 mg of tacrolimus/day (0.024 ± 0.006 mg/kg/day) had tacrolimus C/D ratio larger than 150 ng/mL/mg/kg, and vice versa. When taking into account target tacrolimus concentrations, there are authors who consider that the higher C/D ratio obtained, the slower metabolic efficiency can be expected and, consequently, lower tacrolimus dose is required [5]. Therefore, in all patients who had tacrolimus C/D ratio over 150 ng/mL/mg/kg, the examination of genetic polymorphism was performed in order to show its influence on tacrolimus metabolism.

Most of these patients (84.62%) had CYP3A5 *3*3 allele (gene mutant, non-expressers for enzyme CYP3A5). It is interesting to mention that Đorđević et al. [23] showed that in Serbian population, in 140 healthy volunteers, 84.7% had *3*3 gene variant. It had already been shown that, after the equal dose of this drug, the patients with CYP3A5 *3 allele often have higher blood concentrations of tacrolimus in comparison to the patients who have CYP3A5 *1 allele (CYP3A5 *1*1/*1*3) [9]. Considering that CYP3A5 enzyme is dominant in tacrolimus metabolism, it can be expected that many patients in our population tend to have higher tacrolimus blood concentrations after empirical treatment with its usual dosage in the early period after kidney transplantation. Most of the studies confirmed that carriers of CYP3A5 *3*3 genotype require lower doses of tacrolimus [24, 25]. When the given doses were equal, in order to maintain drug levels in optimal range, it turned out that the carriers of CYP3A5 *1*1/*1*3 genotype had 1.5–2-fold higher TTC in comparison to CYP3A5 *3*3 genotype [24, 25].

All the patients in our study had CYP3A4 *1*1/*1*1B (gene non-mutant, expressers for enzyme CYP3A4), which is associated with the functional state of enzyme CYP3A4. The other authors showed that enzyme CYP3A4 is predominantly active in Caucasians in comparison to Asians, Mexicans and African-Americans. The presence of this polymorphism in Caucasians ranged from 90 to 98% [26, 27]. Some recent studies demonstrated that CYP3A4 polymorphism, resulting from the A > G substitution at position 392, referred to as CYP3A4*1B (CYP3A4 – 392 GG) allele, consequently caused a diminished enzymatic activity and, thus, reduced tacrolimus clearance [26].

Regarding ABCB1 C3435T gene, 30.77% of our patients had a mutant gene (TT variant, which is associated with the diminished activity of P-glycoprotein). P-glycoprotein, which is encoded by the ABCB1 gene, is a large ATP-dependent transmembrane protein involved in the extracellular efflux of tac-

rolimus [10]. The efflux pump is responsible for the efflux of the already absorbed tacrolimus from enterocytes back into the intestinal lumen and, therefore, reduces its bioavailability. The genetic polymorphism of P-glycoprotein is associated with the reduced function of this efflux pump and, consequently, the increased tacrolimus absorption and blood concentration. The most extensively investigated SNPs of ABCB1 are 3435C > T (rs1045642) in exon 26, 1236C > T (rs128503) in exon 12, and 2677G > T/A (rs2032582) in exon 21 [28]. It was shown that the patients who had wild-type genotype ABCB1 3435C > T (CC) had stable tacrolimus blood concentration, while the patients with TT variants ABCB1 had up to 60% higher tacrolimus blood levels [29], because TT genotype a expressed lower intestinal activity of P-glycoprotein. Consequently it could be supposed that better absorption of tacrolimus and lower daily dose would be required in these patients [18]. Gene variants for ABCB1 C3435T CC and CT are associated with normal activity of P-glycoprotein [30].

According to our study, among 211 patients who were subjected to renal transplantation, about 25% had both non-functional CYP3A5 *3*3 and non-functional ABCB1 C3435T (TT) allele [31]. On the other hand, in our previous retrospective case series study, 26.6 % of renal transplant recipients had tacrolimus blood concentration values equal to and lower than 5 ng/mL [13], similar to the results obtained in this study. Since the number of the patients with high tacrolimus C/D ratio was small, definite conclusions cannot be made. Some factors, other than genetic polymorphism, also led to significantly higher tacrolimus C/D ratio. Therefore, we may assume slower elimination efficiency and, consequently, the requirement for lower tacrolimus dose in these patients. It is in accordance with a widely accepted attitude that numerous factors are contributors to the high tacrolimus pharmacokinetic variability in this category of patients.

The limitation of the study relates to the small sample size of renal transplant recipients who had genotyping of CYP3A5 and CYP3A4 enzymes performed, as well as the transporter P-glycoprotein. Also, this study does not taken into account other variables that can affect the TTC and tacrolimus C/D ratio.

Conclusion

The correlation between tacrolimus daily dose per body weight and tacrolimus C/D ratio was very strong in renal transplant recipients in our study. Tacrolimus C/D ratio greater than 150 ng/mL/mg/kg is the cut-off value in patients subjected to renal transplantation which might indicate the patients who are poor CYP3A5 metabolizers and/or with dysfunctional P-glycoprotein. Therefore, genotyping of these genes in renal transplant recipients is beneficial in order to emphasize the necessity of the reduction of the initial tacrolimus dose which would, consequently, decrease the risk of achieving tacrolimus concentrations over the therapeutic range immediately after transplantation. Since numerous factors also contribute to the variability of tacrolimus blood concentrations, a greater number of studies examining the relationship between the polymorphism of genes and clinical endpoints will be needed. As a result, cost/benefit analysis could be done and, therefore, genetic examination, prior transplantation, justified.

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Estimation of the posterior tibial slope on magnetic resonance images in Serbian population

Procena veličine zadnjeg tibijalnog nagiba metodom magnetne rezonance u srpskoj populaciji

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Abstract

Background/Aim. Preservation of an adequate posterior tibial slope (PTS) during total knee arthroplasty is crucial for the biomechanical stability and function of the knee joint. Studies that investigated anatomical features of the tibial plateau found significant gender and inter-population differences in all components of the PTS. The aim of this study was to establish reference values of PTS in Serbian population and to explore if there is any difference in the tibial plateau inclination between genders. **Methods.** We retrospectively reviewed 161 magnetic resonance images (MRIs) of the knee of adult patients examined in Medical Military Academy in Belgrade, Serbia, in a period from November 2011 to September 2014. Measurements of PTS components: medial tibial slope (MTS), lateral tibial slope (LTS), and coronal tibial slope (CTS) were performed through several steps, according to the suggestions in the recent literature. Obtained values for each tibial slope were compared between gender subgroups using appropriate statistical tests. **Results.** Mean values of each component of the posterior tibial slope for male vs. female subgroups were as follows: MTS 3.7° ± 2.8° vs. 5.1° ± 2.9°, LTS 4.2° ± 2.8° vs. 4.3° ± 2.7°, and CTS 3.9° ± 2.4° vs. 3.3° ± 1.9° respectively. The medial tibial slope was significantly higher in females than in males ($p = 0.005$). The mean value of the coronal tibial slope was greater in males without statistically significant difference ($p = 0.105$). **Conclusion.** This study demonstrated significant difference in MTS of the tibial plateau between males and females, being higher in the female subgroup.

Key words:
knee joint; arthroplasty; magnetic resonance imaging; joint instability.

Apstrakt

Uvod/Cilj. Postizanje adekvatnog zadnjeg tibijalnog nagiba (PTS) tokom totalne artroplastike zgloba kolena doprinosi njegovoj biomehaničkoj stabilnosti i očuvanju funkcije zgloba. Studije koje su istraživale anatomske karakteristike tibijalnog platoa ukazuju na to da postoje značajne razlike među polovima i između populacija u svim komponentama PTS. Cilj ove studije bio je da se ustanove referentne vrednosti PTS u našoj populaciji i da se utvrdi da li postoje razlike u komponentama PTS među polovima. **Metode.** Snimci zgloba kolena 161 odraslog bolesnika načinjeni metodom magnetne rezonance (MR) u Vojnomedicinskoj akademiji u Beogradu (Srbija), retrospektivno su analizirani u periodu od novembra 2011. do septembra 2014. godine. Na snimcima su merene komponente zadnjeg tibijalnog nagiba [medijalni tibijalni nagib (MTS), lateralni tibijalni nagib (LTS), koronalni tibijalni nagib (CTS)] na način predložen u novijim publikacijama drugih autora. Dobijene vrednosti tibijalnih nagiba su upoređivane između polova korišćenjem odgovarajućih statističkih testova. **Rezultati.** Srednje vrednosti pojedinačnih komponenti zadnjeg tibijalnog nagiba za ispitanike muškog vs. ženskog pola bile su sledeće: MTS 3.7° ± 2.8° vs. 5.1° ± 2.9°, LTS 4.2° ± 2.8° vs. 4.3° ± 2.7°, i CTS 3.9° ± 2.4° vs. 3.3° ± 1.9°. Srednja vrednost medijalnog tibijalnog ugla bila je značajno veća kod žena nego kod muškaraca visoko statistički značajnu razliku ($p = 0.005$). Ispitanici muškog pola su imali veće srednje vrednosti koronalnog tibijlanog ugla od žena, iako statističkom analizom nije potvrđena značajnost razlike ($p = 0.105$). **Zaključak.** U radu je pokazano da postoji značajna razlika među polovima u vrednostima medijalnog tibijalnog nagiba koji je značajno veći kod žena.

Ključne reči:
koleni zglob; artroplastika; magnetna rezonanca, snimanje; zglob, nestabilnost.

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Introduction

Total knee arthroplasty is widely used surgical procedure for the treatment of degenerative and rheumatologic diseases and certain fractures of the knee joint. Recent epidemiological studies that collected the data from eighteen countries reported 234 total knee replacements/100,000 population per year with the annual increase in incidence from 5.3% to 17%, depending on the country [1]. The long-term outcome of the total knee arthroplasty is highly dependent on the knee joint anatomy, particularly the posterior tibial slope (PTS) [2]. This anatomical feature represents the degree of posterior inclination of the tibial plateau in relation to the line perpendicular to the mid-diaphysis of the tibia [3]. Preservation of an adequate PTS during total and unicondylar knee replacement contributes to the overall biomechanical stability and function of the knee joint including maximal flexion and its resting position [4].

Values of the PTS that has to be set in implanted knee endoprosthesis are the subject of debate among surgeons. Great number of authors emphasize that there are no significant differences in the postoperative range of motion among patients whose tibial cuts were intraoperativly set from 0° up to 5° degrees during the implantation of the posterior cruciate ligament-sacrificed prosthesis model [5, 6]. Concerning cruciate retaining endoprosthesis, other authors argue that a greater extent of flexion can be achieved with cutting the tibia at an inclination of 5° to 7° [7–9].

Studies that investigated anatomical features of the tibial plateau found significant gender and inter-population differences in all components of the PTS [3, 10–12]. Females in general had greater medial (MTS) and lateral tibial slope (LTS) than males [3, 10, 12], while the mean values of the same parameters showed large variability depending on the study population. Furthermore, the lowest values of both MTS and LTS were found in White race [10], whereas Asian race showed the greatest inclination of the tibial plateau [10, 13, 14]. Recent studies suggest that higher values of the PTS may contribute to the anterior cruciate ligament injuries [15–18] and development of meniscal tear [19].

The aim of this study was to establish reference values of the PTS in Serbian population as well as to explore if there is any difference in the tibial plateau inclination between genders. Although the measurement of the PTS in Serbian population was recently performed on cadavers [20], we are unaware of any study that estimates value of the posterior tibial slope in clinical settings.

Methods

We retrospectively reviewed 207 knee magnetic resonance images (MRIs) of adult patients examined in the Medical Military Academy in Belgrade (Serbia), in a period from November 2011 to September 2014 year. Patients with tumors, bony cists, osteoarthritis, fractures of tibial plateaus, and previous knee surgery were excluded from the study. A final study sample consisted of 161 patients: 116 (72%) males aged between 21–53 (average 35.2), 45 (28%) females aged between 21–63 (average 41.8).

Measurement of the PTS components [medial tibial slope, lateral tibial slope, and coronal tibial slope (CTS)] was performed through several steps, according to Hashemi et al. [3]. Representative MRIs for the PTS measurement were selected using Merge e-film 3.4 freeware program. The orientation of the proximal tibial anatomic axis (PTAA) was established on sagittal and coronal section images respectively according to the mid-point method [3, 13]. Sagittal PTAA was determined on the mid-sagittal section that passed through the tibia at the level of the intercondylar notch (Figure 1a). This axis was reproduced on the two adjacent sagittal section images passing through the center of the medial and lateral articular surfaces of the tibial plateau, respectively (Figures 1b and 1c). On these images, a line perpendicular to the PTAA was constructed as well as the line that connected the most superior point of the anterior and posterior half of the tibial plateau. An angle between these two lines represented medial and lateral tibial slope, respectively (Figures 1b and 1c). A similar approach was used for the measurement of the coronal tibial slope. The PTAA in coronal plane was constructed on the section image that passed through the most lateral points of medial and lateral half of the tibial plateau (Figure 2a). Two additional lines were constructed (one perpendicular to the coronal PTAA and another that connected the most superior point on the lateral and medial portion of tibial plateau) and the angle between them was measured (Figure 2b). Corel DRAW X6 program was used for the line construction and angle measurement.

In order to avoid bias in the PTS assessment, the same observer repeated all measurements in randomly selected 50

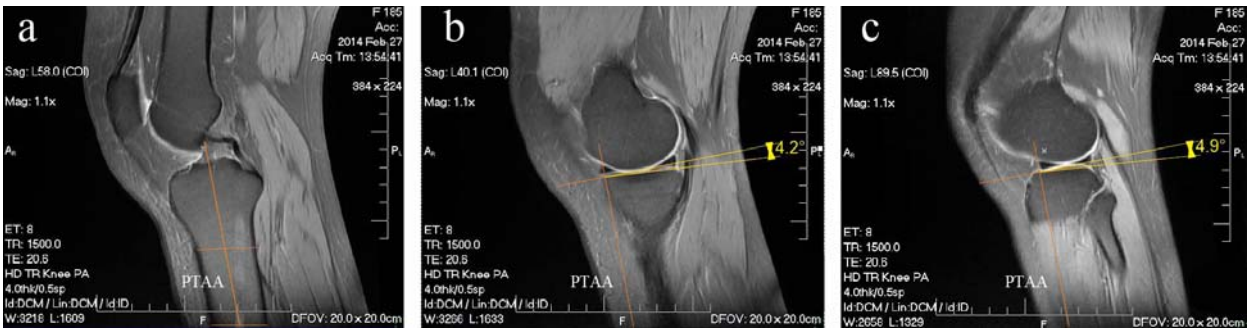

Fig. 1 – Reconstruction of the proximal tibial anatomic axis (PTAA) in the mid-sagittal plane (a) with the measurement of the medial (b) and lateral (c) tibial slope.

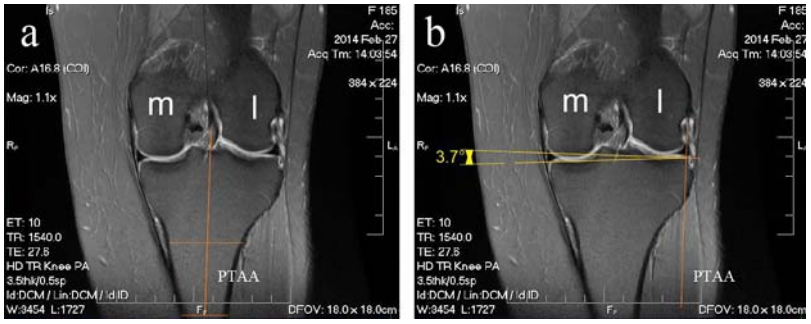

Fig. 2 – Reconstruction of the proximal tibial anatomic axis (PTAA) in the coronal plane (a) with the measurement of the coronal tibial slope (b).

patients after three months. Intraclass correlation analysis showed high reproducibility (Cronbah's alpha coefficient was 0.96 for MTS, and 0.94 for LTS and CTS).

The accuracy of measurement was limited to 0.1°.

Statistical analysis

The database was made in the statistical program SPSS, version 15.0. Analysis included descriptive and analytical statistical methods. Normality of data distribution was tested by Kolmogorov-Smirnov test. Mann-Whitney U tests was applied to compare the medial tibial slope, lateral tibial slope and coronal tibial slope between genders. Wilcoxon Signed Rank Test was performed to explore if there is any difference between medial and lateral tibial slope. The level of statistical significance was set at or below 0.05.

Results

The mean values of each component of the posterior tibial slope were as follows: medial tibial slope 4.1° ± 2.9°, lateral tibial slope 4.2° ± 2.8°, and coronal tibial slope 3.7° ± 2.3°. Angles of the each component of the posterior tibial slope measured in male and female subgroup are displayed in Table 1. The mean values of medial and lateral tibial slope were higher in female than in male subjects, with a significant difference ($p = 0.005$) detected in the former ones (Figure 3). Conversely, coronal tibial slope was greater in males than in females even though the difference was not statistically significant ($p = 0.105$) (Figure 3).

Comparative analysis of medial and lateral tibial slopes within gender subgroups showed the difference at the border of significance only in females ($p = 0.058$), while both angles differed non-significantly in males ($p = 0.128$) (Figure 4).

Table 1

Components of posterior tibial slope measured in male and female individuals (in degrees)

Tibial slope	Gender	Mean	Standard deviation
Medial	male	3.7	2.8
	female	5.1	2.9
Lateral	male	4.2	2.8
	female	4.3	2.7
Coronal	male	3.9	2.4
	female	3.6	1.9

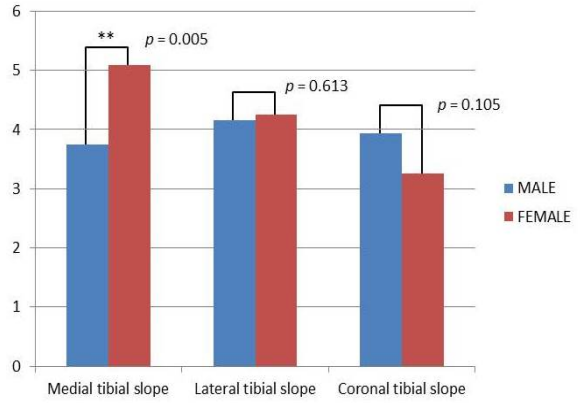

Fig. 3 – Gender differences in medial, lateral, and coronal tibial slope.

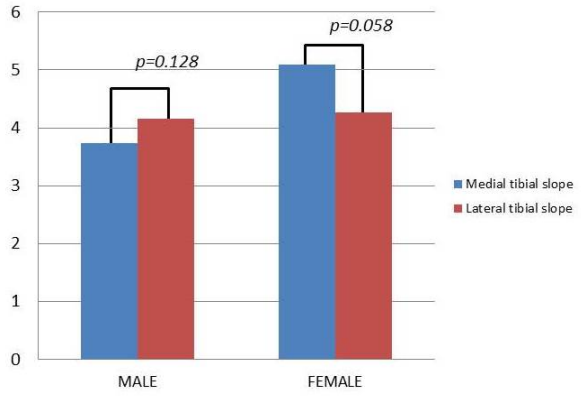

Fig. 4 – Comparative analysis of medial and lateral tibial slopes in gender subgroups.

Discussion

The posterior tibial slope range from zero to fifteen degrees is considered normal in healthy subjects [13, 14]. However, recently published studies reported inter-population differences in each component of the PTS. In European population, Hudek et al. [12] found the mean MTS around 3°, while the same slope was around 15° in Chinese population [13]. Our results of the PTS in both genders were consistent with values detected in Europeans. Additionally, tibial plateau morphology in Asians is characterized by significantly lager posterior slope then in White race [10]. This finding could be interpreted by the real presence of the race-related differences in proximal tibial morphology, but it could be also a consequence of various radiological methods applied for the measurement of the PTS [3, 10, 13, 17, 21, 22]. The plain radiography is the most commonly used imaging technique for this purpose [11, 22–24]. However, it is not considered reliable enough for the PTS assessment due to the superimposition of the medial and lateral slopes on the lateral radiographs. Study by Utzschneider et al. [22] showed that computed tomography (CT) and MR methods are more accurate in evaluation of the inclination of the tibial plateau. These techniques eliminate errors resulting from superimposition, patient position and knee rotation during an examination, and allow multiplanar reconstruction and selection of the most representative section images [16]. In general, authors prefer to use the MR examination for the measurement of the PTS in order to avoid unnecessary exposure of the patient to high radiation doses during CT scanning.

Considering gender differences in tibial plateau inclination, many authors reported that females often have higher MTS than males [3, 10, 14]. In addition, Hashemi et al. [3] described that in their population sample CTS angle was greater in males. Values that we registered in Serbian population showed the same tendency, but statistical significance was confirmed only for the gender difference in the MTS.

Results that we obtained are similar with findings of Haddad et al. [10], who also measured the same parameters of the PTS on MR of the White race European population [10]. In this study, the mean value of the MTS and the LTS for the whole study sample was 4.2° and 4.4° respectively, which is slightly greater than in our population (MTS 4.1°, LTS 4.2°, CTS 3.7°). When compared MTS and LTS values in gender subgroups between studies, we observed that males and females from the UK had higher MTS than Serbian population. In the case of the LTS, greater mean values than our results were reported only for the UK females. We also compared our findings with the study that was conducted among the USA population [3]. The mean value of the PTS in the USA population was slightly different from our findings. In their population sample the mean MTS was 5.02° and the mean LTS was 6.38°, while the mean CTS value was not reported [3]. Values of MTS were similar between genders in both studies. The LTS was greater in the USA study sample for both genders, but CTS was higher just in female subgroup.

Our study is potentially limited by the fact that determination of the tibial shaft axis would seem more reliable on the image of the entire lower leg then on the proximal part of the tibia. However, authors that measured tibial slopes on MR or CT images that included different lengths of the tibia (from proximal part to the entire bone) reported that length of the tibia did not significantly influence the measurement results [22, 25]. These authors were consistent in suggestions that determination of bone axis on proximal 10 cm of the tibial shaft could be accepted as reliable.

Conclusion

The mean value of the medial tibial slope was significantly higher in females than in males. Conversely, the mean value of the coronal tibial slope was greater in males, but without statistically significant difference. Clinicians should be aware about the exact value of each component of the tibial plateau inclination in order to achieve the best surgical outcome during arthroplasty. Additionally, the posterior tibial plateau values are important for anatomical and anthropometrical studies.

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Risk factors for catheter-related infections in patients on hemodialysis

Faktori rizika od nastanka infekcija povezanih sa kateterom kod bolesnika na hemodijalizi

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Abstract

Background/Aim. Catheter-related infections are a significant morbidity and mortality cause in patients on hemodialysis. The objective of this study was to determine the incidence, to analyze risk factors and to identify etiological causes of catheter-related infections in these patients. **Methods**. The study was carried out at the Clinic for Nephrology and Clinical Immunology of the Clinical Centre of Vojvodina, from August, 2012 to May, 2015. One hundred and thirteen patients on chronic hemodialysis participated in the study. The risk factors of catheter-related infections in the infected patients were to those in the control group, as follows: demographic and laboratory parameters, co-morbidities and the use of immunosuppressive therapy, the length of hemodialysis treatment, urgent catheter placement, the position and placement difficulties, the number of insertions and catheter maneuvering, the existence of permanent vascular access in maturation or without a vascular access in the course of catheter positioning, catheter life, surgical procedures (≤ 30 days from catheter placing), the length of hospitalization and isolated infection causes. **Results**. One hundred and ninety-seven catheters were placed in 113 patients, among which 182 of them temporary. The total number of catheter days was 17.842, the incidence of infections was 3.53/1,000 catheter days. During the monitoring period, 63 catheter-related infections were diagnosed, 54 (85.7%) with temporary and 9 (14.3%) with permanent catheters. Multivariate logistic regression analysis (with border values/levels determined by receiver operating characteristic – ROC analysis) determined independent predictors of catheter-related infections in the following order: hemoglobin levels < 95 g/l ($p < 0.001$) and albumin levels < 33 g/l ($p = 0.041$), catheter duration of > 90 days ($p = 0.004$), > 2/day catheter maneuvering ($p = 0.011$) and the duration of hospitalization of > 15 days ($p = 0.003$). The main pathogen was *Staphylococcus spp.* Coagulase negative. **Conclusion**. Intensifying of prevention measures and infection control would significantly reduce the frequency of catheter-related infections and the number of hospitalizations. The timely creation of a native arteriovenous fistula would decrease the use of hemodialysis catheters.

Key words:
renal dialysis; catheter-related infections; risk factors.

Apstrakt

Uvod/Cilj. Infekcije povezane sa kateterom i dalje predstavljaju značajan uzrok morbiditeta i mortaliteta kod bolesnika na hemodijalizi. Cilj ovog ispitivanja je bio da se utvrdi incidencija, analiziraju faktori rizika i identifikuju etiološki uzročnici kateter infekcija kod ovih bolesnika. **Metode.** Ispitivanje je sprovedeno na Klinici za nefrologiju i kliničku imunologiju Kliničkog centra Vojvodine u periodu od avgusta 2012. do maja 2015. godine. Ispitivanjem je bilo obuhvaćeno 113 bolesnika lečenih hroničnim hemodijalizama. Upoređivani su faktori rizika od infekcija povezanih sa kateterom kod bolesnika sa dokazanom infekcijom u odnosu na kontrolnu grupu. Analizirani su demografski i laboratorijski parametri, komorbiditeti i upotreba imunosupresivne terapije, dužina dijaliziranja, urgentno plasiranje, pozicija i otežano plasiranje katetera, broj mesta insercije i manipulacija kateterom, postojanje trajnog vaskularnog pristupa u maturaciji ili bez vaskularnog pristupa tokom plasiranja katetera, dužina trajanja katetera, hiruške intervencije (≤ 30 dana od plasiranog katetera), dužina hospitalizacije i izolovani uzročnici infekcija. **Rezultati**. Kod 113 bolesnika plasirano je 197 katetera, od kojih su 182 bila privremena. Ukupni broj dana katetera iznosio je 17,842, a incidencija infekcija je bila 3.53 slučajeva na 1,000 kateter dana. Tokom perioda praćenja potvrđene su 63 kateter-povezane infekcije, 54 (85.7%) privremenih i 9 (14.3%) trajnih katetera. Multivarijantnom logističkom regresionom analizom (granične vrednosti određene *receiver operating curve* – ROC analizom), kao nezavisni prediktori kateter-povezanih infekcija dobijeni su: vrednost hemoglobina < 95 g/l ($p < 0.001$) i albumina <

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33g/l (p = 0.041), trajanje katetera > 90 dana (p = 0.004), > 2/dan manipulacije kateterom (p = 0.011) i trajanje hospitalizacije > 15 dana (p = 0.003). Najčešći uzročnik je bio *Staphylococcus spp.* koagulaza negativan. **Zaključak**. Intenziviranje mera prevencije i kontrole infekcija bi znatno smanjilo učestalost kateter-povezanih infekcija i broj hospitalizacija. Pravovremeno kreiranje nativne arteriovenske fistule smanjilo bi upotrebu dijaliznih katetera.

Ključne reči:
bubreg, dijaliza; kateter, povezane infekcije; faktori rizika.

Introduction

The number of patients at the end-stage of renal disease in the need of renal replacement therapy is rising in Europe and the world [1]. At the end of 2013, 5,775 patients were on renal replacement therapy, and the majority of them was on hemodialysis – 4,480 [2]. The quality of life and survival expectancy of those patients depend on the duration of functional vascular approaches (VA) [3]. Since the artery-vein fistula (AVF), which was depicted back in 1966 by Braescia and Cimino, has the longest survival rate and the least complication frequency, it should, whenever it is possible, be the first choice for VA [1]. Hemodialysis catheters are used for quick establishing of an adequate VA, when urgent hemodialysis is indicated in time of maturation of AVF and in patients in whom all other VA have been exhausted [1, 4]. Despite the priorities of AVF, nearly 80% of those patients start treatment with the dialysis catheters [3]. The previous papers have shown that VA is the major risk factor for the infection occurrence in the patients undergone hemodialyses. It is concluded that they suffer from a lesser risk of infections with AVF and artery-venous graft (AVG), and from a greater risk with temporary or permanent catheters [5]. The frequency of catheter-related infections ranges from 1.0 to 5.5 episodes in 1,000 catheter days [6–8]. In relation to the patients with the permanent VA, the ones who are dialyzed via catheters are hospitalized two to three times more due to infections [9]. The catheter infections cause a significant morbidity rate as well as the mortality increase for more than 50% in relation to the patients with the native AVF [10]. Besides, metastatic infections are present in 5% to 10% of patients with catheter sepsis in the form of osteomyelitis, endocarditis, septic arthritis and epidural abscess [11]. The risk infection factors related to catheters are the make and the position of catheters, and those related to patients are comorbidities, patients' hygienic conditions [12]. The most frequent etiological culprits of infections are gram-positive microorganisms, although bacteremia can be caused by gram-negative microorganisms as well. These patients are at risk of infections caused by hospital multi-resistant cultures that are less susceptible to many antibiotics [13]. The isolation of etiological agent and determining of antibacterial susceptibility profile are important to obtain a better prognosis [14].

The objective of this research was to determine the incidence rate, to analyze risk factors and to identify etiological causes of catheter-related infections in patients on chronic dialysis.

Methods

The research was conducted retrospectively at the Clinic for Nephrology and Clinical Immunology of the Clinical Centre of Vojvodina from Aug, 2012 to May, 2015. It included 113 hospitalized patients over 18 years of age on chronic dialysis treated for 4 hours, three times a week. The kind of dialysis was bicarbonate on polysulphate capillary membrane of the surface of 1.1 to 1.3 m^2, blood flow of 250–300 mL/min. The research involved the patients who had undergone an urgent temporary catheter placement (with or without AVF) in order to start an active treatment for renal insufficiency and the patients with replaced or permanent catheters due to maturation or loss of the existing permanent vascular access fistula and graft (AVF, AVG). The patients who were excluded from the research are: the patients with acute renal insufficiency, the patients from other hemodialysis centres temporarily dialyzed at our centre, the ones who started dialysis at home, the ones transferred from peritoneum dialysis to hemodialysis as well as transplant patients with no graft.

The risk factors for catheter-related infections were compared between the examined group and the control group of patients with no infections in the course of the research period. Since several patients had more infections on different catheter positions, every new infection of the placed catheter was analyzed. If one catheter in the course of the same hospitalization caused several infections, only the first infection entered the statistical data. Some patients had a temporary catheter placed for a longer period due to inability of permanent vascular placement (VP), and in such cases, the duration of temporary and permanent catheters was determined. Defining of urgently placed catheters meant quick solving of vascular access from vital indications in the patients with or without AVF/AVG in maturation. Placing difficulties caused swelling and hematomas or bleeding at the spot of catheter placement.

The following was analyzed: demographical and laboratory parameters, comorbidities, the use of immunosuppressive therapy, the length of dialyzing, urgent placing, the position and difficulty positioning, the number of insertion spots as well as catheter maneuvering, the existence of permanent vascular access in maturation or without vascular access during catheter placement, the length of catheter use, surgical procedures (≤ 30 days of catheter placement), the duration of hospitalization and the isolated infection causes.

Temporary catheters with two lumens were placed by an anesthesiologist by a modified Seldinger method, whereas the permanent ones were administered by vascular surgeons who, after the placement, drew catheter subcutaneously, i.e. tunneled it along the anterior rib cage wall using the aseptic technique [15]. After placing a non-tunneled catheter, prior turning it on, 10 mL of blood was aspirated from every rod, then every lumen was widened with 10 mL of 0.9% NaCl. In

tunneled catheters, the procedure was the same, only the widening of the catheter was 40 ml 0,9% NaCl (especially in femoral catheters which are longer). At the end of dialysis, after turning off, the procedure was repeated, and then the catheter lumens were aseptically locked with heparin. While determining the heparin volume, the values prescribed by producers and inscribed at every catheter lumens were carefully taken into account. Then, the exit was cleaned by benzine, Codane (uncolored alcohol solution) and Octanisept (Octenidine tenoxi ethanol) along with bandaging.

In the monitored period, all places of catheter insertion were checked, the swabs of the exit place and blood cultures were examined. Every time when infection was suspected, 3 sets of 7–10 mL of blood cultures were taken by vein puncture and from the lumens of dialytic catheters. Himedia Hi-Combi Dual Performance Medium HiSafe Blood Culturing System 40 mL was used for hemocultures. They were processed by standard laboratory technique and with the help of BacT/Alert 3D (Bact/Alert, bioMerienx, Marcy l Etoile France) of the automatic system for continual monitoring of hemocultures. After aseptic catheter removal, the tip of catheter of 5 cm was processed in a sterile lab container by standard microbiological method.

The diagnosis of catheter-related infections was defined by at least one blood culture and the culture from the catheter tip with the same pathogen along with clinical manifestations of the infection with no evidence of the other infection source [16].

If it was a bacteria that makes normal skin flora (*Staphylococcus spp*. Coagulase negative, etc.), the infection was included in the research only when the same microorganism was isolated in two blood cultures along with clinical manifestation of infection [17]. Demographic and laboratory data were collected along with medical documentation of the Clinic for Nephrology and Clinical Immunology. The following laboratory parameters were analyzed: hemoglobin, iron, ferritin, albumin, calcium and phosphorus. The analyses (Hg, Erci and Het) were done on the BECKMAN COULTER machine by HmX method impendence and flow citometry. The serum concentrations of calcium and phosphorus were processed by photometric method. Albumin serum was determined by a photometric color test on the OLYMPUS analyzer by using Beckman Coulter kits (Ireland). Iron levels and ferritin were processed on the Architech c8000 analyzer by Abbott company with commercial sets of the same company (Wiesbaden, Germany). Ferritin leves were obtained by the immunoturbimetric method, while iron by the colometric method.

In the course of this analysis, numerical data were shown by the mean value, standard deviation and median, whereas descriptive variables by absolute and relative numbers. The following statistical methods were used: χ^2, *t*-test, Mann-Whitney U test, receiver operating characteristic (ROC) analysis and multivariate logistic regression analysis.

Results

One hundred and ninety-seven catheters were placed in 113 patients, 182 of which were temporary. The total number of catheters was 17,842, with the infection incidence of 3.53 cases in 1,000 catheter days (the number of days of temporary ones was 14,521, and of permanent ones was 3,321, with the infection incidence of 3.72 and 2.71 cases in 1,000 catheter days). In the course of the monitoring period, 63 catheter-related infections were diagnosed, 54 (85.7%) temporary and 9 (14.3%) permanent catheters. Thirty-five patients (19.2%) had one infection, while 19 (10.4%) two or more infections. Nine (60%) patients using permanent catheters had two or more infections. During the monitoring period, 9 patients had their temporary catheters replaced by permanent ones, 3 patients had two permanent catheters grafted. Univariate analysis of catheter-related infection risk factors showed that the infection catheter group in relation to the control group had statistically significantly lower levels of hemoglobin, iron, albumin ($p < 0.005$): longer period of dialyzing, higher mean value of catheter duration (median 85:79), more insertion spots, a greater number of catheter maneuvering and a bigger number of hospital days (Tables 1 and 2). Multivariate logistic regression analysis (forward conditional model) of univariate significant risk factors for infection occurrence (border values determined by ROC analysis), as independent predictors of catheter-related infections, were singled out as follows: hemoglobin levels < 95 g/L ($p < 0.001$), albumin levels < 33g/L ($p = 0.041$), catheter life of > 90 days ($p = 0.004$), > 2/day catheter maneuvering ($p = 0.011$) and the length of hospitalization of > 15 days ($p = 0.003$) (Table 3).

Table 4 shows isolated causes of catheter infections. Gram-positive microorganisms were isolated in 55 (87,3%) patients. The most common pathogen was Coagulase-negative staphylococci 42.8%, then *Staphylococcus aureus* 31.7%, *Enterococcus faecalis* 7.9%, *Enterococcus spp*. 3.2% and *Enterococcus faecium* 1.6%. Gram negative (*Proteus mirabilis* and *Klebsiela pneumonia* 2%) and polymicrobic microorganisms were isolated in 6.3% patients.

Discussion

Catheter-related infections and sepsis are linked to a high morbidity rate and hospitalization, high treatment costs and a poor survival rate [17]. The previous studies dealt with diverse numerous risk factors related to catheter infections. According to literature, the use of central venous catheter, the most frequent risk factors for catheter-related infections in hemodialytic patients were: female gender, diabetes, anemia, hipoalbuminemia, urgent catheter placing, inadequate hygiene of hands prior catheter maneuvering and previous hospitalization [7, 18–20]. The infection incidence in our study was 3.53 cases in 1,000 catheter days (temporary 3.72; permanent 2.71 cases in 1,000 catheter days), similar to the results of certain previous studies [6, 7, 21].

The patients of both groups were of average age > 60 and did not differ in age in relation to catheter-related infections, which was confirmed by some studies opposed to the study by Murea et al. [22] in which the patients aged 75 had 60% less frequent infections [22–24]. However, our patients diagnosed with infection suffered from a significantly higher

Table 1

Risk factors with dialysis catheter infections (univariate analysis)

Risk factors	Catheter infections n = 63	No catheter infections n = 134	*p*
Gender (M/F), n/n	24/21	48/20	0.095
Age (years), $\bar{x} \pm SD$	63.4 ± 12.7	63.1±10.04	0.912
< 65, n (%)	34 (31.5)	74 (68.5)	
65–75, n (%)	12 (26.1)	34 (73.9)	0.392
≥ 75, n (%)	17 (39.5)	26 (60.5)	
DD (months), $\bar{x} \pm SD$	43.0 ± 44.6	23.4 ± 26.1	0.004*
Co-morbidities, n (%)			
hypertension	58 (92.1)	120 (89.6)	0.766
coronary disease	7 (11.1)	22 (16.4)	0.444
cardiomyopathy	43 (68.3)	79 (59.0)	0.273
acute brain stroke	14 (22.2)	18 (13.4)	0.176
diabetes mellitus	28 (44.4)	50 (37.3)	0.425
DSD	32 (50.8)	58 (43.3)	0.405
PAOD	6 (9.5)	6 (4.5)	0.288
HOPD	3 (4.8)	7 (5.2)	0.597
malignant disease	1 (1.6)	11 (8.2)	0.059
Hemoglobin (g/L), $\bar{x} \pm SD$	93.1 ± 9.1	106.2 ± 10.5	< 0.001*
Iron (µg/L), $\bar{x} \pm SD$	9.5 ± 5.9	11.2 ± 6.1	0.026*
Ferritin (µg/L), $\bar{x} \pm SD$	742 ± 594	668 ± 605	0.211
Calcium (mmol/L), $\bar{x} \pm SD$	2.21 ± 0.23	2.27 ± 0.21	0.055
Phosphorus (mmol/L), $\bar{x} \pm SD$	1.83 ± 1.37	1.77 ± 0.41	0.328
Albumin (g/L), $\bar{x} \pm SD$	32.4 ± 5.8	35.8 ± 5.4	< 0.001*

DD – dialysis duration; DSD – digestive system diseases ; PAOD-peripheral artery occlusive disease; HOPD – chronic obstructive pulmonary disease; M/F – male/female; *$p < 0.05$.
$\bar{x}$ – mean; SD – standard deviation; n (%) – number (percentage) of patients.

Table 2

The risk factors in dialysis catheter infections (univariate analysis)

Risk factors	Catheter infections n = 63	No catheter infections n = 134	*p*
Position of temporary/permanent catheter, n/n			
v. jugularis	35/5	93/2	0.201/0.174
v. subclavia	10/2	25/4	
v. femoralis	9/2	10/0	
Catheter duration (days), $\bar{x} \pm SD$	120.8 ± 98.0	77.3 ± 74.0	
< 30, n (%)	11 (22.9)	37 (77.1)	
30–90, n (%)	24 (27.3)	64 (72.7)	0.017*
> 90, n (%)	28 (45.9)	33 (54.1)	
Number of catheter insertions			
1, n (%)	38 (33.9)	74 (66.1)	
2, n (%)	6 (15.8)	32 (84.2)	
≥ 3, n (%)	19 (40.4)	28 (59.6)	
Urgent catheter placement , n (%)	19 (30.2)	55 (41.0)	0.189
AVF[a] , n (%)	26 (26.0)	74 (74.0)	
AVG[a] , n (%)	3 (25.0)	9 (75.0)	
No access[b] n (%)	34 (40.0)	51 (60.0)	
Catheter maneuvering difficulties, n (%)	13 (20.6)	13 (9.7)	0.059
Catheter maneuvering ≥ 3/days, n (%)	14 (22.2)	14 (10.4)	0.026*
Surgical procedures, n (%)	25 (39.7)	46 (34.3)	0.283
Length of hospitalization, (days) $\bar{x} \pm SD$	34.62 ± 33.7	23.37 ± 25.2	0.010*
Immunosuppressive therapy, n (%)	0 (0.0)	7 (5.2)	0.064

AVF – arteriovenous fistula; AVG-arteriovenous graft; [a] Permanent vascular access in maturation; [b] No permanent vascular access or the loss of it; *$p < 0.05$.
$\bar{x}$ – mean; SD – standard deviation; n (%) – number (percentage) of patients.

Table 3

Risk factors with dialysis catheter infections (multivariate analysis)

Risk factors	Beta	SD	*p*	OR
Hemoglobin < 95 g/L	2.505	0.410	< 0.001	12.2
Albumin < 33 g/L	0.808	0.395	0.041	2.2
Catheter duration > 90 days	1.188	0.418	0.004	3.3
Catheter maneuvering > 2/day	1.403	0.551	0.011	4.1
Hospitalization duration > 15 days	1.177	0.402	0.003	3.2
Model constant	-3.683	0.526	< 0.001	0.02

SD – standard deviation; *p* – probability; OR – odds ratio.

Tabela 4

Frequency of isolated pathogens

Pathogen	n (%)
Gram positive microorganisms	55 (87.3)
Staphylococcus species Coagulase-negative	27 (42.8)
Staphylococcus aureus	20 (31.7)
Enterococcus faecalis	5 (7.9)
Enterococcus species	2 (3.2)
Enterococcus faecium	1 (1.6)
Gram negative microorganisms	4 (6.3)
Proteus mirabilis	2 (3.2)
Klebsiela pneumoniae	2 (3.2)
Polymicrobic flora	4 (6.3)

medium length of hospitalization, which can be explained by exhaustion of permanent vascular approaches along with a more frequent need to replace temporary catheters and eventually the need to replace them with permanent ones. The frequency of the monitored stages and disorders was similar to other developed countries [25]. The patients of the advanced age have complex changes of the immune system and very often conjoint chronic diseases such as systemic hypertension and diabetes [26]. Grothe et al. [27] have shown that diabetic and hypertonic patients have 22% more chances to develop catheter infection. Since it is well-known that diabetes leads to impaired immune system and in combination with immunosuppressive uremia, it can lead to a higher risk of bacteremia; the link between diabetes and catheter infections is confirmed in certain studies [7, 27]. While some authors highlight that simultaneous renal insufficiency and surgical procedure, hematological malignancies, neutropenia that lasts longer than 8 days and presence of coalesced immunodeficiency have a role in infection development, some others did not validate this connection [23, 28]. In our study, we did not link comorbid states and the use of immunosuppressive therapy with dialytic catheter infections, as opposed to significantly lower values of hemoglobin, iron and albumin in relation to the control group of patients, which corresponds with previous study results [7, 14, 20]. The lower hemoglobin levels can be related to the risk of iron overload, which increases the colonization of bacteria and weakens the phagocyte function [29]. Hypoalbuminemia is frequent in dialytic patients due to malnutrition and it contributes to the occurrence of bacterial infection [30]. Lukowsky et al. [31] have shown that one third of deaths in the first 90 days of hemodialysis was related to hypoalbuminemia < 35 g/l. Multivariate analysis showed that the albumin levels of < 33 g/L and hemoglobin levels of < 95 g/L are significantly independent predictors of temporary catheter infection, which refers to a compulsory hypoalbuminemia and anemia correction in order to decrease infections. We have not found the difference between the patients with or without catheter infection in relation to ferritin values, which corresponds with the study results in which the infections of temporary and permanent vascular approaches were analyzed [7, 21].

In terms of the planned VA treating, the risk of catheter-related bacterial infections increases 2.21 times in urgent cases when it is necessary to place or replace a catheter within 24 hours [20]. In 37.6% of catheters that were urgently placed (with or without AVF/AVG in maturation), we did not diagnosed significantly more infections, which is a slightly lower percentage in relation to the same results of other studies [14, 32, 33].

According to the literature data, the most widespread position of temporary catheters is in interior jugularis vein, which is shown in both groups of our patients [14, 34]. Although vascular access societies and the European Best Practice Guidelines (EBPG) for hemodialysis advise on using the right jugularis vein as the way to place tunneled vascular catheters, the most widespread of these catheters in our infected patients was the jugularis vein, as opposed to subclavia vein in the control group [35]. It was not established that the catheter position was a significant risk factor for temporary or permanent catheter-related infections. In earlier studies, the greatest frequency of infections was with temporary femoral catheters, followed by jugularis then the access under clavicle. According to Caylan et al. [20] the position of temporary catheters in femoral vein creates 2.14 times bigger risk for catheter-related infections occurrence. However, the results of recent studies do not link the insertion spot of temporary and permanent catheters to a higher risk of infections [36–38].

Other analyzed risk factors include, according to univariate analysis, a greater number of insertion spots and frequent catheter maneuvers. Catheter maneuvering > 2/day was established by multivariate analysis, creates 4.1 times bigger risk of catheter-related infection occurrence. Caylan et al. [20] concluded that inadequate hygiene of hands immediately before catheter maneuvering created higher risk that maneuvering itself ≥ 3/day. Catheter placing difficulty was not a significant factor for catheter infections in our patients, which was shown by Gauna et al. [14]. During hospitalization of the catheter placed patients, we have not found that a permanent VP in maturation or its loss influenced infection occurrence. A big comprehensive recent analysis has shown that starting catheter dialysis long before AVF predicts, that a continual use of catheter as a dialytic access during one year increasea a infection risk. Over 13% of all patients had at least one positive blood culture in the first year of starting dialysis, and the risk infection was 3 times higher in the patients with catheter in relation to AVF [39].

The use of vascular catheters in Serbia is smaller the one in Dialysis Outcomes anal Practice Patterns Study

(DOPPS) countries, where they are used as a permanent access in 4–18% patients, if we exclude Japan with 1% of the patients, although it is bigger than those recommended by the Vascular Access Society and European Guide [35, 40]. The explanation was similar to the findings from previous years: permanent catheters are positioned in a small number of reference centres, procurement discontinuity, the existence of patients with exhausted vascular access, numerous comorbidities and a short survival expectancy period [2]. In relation to the above mentioned, it is not unexpected that infection occurs in cases of significantly shorter catheter use in comparison to the control group, which is depicted in most studies, except for one where no statistical significance was found [5–7, 41, 42]. Fram et al. [19] analyzed the duration of temporary and permanent vascular access by using three periods (0–30, 30–180, > 180 days) and concluded that the infection incidence is significantly higher up to 30 days, which highlights the need to careful implementation of prevention measures in the course of catheter positioning [19]. Napalkov et al. [43] showed that most infections caused by temporary catheters occurred in the first 90 days, notably, that the incidence rate was 5.1/1,000 catheter days, and that the infection risk was higher up to 6 months from catheter positioning, which leads to a conclusion that catheter treatment should be intensified in the mentioned period. It was concluded by multivariate analysis that in the period of > 90 days, the risk of infection is 3.3 times higher, but the sample of permanent catheters in our patients was relatively small and some temporary catheters were used as permanent vascular access. According to the valid recommendations, catheters should not be changed in order to prevent infections, but it is necessary to decrease their use by ensuring matured AVF. Besides a smaller infection risk, we are fully aware of other advantages of permanent catheters in relation to temporary ones. Permanent catheters are better than temporary ones even in intensive care units if catheter is expected to stay longer than 3 weeks [44].

The implementation of immunosuppressive therapy and surgical interventions (≤ 30 days from catheter positioning), were not related to infections in our patients, which corresponds with the results of two other studies [14, 20].

The duration of hospitalization was a significant risk factor for the occurrence of infections in our patients, notably the infection risk increased 3.2 times in the period of hospitalization of > 15 days. The previously mentioned risk factor for dialytic catheter infections was confirmed in certain studies [42, 45–47].

Most infections were a result of gram positive microorganisms (87.3%), which is confirmed by most studies, although the presence was from 33% to 72.8% [7, 8, 19–21]. Two most common isolated gram-positive pathogens were *Staphylococcus Spp*. Coagulase negative and *Staphylococcus aureus*, which was not surprising considering that both of them have skin origin. The sum data from 1992 to 1999 indicate that *Staphylococcus Spp*. Coagulase negative is the most isolated pathogen for hospital-catheter-related infections (37%), followed by (13.5%) [48]. In comparison to more recent studies, the most common isolated pathogens were *Staphylococcus aureus* and *Staphylococcus epidermis* [5, 7–9, 13, 14]. In our patients, *Staphylococcus* was isolated in (31.7%) cases after *Staphylococcus spp*. Coagulase negative (42.8%), and according to the percentage of incidence, it corresponds with the results of most studies [7, 19–21]. Isolated *Staphylococcus aureus* is potentially lethal, and the annual frequency of its incidence in dialysis patients is between 6 and 27% [49, 50]. Mokrzycki et al. [51] have shown that the infection of tunneled catheters caused by this pathogen causes more than 3 times higher risk of complications due to infection, as well as 4 times higher risk of recurrent bacterial infection or death within 3 months in relation to other pathogens. However, two studies have shown that *Staphylococcus aureus* is the most frequent gram positive microorganism, although the most present ones were gram-negative bacteria isolated from blood culture [14]. Gram-negative microorganisms were isolated in 26.9 (56%) cases, while fungus was less common [7, 8, 19–21]. The infections caused by gram-negative bacteria are difficult to treat due to high resistance and they can cause nosocomial infections and are frequently linked with high mortality rate [52]. Polymicrobial pathogens can be present in 9.5–11% of catheter-related bacterial infections [22]. In our study, a slightly lower percentage of gram-negative bacteria and polymicrobial pathogens were isolated (6.3%). This study had certain limitations that were typical in retrospective data collecting character. Since it is an intersection study, the data on VA patients that had been treated by chronic dialyses were not included.

Conclusion

The levels of hemoglobin of < 95g/L, and albumin of < 33 g/L, the duration of catheter treatment of > 90 days, > 2/day manipulations and the length of catheter hospitalization of > 15 days are independent predictors of catheter-related infections. Anemia correction, better nourishment, reduced rate of dialysis catheters by timely AVF introducing along with enhancing prevention measures and infection control recommended by official guidelines and adjusted to the conditions of hemodialysis unit by multidisciplinary team supervised by epidemiologists would significantly lower the frequency of catheter-related infections and the number of hospitalizations.

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Association between serum concentration of parathyroid hormone and left ventricle ejection fraction, and markers of heart failure and inflammation in ST elevation myocardial infarction patients treated with primary percutaneous coronary intervention

Udruženost serumske koncentracije paratireoidnog hormona i ejekcione frakcije leve komore, markera srčane insuficijencije i inflamacije u akutnom infarktu miokarda sa ST elevacijom lečenim primarnom perkutanom koronarnom intervencijom

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Abstract

Background/Aim. Previous studies have shown increased serum concentration of parathyroid hormone (PTH) in acute myocardial infarction and heart failure. In this study we examined the relationships between parathyroid hormone status and biochemical markers of myocardial injury and heart failure, as well as electrocardiographic (ECG) and echocardiographic indicators of infarction size and heart failure. **Methods.** In 390 consecutive patients with ST segment elevation myocardial infarction (STEMI), average age 62 ± 12 years, laboratory analysis of serum concentrations of creatine kinase MB isoenzyme (CK-MB), C-reactive protein (CRP) and intact PTH and plasma concentration of brain natriuretic peptide (BNP) were done during the first three days after admission. All patients were treated with primary percutaneous coronary intervention (PCI). Exclusion criterion was severe renal insufficiency (glomerular filtration rate ≤ 30 mL/min). Serum concentration of PTH was measured on the 1st, 2nd and, in some cases, on the 3rd morning after admission and maximum level of PTH was taken for analysis. Patient cohort was divided into four groups according to quartiles of PTH maximum serum concentration (I ≤ 4.4 pmol/L; II > 4.4 pmol/L and < 6.3 pmol/L; III ≥ 6.3 pmol/L and < 9.2 pmol/L; IV ≥ 9.2 pmol/L). Selvester's ECG score, left ventricle ejection fraction and wall motion index (WMSI) were determined at discharge between 5–14 days after admission. **Results.** We found that LVEF at discharge significantly decreased ($p < 0.001$) and WMSI at discharge and ECG Selvester´s score significantly increased across the quartiles of PTH max. level ($p < 0.001$ for both parameters). BNP, CRP and CK-MB isoenzyme level significantly increased across the quartiles of PTH max. level ($p < 0.001$; $p < 0.001$ and $p = 0.004$, retrospectively). **Conclusion.** The patients in the 4th quartile of PTH had significantly lower LVEF and higher WMSI and Selvester's ECG score at discharge. This group of patients also had higher levels of BNP, CRP and CK-MB in blood in the early course of STEMI.

Key words:
parathyroid hormone; st elevation myocardial infarction; heart failure; biomarkers.

Apstrakt

Uvod/Cilj. U prethodnim studijama pokazano je povećanje serumske koncentracije paratireoidnog hormona (PTH) u akutnom infarktu miokarda i srčanoj insuficijenciji. U ovom istraživanju ispitali smo odnos između paratireoidnog statusa i biohemijskih, elektrokardiografskih i ehokardiografskih pokazatelja veličine infarkta i srčane insuficijencije. **Metode.** Kod 390 bolesnika sa akutnim infarktom miokarda sa elevacijom ST segmenta (STEMI), prosečne dobi 62 ± 12 godine, učinjene su laboratorijske analize serumske koncentracije kreatin kinaza MB frakcije (CK-MB), C-reaktivnog proteina (CRP) i intaktnog PTH i koncentracija u plazmi moždanog natriuretskog peptide (BNP) tokom prva tri dana od prijema. Svi bolesnici su lečeni primarnom perkutanom koronarnom intervencijom (PKI). Bolesnici sa težom bubrežnom insuficijencijom isključeni su iz studije (klirens kreatinina ≤ 30 mL/min). Serumska koncentracija PTH određivana je prvog, drugog i, u nekim slučajevima, trećeg dana posle prijema i najveća dobijena koncentracija je uzeta za analizu.

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Kohorta bolesnika je podeljena u četiri grupe na osnovu kvartila maksimalne izmerene serumske koncentracije PTH (I ≤ 4.4 pmol/L; II > 4.4 pmol/L i < 6.3 pmol/L; III ≥ 6.3 pmol/L i < 9.2 pmol/L; IV ≥ 9.2 pmol/L). Selvesterov EKG skor, ejekciona frakcija leve komore (EFLK), i indeks pokretljivosti zidova leve komore (WMSI) su određivani na otpustu bolesnika, između 5–14 dana hospitalizacije. **Rezultati.** Ustanovljeno je da se na otpustu EFLK statistički značajno smanjuje ($p < 0.001$), WMSI i EKG Selvesterov skor statistički značajno povećavaju sa većim kvartilima PTH max. koncentracije ($p < 0.001$ za oba parametra). BNP, CRP i CK-MB nivoi značajno se povećavaju sa većim kvartilima max. koncentracije PTH ($p < 0.001$; $p < 0.001$ $p = 0.004$, retrospektivno). **Zaključak.** Bolesnici u četvrtom kvartilu PTH imaju manju EFLK i veći Selvesterov EKG skor i WMSI od ostalih bolesnika. Ovi bolesnici takođe imaju i značajno veću koncentraciju BNP, CRP i CK-MB u ranoj fazi akutnog infarkta miokarda sa ST elevacijom (STEMI).

Ključne reči:
paratireoidni hormon; infarkt miokarda sa st elevacijom; srce, insuficijencija; biološki pokazatelji.

Introduction

Parathyroid hormone (PTH) has some cardiovascular effects which can be important for response to acute myocardial injury and acute heart failure [1–4]. Through the receptors on smooth mucle cells, PTH induces systemic arterial and coronary vasodilatation [5–7] and through the receptors on cardiomyocytes it produces chronotropic and inotropic effect on the heart [5–7]. It is one of the main messengers for the mobilization and homing of bone marrow derived stem cells which can partly regenerate the damaged myocardial muscle [8–11].

The serum concentration of PTH increases in acute ST elevation myocardial infarction (STEMI) [12, 13] and can even predict mortality in critically ill patients [14]. Cathecholamine stress and autonomic nervous system are probably the main inducers of the increased PTH release in circulation during acute myocardial infarction [15–17]. Large myocardial infarction causes haemodynamic compromise and huge reaction of supra-adrenal gland and sympatic nervous system which activate several hormonal systems including PTH which play an important role in the struggle to maintain sufficient circulation and perfusion of the vital organs [18].

We have previously shown that PTH can be a marker of acute heart failure in patients with STEMI treated with primary percutaneous coronary intervention (PCI). The aim of this study is to establish correlations between serum concentration of PTH and several parameters which are associated with the prognosis of STEMI patients such as Selvester's ECG score, left ventricular ejection fraction (LVEF), wall motion score index (WMSI), and conventional biomarkers of heart failure, brain natriuretic peptide (BNP), C-reactive protein (CRP) and creatine kinase MB isoenzyme (CK-MB).

Methods

Study population and design

In this study, we included 390 consecutive patients admitted to the Coronary Care Unit of the Military Medical Academy (MMA) in Belgrade between Decembar 2008 and June 2015 because of the STEMI. The diagnosis of STEMI was established according to current guidelines of the European Society of Cardiology (ESC) and the American College of Cardiology/American Heart Association (ACC/AHA) (typical chest pain lasting > 20 minutes, electrocardiographic (ECG) changes consisting of ST-segment elevation in at least two contiguous precordial leads ≥ 2 mm in men over the age of 40 years, ≥ 2.5 mm in men below the age of 40 years, ≥ 1.5 mm in women in leads V2–V3 and/or ≥ 1.0 mm in other leads, or horizontal or descending ST depression ≥ 0.5 mm and/or T inversion ≥ 0.1 mV in V1–V3 precordial leads with prominent R wave or R/S ratio > 1 with hypokinesis or akinesis of the posterior left ventricle wall at admission echocardiography examination, confirmed with plasma CK-MB or troponin serum concentration elevation. All patients were treated with primary PCI (less than 12 hours after beginning of the chest pain) with adjunctive drug therapy and according to the ESC and the ACC/AHA guidelines. There was no age limit for study enrollment.

At admission, detailed medical history was taken for all patients, especially with regard to risk factors for ischemic heart disease and a complete physical examination with 12-lead ECG. Urgent laboratory analysis were also done for all patients at admission, including troponins and CK-MB. CK-MB was taken every 6 hours during the first 24 hours and maximum concentration was taken for analysis. Blood samples for PTH, BNP, CRP were taken from an antecubital vein at 8 am, i.e. before a meal. PTH serum concentration was measured on the first and second morning after admission, if the second measurement was higher than the first one, the third measurement was done. The highest concentration was taken for analysis. CRP was taken on the first and second morning and highest concentration was taken for analysis. BNP was taken on the first morning after admission. Echocardiographic assessment was done before discharge for all patients, usually on the days 5 to 8 of hospitalization.

The main exclusion criterion was low creatinine clearance (less than 30 mL/min). Beside renal dysfunction, other exclusion criteria were the presence of known malignant, infectious or autoimmune disease and death during the first 24 hours of the first hospitalization day.

The study was conducted according to the Declaration of Helsinki and was approved by the MMA Ethical Committee. Written informed consent was obtained from all participating patients.

Clinical and echocardiographic assesment

For all patents detailed history of risk factors for coronary artery disease was taken and complete physical examination at the admission to asses hemodynamic stability.

Selvester´s ECG score was done as an ECG metod for estimating myocardial infarction size. We used simplified score of 37 criteria, 29 point system for scoring ECG at discharge. All patients underwent a two-dimensional Doppler echocardiography examination at discharge (GE Vivid 7 and Philips iE 33) which was performed in the left lateral position. Left ventricle (LV) systolic function was assessed by ejection fraction and WMSI. Left ventricular ejection fraction was quantified by the Simpson method according to the American Society of Echocardiography (ASE) and the European Association of Echocardiography (EAE) guidelines. Wall motion score index was calculated according to 17-segments model (ASE and EAE guidelines).

Coronary angiography

All coronary angiograms were done in MMA. Angiographic thrombolysis in miocardial infarction (TIMI) flow grade of the infarction artery was estimated before and after completion of PCI according to four grades of flow according to standard TIMI criteria [19]. Multivessel disease was defined as 70% or greater stenoses in at least one major epicardial artery and 50% or greater stenoses in at least one other major coronary artery [20]. All angiograms were reviewed by two independent interventional cardiologists.

Laboratory testing

Creatine kinase MB was determined in the serum of patients by immunoinhibition method on the commercial Dimension Clinical Chemistry System (Siemesn Heathcare Diagnostics), at admission and every 6 hours during the next 24 hours. C-reactive protein extended range was determined in the serum of patients with a particle enhanced turbidimetric immunoassay on commercial Dimension system on the first and second day in the morning before a meal. Brain natriuretic peptide was detemined in plasma by chemiluminescence immunoassay (ADVIA Centaur XP analyzer; Siemens Medical Solutions, Fernwald, Germany) on the first day of hospitalization in the morning before a meal. Intact PTH serum levels was determined from the venous blood sample withdrawn on the first, second and third (if the patient had the second level of PTH lower than the first one and was clinically stable, we did not take the 3rd sample) morning after admission, before a meal (15±8 hours from the admission). Intact PTH was measured in fresh serum during 4 hours from sampling by a commercial two-site sandwich immunoassay using chemiluminometric detection technology. Intact PTH was measured on ADVIA Centaur analyzer (Siemens Medical Solutions, Fernwald, Germany). The reference range from the test is 1.60–7.00 pmol/L and intra-assay coefficient of variation is 2.7%.

Stastitical analysis

In the study we used the methods of descriptive statistics: continuous variables are presented as a mean with standard deviation (SD), or as a median with interquartile range (IQR) depending on the distribution of data. Categorical variables are reported as counts with percentages. According to the quartiles of maximum PTH serum concentration, the patients were split into 4 groups. Differences between the distribution of categorical variables among the 4 groups of patients were estimated by the χ^2 test. Differences in age, systolic blood pressure and heart rate were calculated with two-way ANOVA. The differences among the levels of LVEF, WMSI, plasma levels of BNP, serum levels of CK-MB and CRP and Selvester's score were compared across the quartiles of PTH maximum with Kruskal Wallis test. *P* value less than 0.05 was considered significant. The differences between quartile 1 and 2, quartile 2 and 3 and quartile 3 and 4 for all variables were tested by the Mann-Whitnney test (*p* value less than 0.05 was also considered significant). All analyses were performed by using the SPSS version 21 (SPSS Inc, Chicago, IL, USA).

Results

There were 390 patients included in the study, average age 62 ± 12 (ranging ftom 32 to 87 years), where 108 (27.7%) were females and 282 (72.3%) were males.

We divided all patients in quartiles according to serum concentration of parathyroid hormone (I ≤ 4.4 pmol/L; II > 4.4 pmol/L and < 6.3 pmol/L; III ≥ 6.3 pmol/L and < 9.2 pmol/L; IV ≥ 9.2 pmol/L). The demographic and clinical characteristics of the patient population are shown in Table 1.

The demographic and clinical characteristics of the patients

We studied 390 patients and three quarters of them were men ($p = 0.050$). There was a statistically significant difference between quartiles of the patients by the age (58 ± 11 vs. 60 ± 12 vs. 63 ± 12 vs. 67 ± 12) respectively $p < 0.001$. Also, there was a significant difference among quartiles of the patients in the data of arterial hypertension ($p = 0.038$) and diabetes mellitus ($p = 0.008$). With the higher PTH max. level, the incidence of hypertension was higher. However, information about diabetes was questionable because diabetes was less common in the 2nd quartile of PTH max. which is probably an incidental finding. We did not find a significant difference between quartiles of the patients in the data of active smoking ($p = 0.166$) and hypercholesterolemia ($p = 0.688$).

There were significantly larger number of patients with Killip class greater than I in the 4th quartile of PTH max. level than in the 1st (11.5% vs. 1.0%; $p < 0.001$). In accordance with that data, we found that heart rate on the admission was significantly higher in the 4th quartile of PTH max. level than in the 1st quartile (84.9 ± 24.7 beats/min vs. 74.2 ± 18.8 beats/min; $p = 0.001$), but, on the contrary, systolic blood pressure on the admission showed no significant difference among quartiles of PTH max. level (132.2 ± 23.3 mmHg vs. 132.6 ± 25.3 mmHg vs. 137.0 ± 29.8 mmHg vs. 126.2 ± 35.3 mmHg; $p = 0.078$).

Table 1

The demographic, clinical and procedure related characteristics of the patients

	All	Q1	Q2	Q3	Q4	*p*
Age (years), mean ± SD	61.57 ± 12.016	58.42 ± 10.915	59.98 ± 12.205	62.94 ± 11.667	66.63 ± 11.778	< 0.001
Female, n (%)	108 (72.3)	23 (5.8)	22 (5.6)	26 (6.7)	37 (9.57)	0.050
Risk factors, n (%)						
active smokers	197 (51.4)	55 (14.4)	53 (13.8)	50 (13.1)	39 (10.1)	0.166
arterial hypertension	282 (72.3)	63 (16.2)	66 (16.9)	75 (19.2)	78 (20.2)	0.038
diabetes mellitus	101 (26.0)	34 (8.7)	14 (3.8)	24 (6.2)	29 (7.5)	0.008
hypercholesterolemia	217 (56.4)	50 (13.0)	57 (14.8)	58 (15.1)	52 (13.5)	0.688
Infarction related artery territory, n (%)						0.739
LM	2 (0.5)	1 (0.3)	1 (0.3)	0 (0.0)	0 (0.0)	
LAD	170 (43.6)	40 (10.3)	42 (10.8)	41 (10.5)	47 (12.1)	
RCx	61 (15.6)	12 (3.1)	15 (3.8)	17 (4.4)	17 (4.4)	
RCA	157 (40.3)	45 (11.5)	39 (10.8)	41 (10.5)	32(8.2)	
Killip class > 1 at admission, n (%)	66 (16.9)	4 (1.0)	7 (1.8)	10 (2.6)	45 (11.5)	0.000
Previous infarction, n (%)	56 (14.4)	15 (3.8)	11 (2.8)	8 (2.1)	22 (5.6)	0.021
Multivesel disease, n (%)	256 (65.8)	52 (13.4)	65 (16.7)	72 (18.5)	67 (17.2)	0.017
Systolic arterial pressure at admission, mean ± SD	132.07 ± 28.961	132.24 ± 23.332	132.64 ± 25.371	137.00 ± 29.798	126.23 ± 35.327	0.078
Heart rate at admission, Mean ± SD	77.83 ± 20.938	74.23 ± 18.842	74.62 ± 18.842	74.65 ± 18.951	84.93 ± 24.710	0.001
Total ischemic time in hours – median (IQR)		3.00 (2.00-6.00)	4.00 (2.50-7.50)	3.00 (2.88-6.00)	5.00 (3.00-9.00)	0.002
TIMI flow before PCI, n (%)						
0/1	297 (76.2)	74 (19.0)	78 (20.0)	72 (18.5)	73 (18.5)	0.799
0/3	48 (12.3)	10 (2.6)	11 (2.8)	14 (3.6)	13 (3.3)	
TIMI flow after PCI, n (%)						0.001
0/1	14 (3.6)	2 (0.5)	1 (0.3)	3 (0.8)	8 (2.1)	
0/3	318 (81.5)	92 (23.6)	79 (20.3)	80 (20.5)	67 (17.2)	
Implantation of stents, n (%)	337 (86.4)	89 (22.8)	85 (21.8)	87 (22.3)	76 (19.5)	0.102
GP inhibitors, n (%)	97 (24.9)	25 (6.4)	23 (5.9)	27 (6.9)	22 (5.6)	0.898
Clopidogrel before PCI, n (%)	321 (82.3)	70 (17.9)	82 (21.0)	85 (21.8)	84 (21.5)	0.12
Ticagrelor before PCI, n (%)	69 (17.7)	28 (7.2)	15 (3.8)	14 (3.6)	12 (3.1)	

LM – left main coronary artery; LAD – anterior descending artery; RCx – right circumflex artery; RCA – right coronary artery; IQR – interquartile range; TIMI – thrombolysis in myocardial infarction; PCI – primary percutaneous coronary intervention; SD – standard deviation; Q1 – first quartile; Q2 – second quartile; Q3 – third quartile; Q4 – fourth quartile.

Every seventh patient had a previous myocardial infarction, i.e. 56 (14.4%) patients and we found that the number of patients with a previous myocardial infarction was significantly higher in the quartile 4 ($p = 0.021$).

The time from symptom onset until reperfusion occured was defined as total ischemic time (TIT). It was significantly different among quartiles of PTH max. level ($p = 0.002$). In the 1st quartile of PTH max. TIT was 3.00 (IQR 2.00–6.00) hours and in the 4th quartile TIT was 5.00 (IQR 3.00–9.00) hours.

Procedure related characteristics of the patients

As described in the methodology of this study, all patients were treated with primary PCI. About two thirds of the patients had multivessel disease, i.e. 256 (65.8%) patients. There were statistically significant differences among quartiles of the patients according to the presence of multivessel disease (52, 13.4% vs. 65, 16.7% vs. 72, 18.5% vs. 67, 17.2%; $p = 0.017$). Also, we analyzed which artery was most commonly infarction related artery (IRA). Infarct related artery in almost half of the cases was left anterior descending (LAD) artery, i.e. in 170 (43.6%) patients LAD was IRA. Right coronary artery (RCA) was IRA in 157 (40.3%) patients, left circumflex artery (LCx) was IRA in 61 (15.6%) patients and left main (LM) coronary artery in 2 (0.5%) patients. Statistically significant differences among quartiles of the patients according to the IRA were not found ($p = 0.739$).

TIMI flow grade of the infarction-related artery was assessed before and after PCI by two interventional cardiologist. Before PCI, majority of the patients (76.2%) had TIMI flow grade 0 or 1 and no statistically significant differences among quartiles of the patients were found ($p = 0.799$). Majority of patients (81.5%) had TIMI flow grade 3 after PCI and no statistically significant differences among quartiles of the patients according to TIMI flow grade after PCI were found ($p = 0.001$).

Majority of patients (86.4%) had stent implantation during PCI and there were no statistically significant differences among quartiles according to the stent implantation ($p = 0.102$). One-quarter of the patients received glycoprotein (GP) inhibitors before PCI with no statistically significant differences among quartiles ($p = 0.898$). All patients received oral antiplatelet therapy with aspirin and a $P2Y_{12}$ receptor blocker. Majority of the patients received clopidogrel (82.3%) and ticagrelor was

used in 17.7% of all patients. There were no statisticaly significant differences found among quartiles of the patients according to the oral antiplatetet therapy ($p = 0{,}120$).

Relation between Selvester´s ECG score, LVEF and WMSI with quartiles of PTH

Selvester´s ECG score significantly increased in the 4th quartile of PTH level ($p < 0.001$), as shown in Figure 1. As a continuous variable Selvester´s ECG score is presented as the median with IQR. Quartile 1 had value 9.00 (IQR 4.50–18.00), quartile 2 had value 9.00 (IQR 3.00–15.00), quartile 3 had value 12.00 (IQR 6.00–21.00) and quartile 4 had value 15.00 (IQR 9.00–24.00). It could be concluded, that the 4th quartile of PTH max. level, i.e. PTH level above the reference limit, had the significantly higher Selvester´s ECG score.

Ejection fraction significantly decreased in the fourth of PTH level ($p < 0.001$) as shown in Figure 2. Medians with IQR for ejection fracton were: for the 1st quartile 50.00% (IQR 45.00–55.00%), for the 2nd quartile 50.00% (IQR 43.00–55.00%), for the 3rd quartile 48.00% (IQR 45.00–55.00%) and for the 4th quartile 40.00% (IQR 32.25–50.00%). The 4th quartile of PTH max. (PTH serum concentration above reference range) had significantly lower ejection fraction.

WMSI at discharge significantly increased in the 4th quartile of PTH level ($p < 0.001$), Figure 3. Medians with IQR for WMSI were: for the 1st quartile 1.31 (1.19–1.50), for the 2nd quartile 1.37 (1.19–1.61), for the 3rd quartile 1.38 (1.25–1.56) and for the 4th quartile 1.6250 (1.3100–1.8100). The patients in higher PTH max. quartiles had more pronounced regional myocardial dysfunction.

Relation between BNP, CRP and CK-MB with quartiles of PTH

Brain natriuretic peptide level significantly increased in the 4th quartile of PTH level ($p < 0.001$; Figure 4). Medians with IQR for BNP are: for the 1st quartile 199.49 pg/mL (87.43–296.55 pg/mL), for the 2nd quartile 163.90 pg/mL (80.20–320.00 pg/mL), for the 3rdt quartile 251.50 pg/ml (111.30–449.10 pg/mL) and for the 4th quartile 580.63 pg/ml (232.33–1007.90 pg/mL). Thus, the patients in all 4 quartiles had median BNP level higher than a cut-off point recommended by the ESC guidelines for acute heart failure.

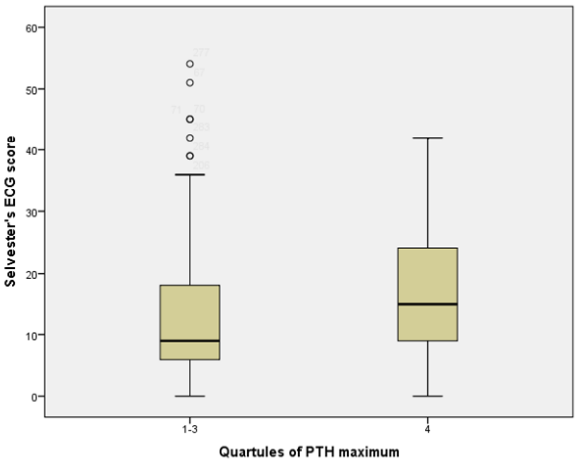

Fig. 1 – Value of Selvester's electrocardiography (ECG) score, the 4th versus other three quartiles of parathyroid hormone (PTH) maximum concentration, $p < 0.001$.

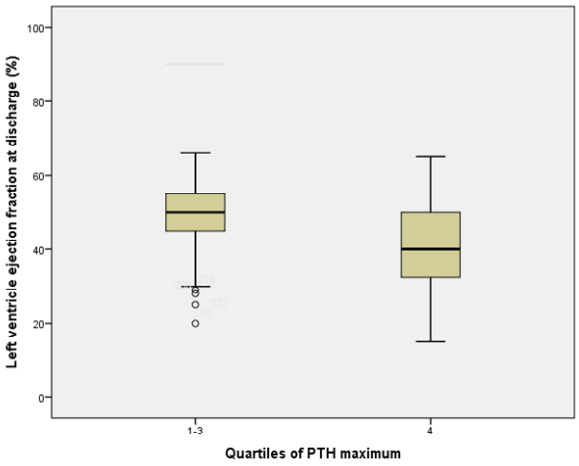

Fig. 2 – Left ventricle ejection fraction (LVEF), the 4th versus other three quartiles of parathyroid hormone (PTH) maximum concentration, $p < 0.001$.

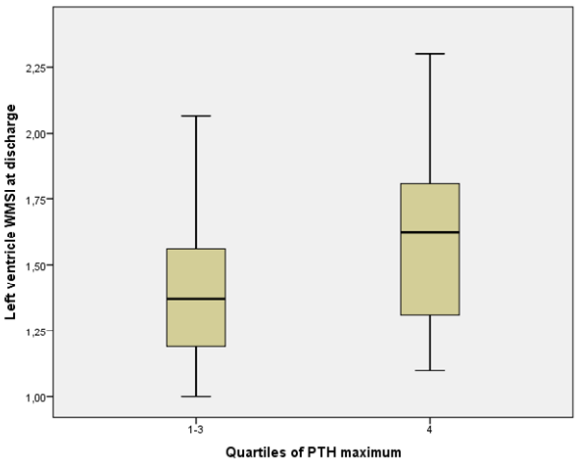

Fig. 3 – Value of wall motion score index (WMSI), the 4th versus other three quartiles of parathyroid hormone (PTH) maximum concentration, $p < 0.001$.

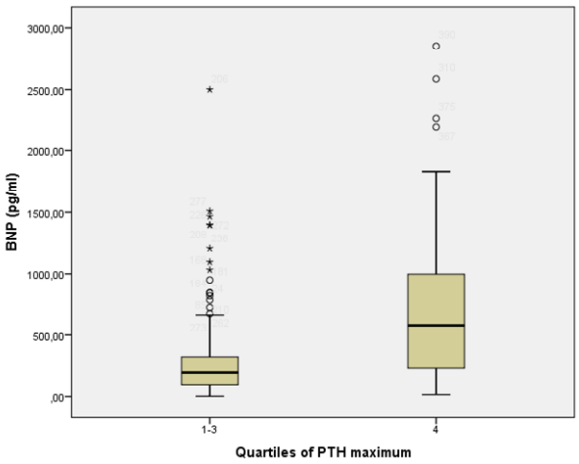

Fig. 4 – Brain natriuretic peptide (BNP) concentration, the 4th versus other three quartiles of parathyroid hormone (PTH) maximum concentration, $p < 0.001$.

C-reactive protein also significantly increased in the 4th quartile of PTH level ($p < 0.001$, Figure 5.) CRP serum level had median value with IQR for the 1st quartile 14,000 mg/L (8.200–26.250 pg/mL), for the 2nd quartile 16.000 mg/L (8.780–43.000), for the 3rd quartile 24.330 mg/L (9.938–49.725 pg/mL) and for the 4th quartile of PTH max. level value of 66.750 mg/L (25.000–120.500 pg/mL). The 4th quartile of PTH max. (PTH serum concentration above reference range) had a very high median CRP level of 66.750 mg/L.

Finally, CK-MB level significantly increased in the 4th quartile of PTH level ($p = 0.004$, Figure 6). Medians with IQR for CK-MB were: for the 1st quartile 160.50 IU/L (85.75–269.75 IU/L), for the 2nd quartile 192.00 IU/L (97.50–355.50 IU/L), for the 3rd quartile 209.50 IU/L (112.75–341.50 IU/L) and for the 4th quartile 237.00 IU/L (132.50–437.00).

There was also significant correlation between serum PTH maximum levels and serum creatinine concentrations at admission (Figure 7). However, there were no significant differences among the values of LVEF, WMSI, Selvester's score and CK-MB across the quartiles of creatinine (data not shown). This means that PTH has independent from creatinine level, an association with mentioned parameters.

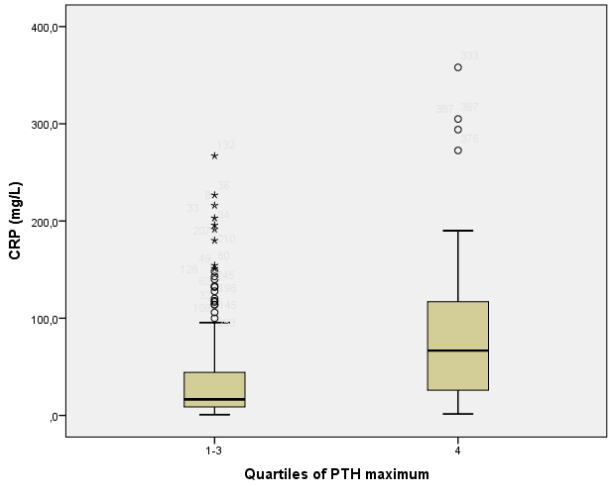

Fig. 5 – C-reactive protein (CRP) concentration, the 4th versus other three quartiles of parathyroid hormone (PTH) maximum concentration, $p < 0.001$.

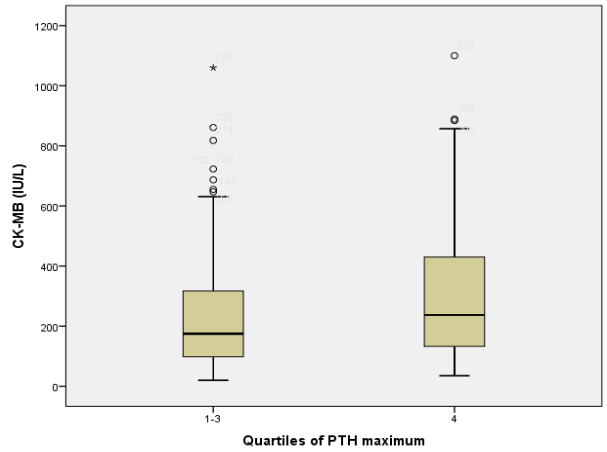

Fig. 6 – Creatine kinase-MB isoenzyme (CK-MB) concentration, the 4th versus other three quartiles of parathyroid hormone (PTH) maximum concentration, $p < 0.001$.

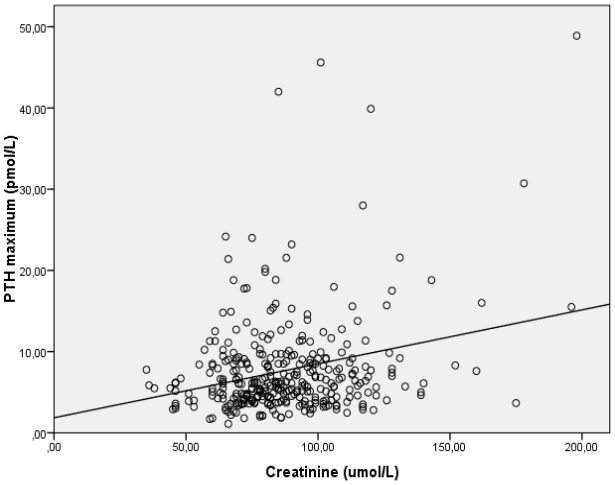

Fig. 7 – Relationship between the maximum serum concentration of parathyroid hormone (PTH) and serum creatinine at admission in ST elevation myocardial infarction (STEMI) patients.

Analysis of the statistical significance of differences among the individual quartiles for the ECG, echocardiography and biochemical markers of prognosis of acute myocardial infarction

When we analyzed whether there was a statistically significant difference (Mann-Whitney test) between the individual quartiles of the above listed markers of prognosis in acute myocardial infarction, we noticed that there was no statistically significant difference between quartiles 1 and 2, nor 2 and 3, but only between quartiles 3 and 4 of PTH max. levels for all of these markers except for CK-MB max. concentration (Table 2). The 4th quartile of PTH max. serum concentration, therefore, had significantly higher levels of all markers of poor prognosis in acute myocardial infarction (Figures 1–6). For CK-MB max. concentration, we did not find a statistically significant difference between the 1st and 2nd or between the 2nd and 3rd or between the 3rd and 4th quartile, but this does not mean that there was no difference between the 4th and the 1st quartile or the 3rd and the 1st quartile, and therefore, as stated above, there was a statistically significant increase in CK-MB max. concentration with higher quartiles of PTH max.

Discussion

In our patients with STEMI, who were treated with primary PCI, PTH maximum levels, which were on the upper limit of reference range and above this limit, were significantly associated with markers of larger infact size and heart failure. Half of the study patients were in the 3rd and 4th quartile of PTH levels (the upper limit of reference range and above this limit) and all of them had the clinical, ECG, echocardiographic and biochemical markers of larger myocardial infarction size and heart failure.

Clinically, patients in the 3rd and 4th quartile of PTH max. had higher Killip class at admission. They also had a

Table 2

The statistical significance of differences between quartiles of parathyroid hormone (PTH) maximum levels for analyzed electrocardiographic (ECG), echocardiographic and biochemical markers of prognosis of acute myocardial infarction

Paremeter	I vs. II quartile *p*	II vs. III quartile *p*	III vs. IV quartile *p*
Selvester's QRS score	0.391	0.094	0.008
LVEF	0.694	0.273	0.001
WMSI	0.517	0.422	0.001
BNP	0.968	0.105	0.001
CRP maximum	0.438	0.193	0.001
CK-MB maximum	0.099	0.676	0.100

LVEF – left ventricle ejection fraction; WMSI – wall motion score index; BNP – brain natriuretic peptide; CRP-C-reactive protein; CK-MB – creatine kinase-MB isoenzyme; *p* – probability.

history of previous myocardial infarction and a longer total ischemic time. On coronary angiography, patients in the 3rd and 4th quartile of PTH max. level had significantly higher incidence of multivessel disease and TIMI flow after PCI in these patients was significantly less frequently grade 3. Accordingly, echocardiographic prognostic factors in myocardial infarction correlated with the PTH serum level, ejection fraction significantly decreased and WMSI significantly increased across the quartiles of PTH max. level. Selvester´s ECG score was shown to correlate with infarction size. In our study Selvester´s ECG score was significantly higher in higher PTH max. quartiles. The most widely used biochemical prognostic markers in acute myocardial infarction are CKMB, CRP and BNP. All these three markers in our study correlated with the PTH level and increased across the quartiles of PTH max. level.

Possible pathogenesis of PTH level increase in acute myocardial infarction (AMI)

Several studies have shown that there is an increase in PTH serum level in acute myocardial infarction. In study of Joborn et al. [13] serum concentration of PTH was increased in early course of acute myocadial infarction (AMI) as compared to the reference day number 7. In the same period, mean values of total and ionized calcium did not change significantly, but PTH correlated negatively with serum calcium. Conversely, PTH correlated positively with plasma and platelets epinephrine. That is one possible explanation for significant elevation of PTH in large AMI. It is well known that AMI increases sympathoadrenal activity [15]. *In vivo* and *in vitro* studies have shown that epinephrine can directly stimulate secretion of PTH and indirectly by lowering plasma total and ionized calcium [16, 17]. Carlstedt et. al. [14] reported that PTH was stronger predictor of mortality than APACHE II score in patients in emergency department. The highest PTH levels were observed in patients with myocardial infarction and congestive heart failure. The reason for this observation remained unclear. Hypocalcaemia was not found in these patients and there was no clear association with proinflammatory cytokines. They also assumed that catecholamines could be responsible for elevation of PTH.

To the best of our knowledge, there is only one study of PTH secretion in acute heart failure. Sugimoto et al. [20] included in a study 266 patients admitted for acute decompensated heart failure (HF) without acute coronary syndrome. The authors in this study, contrary to the findings in chronic heart failure, found that the low-normal levels of PTH were associated with higher 1-year all-cause mortality. They concluded that the PTH was somehow necessary in the acute heart failure. In experimental studies it was demonstrated that PTH exerted vasodilating effect on blood vessels and positive chronotropic and inotropic effect on the heart [7]. There is evidence that this might be beneficial in heart failure even when PTH level increase is small and close to the upper limit of the normal range [21]. Also, since the authors of this study excluded from the trial the patients with acute coronary syndrome, where it was observed in the earlier, as well as in our study that PTH serum concentration was elevated, it could be concluded that ischemic myocardium in some way contributes to the increased secretion of PTH. In experimatal studies of the application of PTH in myocardial infarction, PTH contributes to mobilization and homing of stem cells from bone marrow in ischaemic myocardium and has a regenerative role [8–11]. There is a need to investigate whether PTH serum concentrations in myocardial infarction is sufficient for this effect.

Selvester's ECG score is an ECG metod for estimating myocardial infarction size and thus provides prognostic information after myocardial infarction [22]. We used simplified Selvester´ score of 37 criteria, 29 point system [23]. Selvester´s score kept the same predictive value in the era of PCI as it used to in the era of thrombolytic therapy. Roubin et al. [23] showed that QRS score correlated well with survival rate, ejection fraction and Killip class. As the score increased, survival rates and ejection fraction decreased. In a study of Tjandrawidjaja et al. [24] with STEMI patients treated with primary PCI, higher QRS scores were associated with male gender, higher heart rate, worse Killip class, noninferior infarction location, greater ST-segment deviation, and longer times to reperfusion and also with impaired culprit artery flow before and after PCI and more frequent multivessel disease. These findings are consistent with our findings; Selvester QRS score was higher with higher serum PTH concentration and that group of patients had higher Killip class, longer ischemic time, faster heart rate on admission and also poorer TIMI flow after PCI and more often multivessel disease.

After AMI, LVEF is of a prognostic significance. It has been shown in numerous studies that ejection fraction below

30–40% is strong predictor of all-cause cardiovascular mortality and sudden cardiac death (SCD) [25–27]. Also, there is a good correlation between LVEF and infarction size [28, 29]. Pride et al. [28] showed that there was a linear relation between infarction size and LVEF only for moderate to large infarcts, but not for small infarcts. In our study, patients in the 4th quartile of PTH max. level had median LVEF 40%, therefore patients with PTH serum level above the upper limit of the normal range had the EF that indicated worse prognosis and larger infarction size.

LVEF is a marker of global LV systolic function, whereas WMSI allows regional wall motion analysis, i.e. evaluation of LV regional dysfunction. Similar to the LVEF, WMSI is a strong predictor of all-cause mortality after AMI [27]. Mortality increases exponentially with decreasing LVEF, whereas it increases linearly with increasing WMSI [27]. In the study of Eek et al. [29] a good correlation was found between infarction size and WMSI and global longitudinal strain. They found that wall motion score index > 1.30 accurately identified patients with substantial infarction (≥ 12% of myocardium). In our study, 4th quartile of PTH max. level had median WMSI 1.68, indicating in that way a larger infarction size and worse prognosis.

In acute STEMI, different biomarkers are used in short term and long term disease prognosis [30]. We investigated association of PTH with three most commonly used biomarkers: CK-MB which indicates myocardial necrosis, CRP which is a marker of inflammation and BNP which is elevated mainly in response to left ventricular overload, but also in conditions of myocardial ischemia.

It was shown that BNP had predictive value for adverse events in STEMI infarction as well as in non-STEMI and unstable angina pectoris. Similarly, both of these markers are used in different studies of its prognostic value in acute coronary syndrome. Richards et al. [31] examined the predictive value of BNP, NT-proBNP and radionuclide LVEF for adverse events (death, heart failure and new myocardial infarction) in AMI. LVEF and the B-type natriuretic peptides were proved to be powerful independent predictors for adverse outcomes and the combination of NT-proBNP (or BNP) with LVEF (< 40%) substantially improved risk stratification. Besides, investigators concluded that both B-type peptides had similar utility as prognostic markers when measured early in the course of a broad spectrum of acute coronary syndromes. Velders et al. [32] evaluated the prognostic value of high-sensitivity cardiac troponin T, NT-proBNP and growth differentiation factor-15 (GDF-15) in STEMI treated with primary PCI. They concluded that biomarkers provided additional prognostic value for CVD beyond clinical risk factors and extent of coronary artery disease with NT-proBNP and GDF-15 being most valuable. In these studies, BNP/NT-pro BNP were measured at admission or in the first 24 hours up to 96 hours after the onset of symptoms depending on the type of acute coronary syndrome. We measured BNP value in 24 hours from the onset of symptoms. We found that the BNP plasma concentration significally increased with higher quartiles of PTH max. level. Interestingly, median value of BNP in the 1st quartile was 199.49 pg/mL (IQR 87.43–296.55 pg/mL) which is a high level, i.e. 100 pg/mL is recommended cut-off value for acute onset of heart failure. The 1st quartile, however, involves a stable patients with smaller infarction size. This could be explained by the role of ischemia on BNP release from ventricular myocardium [33, 34].

CRP is well established risk factor in the pathogenesis of atherosclerosis. The role of CRP in acute myocardial infarction was investigated in several studies. Sano et al. [35] showed that elevated CRP serum concentration within 6 hours after the onset of symptoms of AMI might reflect the inflammatory activity of a ruptured plaque. Later in the course of AMI, elevated serum CRP levels may be caused by a inflammatory response to myocardial necrosis. Lagrand et al. [36] have concluded in experimatal study that CRP localizes in infracted, necrotis tissue and promotes local complement activation and subsequently tissue damage. Nikfardjam et al. [37] found that elevated CRP level on admission in patients with AMI predicted short term and long term mortality. Ohlmann et al. [38] in their study of patients with STEMI treated with primary PCI found that plasma concentrations of CRP measured 48 hours and 72 hours after PCI correlated with infarction size. Admission CRP level as well as level at 48 hours after PCI predicted 6-month mortality. They found that CRP level in this patients reached a peak value after a median interval of 49 hours after the admission. Sanchis et al. [39] have also found that CRP level increased after admission and reached its peak after 48 hours. It could be concluded, that CRP level in AMI depends on time from the pain onset until admission [40] and reperfusion therapy. We measured CRP on the first and second day after admission and a peak CRP serum value was reached around 48 hours after admission, what is in concordance with previous studies. We found that CRP level was significantly higher with higher PTH max. quartiles.

There is a number of studies which confirmed correlation between serum levels of CK-MB and infarction size and prognosis after AMI. Most of these studies are from the time before the thrombolytic therapy or from the thrombolytic therapy era [41, 42]. Only Nienhuis et al. [43] showed that peak CK-MB was independent predictor of LVEF and 1-year mortality in patients after primary PCI for STEMI. In current guidelines, troponin is the preferred biomarker to use in patients with acute coronary syndrome (ACS), but CK-MB is considered as the best alternative when troponin assay is not avilable. We used CK-BM because of better availability of this assay in our hospital. In our study the 4th quartile of PHT max. level had significantly higher CK-MB level (median value 237.00 IU/L, interquartile range 132.50–437.00 IU/L). This finding is very similar to finding of Nienhuis et al. [43] where CK value in the 3rd quartile was > 281 IU/L.

Study limitation

The present study is part of our larger study of PTH concentration and kinetics in STEMI. The relationship of PTH and other factors of mineral metabolism was described in our previous paper. In this paper we wanted to point out association of PTH and clinical, echocardiographic and biochemical markers of infarction size and heart failure. We

didn´t take biochemical markers on admission, although in some studies this was done. This was not always possible to make and, beside that, total ischemic time was significantly different among quartiles of patients and since biochemical markers are dependent on this time, we decided to take samples for biochemical markers on the 1st, 2nd and 3rd day after the admission and to take maximum level for analysis as it was done in some other studies.

Conclusion

Patients with STEMI and elevated concentration of PTH (the lower cut-off for the 4th quartile of PTH was 9.2 pmol/L) in the early phase of disease had several markers of worse prognosis, high Selvester's ECG score, low LVEF, increased WMSI, and higher blood concentrations of BNP, CRP and CK-MB.

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Quality of life and depression in elderly persons engaged in physical activities

Kvalitet života i depresija starih osoba koje se bave fizičkom aktivnošću

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Abstract

Backgroung/Aim. Since the number of elderly persons is gradually increasing worldwide, there is a need to identify the factors that affect the quality of healthy ageing. On the other hand, depression is the most common psychiatric disorder in the elderly and one of the most serious health problems that modern society is facing. Considering the importance of physical activity for healthy ageing, the question is whether there are differences in quality of life and depression in the elderly in relation to the certain characteristics of physical activities practicing. **Methods**. Differences in the quality of life and occurrence of depression in elderly were examined in relation to duration of a single training session and frequency of physical activities per week. This non-experimental, descriptive and comparative cross-sectional study involved a total of 188 persons aged 65–84 years, where 90 persons are engaged in a physical activity while 98 persons are not. The Older People's Quality of Life Questionnaire and the Geriatric Depression Scale were used. **Results.** Statistically significant difference was found in the following domains of quality of life: health, social relationships and psychological and emotional well-being as well as in the total score of quality of life and in the occurrence of depression. The highest values of quality of life and the lowest level of depression manifestation were observed in the group of persons whose single training session lasted for 60 minutes, and in the group of persons engaged in a physical activity twice a week. **Conclusion.** The main finding indicates that the differences in the duration of a single training session and the frequency of physical activities per week reflect on the overall quality of life, individual domains of quality of life and the occurrence of depression in the elderly persons.

Key words:
aged; aged, 80 and over; quality of life; depression; motor activity; surveys and questionnaires.

Apstrakt

Uvod/Cilj. S obzirom na to da se poslednjih decenija broj starih osoba u svetu postepeno povećava, postoji potreba za pronalaženjem faktora koji utiču na kvalitet zdravog starenja. S druge strane, depresija je najčešći psihijatrijski poremećaj kod starih osoba i jedan od najozbiljnijih zdravstvenih problema sa kojima se savremeno društvo suočava. Razmatrajući značaj fizičke aktivnosti za zdravo starenje, postavlja se pitanje da li postoje razlike u kvalitetu života i pojavi depresije kod starih osoba u odnosu na određene karakteristike bavljenja fizičkim aktivnostima. **Metode**. Razlike u kvalitetu života i pojavi depresije kod starih osoba su ispitane u odnosu na trajanje pojedinačnog treninga i učestalost bavljenja fizičkim aktivnostima na nedeljnom nivou. U ovoj neeksperimentalnoj, deskriptivnoj i komparativnoj studiji poprečnog preseka učestvovalo je 188 osoba starosti od 65 do 84 godina života, i to 90 osoba koje se bave i 98 osoba koje se ne bave fizičkom aktivnošću. Primenjene su Skala kvaliteta života starih ljudi i Gerijatrijska skala depresije. **Rezultati**. Statistički značajna razlika uočena je u sledećim domenima kvaliteta života: zdravlje, socijalni odnosi i psihičko i emocionalno blagostanje, kao i u ukupnom skoru kvaliteta života i pojavi depresije. Najviše vrednosti kvaliteta života i najniži stepen ispoljavanja depresije zabeleženi su u grupi osoba čiji pojedinačni trening trajao je 60 minuta ili koji su vežbali dva puta nedeljno. **Zaključak.** Glavni nalaz ove studije pokazuje da se razlike u trajanju pojedinačnog treninga i u učestalosti bavljenja fizičkim aktivnostima na nedeljnom nivou odražavaju na ukupan kvalitet života, na pojedine domene kvaliteta života i na pojavu depresije kod starijih osoba.

Ključne reči:
stare osobe; stare osobe, 80 i više godina; kvalitet života; depresija; motorna aktivnost; ankete i upitnici.

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Introduction

The number of elderly persons is gradually increasing, as well as their relative share in total world population; therefore, the recognition of the factors that are important for successful ageing is of the crucial concern for society [1, 2]. Relationship between healthy ageing and quality of life and mental health are the subject of many studies [3–5].

The concept of quality of life refers to the overall well-being within a society. It is the perception of the individual's position in life, expectations, standards and concerns [6]. There are many definitions of this term and they include social, cultural and environmental individuality into consideration [7]. In older population, quality of life is used to describe a number of results that clinicians consider important in the life of elderly [8].

Mental health is another important indicator of healthy ageing. Depression is widespread among the elderly, thus representing the most frequent psychiatric disease and one of the most serious health problems which modern society faces with [9, 10]. Some authors believe that the occurrence of depression in late period of life can actually represent a precursor to dementia, increased risk of suicide or morbidity [11, 12].

Promoting physical and mental functioning and independence in the elderly population represents a key strategy for healthy ageing. Therefore, physical activity may be one of the means of maintaining or improving a quality of life in elderly persons [13]. The need for programs, containing a combination of aerobic, muscle strength, flexibility, and balance training, is highlighted in order to get a stronger influence of physical activity during the old age [14].

Despite numerous studies that have related physical activities and quality of life in elderly [7, 15, 16] and the reduced occurrence of depression [17, 18], the need for finding the best aspects and characteristics of a physical activity still continue to exist. Valuable data could be obtained by perceiving a physical activity through its own characteristics (number of training sessions within a week, duration of a training session, the type of a physical activity, regular training, etc). These data could improve both planning and implementation of physical activity for the ageing population. Recognizing the importance of physical activity for healthy ageing, the question is whether there are differences in quality of life and occurrence of depression between the elderly persons engaged in a physical activity and elderly persons not engaged in a physical activity, but also whether there are differences in relation to the certain characteristics of physical activities practice.

Therefore, the aim of this study was to examine the differences in the overall quality of life, individual domains of quality of life and the occurrence of depression between the elderly persons not engaged in a physical activity and elderly persons engaged in a physical activity in relation to two selected characteristics of the physical activity. The first selected characteristic was the duration of a single training session, and the second one was the frequency of physical activities practice per week. We have assumed that there are differences in quality of life and occurrence of depression among elderly persons related to these characteristics of a physical activity.

Methods

The research was realized in accordance with the terms of the Declaration of Helsinki and with the approval and consent of the Ethics Committee of the Faculty of Sport and Physical Education, University of Belgrade.

Participants

This non-experimental, descriptive and comparative cross-sectional study included 188 participants, aged from 65 to 84 years. General criteria for the inclusion of all participants were as follows: persons over 65 years of age, and the negative history of severe chronic, psychiatric or somatic diseases.

The total sample was initially divided into two groups. The first group (G-PA) consisted of 90 participants who were engaged in a physical activity, at least for the previous six months. There were 62 female and 28 male participants in this group. The second group (G0) included 98 participants who were not engaged in a physical activity. There were 69 female and 29 male participants in this group.

In order to make a complete analysis, G-PA group was subsequently divided by following two criteria. The first criterion involved the duration of a single training session. Therefore, three groups were formed: the first group of participants whose single training lasted for 30 minutes (G30), the second group of participants whose single training lasted for 60 minutes (G60) and the third group of participants whose single training lasted for 90 minutes (G90). The second criterion was related to the number of training sessions per week (frequency). The same sample of participants (G-PA group) was divided into different three groups: the first group of participants who had two training sessions per week (G2), the second group of participants who had three training sessions per week (G3) and the third group of participants who had more than three training sessions per week (G3+). The group of participants not engaged in a physical activity (G0) was not subsequently divided.

The minimum required sample size of 128 participants was computed by using an a priori analysis available through Gpower 3.1 with the effect size set at 0.3, alpha level at 0.05 and power at 0.8. In this regard, a total of 188 participants who had participated in this study was sufficient to examine the differences in quality of life and level of depression in the elderly in relation to selected characteristics of physical activities practicing.

Procedures

The study was conducted during 2015 in several sports and recreation centers in Belgrade, Serbia. Most of the participants from G-PA group were members of different sports and recreational clubs, with licensed instructors, while a smaller number of participants were engaged in a physical

activity individually. Participants engaged in a physical activity were members of the following clubs: the City Center for Physical Culture in Belgrade, University for the Third Age, Yoga Center Belgrade, International Martial Arts Association of Serbia (IMAAS), and the Qigong Association of Serbia. Physical activity of participants included in this study consisted of the following activities: Tai Chi, Qigong, swimming, volleyball, recreational walking and yoga.

The study was conducted in two phases. In the first phase of this study, through the cooperation with the aforementioned sports and recreational clubs, an initial triage was conducted. A total of 194 persons was included. On the basis of the outcomes of regular annual preventive medical examinations, 64 persons were excluded from the further procedure due to their compromised health status. Out of the remaining 130 persons, 90 persons accepted to participate in the study and they were included in the G-PA group. In the second phase of this study, G0 group was formed by using snowball sampling [19]. This group consisted of retired persons not engaged in a physical activity. For the purpose of forming this group, 200 questionnaires were distributed. In order to avoid statistically significant differences between initial groups according to gender and age of the participants, 98 questionnaires were taken into consideration. Participants from both groups responded to questions individually by filling in written forms and circling the number next to the question or the proposition (yes/no) during one session that took no more than 20 minutes. The assistance of the first author was available all the time.

Measures

The Older People's Quality of Life Questionnaire (OPQOL) was used [20] for assessment of the quality of life of elderly, while Geriatric Depression Scale (GDS) was used for assessment of depression [21].

The original version of OPQOL consists of 35 items. It has a five-point scale (from very good to very bad). The scoring scale is ranked from 35 to 175. A higher score indicates a greater number of positive responses on the test. The questionnaire covers the following areas: life overall, health, social relationships and participation, independence, control over life, freedom, home and neighbourhood, psychological and emotional well-being, financial circumstances, leisure, activities and religion as well as total score of quality of life (i.e. K-scor). In this study, the Serbian version of OPQOL was used. Its validity and reliability have been tested on 497 subjects [22]. Reliability of the scale in this study, expressed through Cronbach's α coefficients, is 0.87.

GDS is designed as a self-assessment tool, and it is used for the detection of depression in the elderly. On a scale of 30 questions (longer version), a score of 11 and more, indicates the existence of depression with 84% sensitivity and 95% specificity. Answers are dichotomous (yes/no). Reliability of the scale in this study, expressed through Cronbach's α coefficients, is 0.89.

The questionnaire designed for this study was used to collect general information and characteristics of the participants. The questionnaire contained questions related to age, gender, previous engagement in a physical activity, the time period of exercising, the duration of a single training session, the number of weekly training sessions and the type of a physical activity practiced.

Statistics

First, descriptive statistics was calculated, within which absolute frequency, percentage, mean, median, standard deviation and interquartile range were used. In order to test the uniformity of the group in relation to age, *t*-test for independent samples was used, whereas χ^2 test was used for the uniformity of groups in relation to gender. Cronbach's coefficient alpha was calculated in the part of the preliminary analysis. Morover, prior to all further statistical analysis, Kolmogorov-Smirnov test was performed in order to test the normality of data distribution. Since the data was not normally distributed, nonparametric statistical techniques were performed. Kruskal-Wallis H-test was used in order to detect whether there was a statistically significant difference between the groups (i.e. G0, G30, G60 and G90; i.e. G0, G2, G3 and G3+) in the quality of life domains and level of depression. When the aforementioned test had showed statistical significance, Mann-Whitney *U*-test was subsequently applied in order to further examine differences between groups for each domain and level of depression. Tests for detection of differences were applied on the median values. For all statistical analysis, α level was set at 0.05. The effect size was expressed by *r* coefficient. Analysis and data processing were performed using Statistical Package for the Social Sciences for Windows (SPSS version 21.0).

Results

The study included a total of 188 participants, 131 women and 57 men. Within the G-PA group, 10 (11.1%) of participants were engaged in a physical activity from six months to one year, 7.8% of participants were engaged in a physical activity from one to two years, whereas the largest percentage of participants (81.1%) was practicing a physical activity for more than two years. In relation to the type of a physical activity, by examining the distribution, it was observed that 26.7% was engaged in recreational walking, 13.3% in swimming, 11.1% in volleyball, 17.7% in Tai Chi, 16.6% in Qigong, and 14.4% in yoga.

Characteristics of participants according to their age and gender are presented in Table 1. There was no statistical difference between all groups in relation to age of participants ($p > 0.09$). In addition, there was no statistical difference between the initially formed groups in relation to gender, as well ($\chi^2 = 0.01$; $p = 0.95$).

The difference between groups in relation to the division according to the duration of a single training session was examined first. Applying the Kruskal-Wallis test, differences were confirmed in the quality of life domains and the total score on the depression scale among the four groups examined. Statistically significant difference was found in K-

Table 1

Socio-demographic characteristics of participants – age and gender

Group	Age (years)	Sex	
		male	female
Initial			
G-PA (*n* = 90)	67.8 (5.99)	28 (31.1%)	62 (68.9%)
G0 (*n* = 98)	69.15 (6.97)	29 (29.6%)	69 (70.4%)
DUR			
G30 (n = 29)	68.14 (1.10)	10	19
G60 (n = 47)	67.13 (0.92)	13	34
G90 (n = 14)	66.00 (1.40)	5	9
WT			
G2 (n = 35)	66.29 (1.01)	5	30
G3 (n = 27)	66.37 (1.10)	11	16
G3+ (n = 28)	69.39 (1.12)	12	16

***all data are shown as mean (SD) or as n (%).**
G-PA – group engaged in a physical activity; G0 – group not engaged in a physical activity; G30 – group whose training lasted for 30 min; G60 – group whose training lasted for 60 min; G90 – group whose training lasted for 90 min; G2 – group that had 2 training sessions per week; G3 – group that had 3 training sessions per week; G3+ – group that had more than 3 training sessions per week; DUR – duration of a single training session; WT – weekly training schedule.

score ($\chi^2 = 12.9$; $p < 0.01$), as well as in the following domains: health ($\chi^2 = 13.6$; $p < 0.01$), Social relationships and participation ($\chi^2 = 23.3$; $p < 0.01$) and psychological and emotional well-being ($\chi^2 = 10.8$; $p = 0.013$). In other domains, no statistically significant differences were found: life overall ($\chi^2 = 2.37$; $p = 0.500$), independence, control over life, freedom ($\chi^2 = 4.37$; $p = 0.224$), home and neighborhood ($\chi^2 = 3.67$; $p = 0.299$), financial circumstances ($\chi^2 = 5.31$; $p = 0.151$), and leisure, activities and religion ($\chi^2 = 1.46$; $p = 0.692$).

Subsequently, using the Mann-Whitney *U*-test, differences among the four groups were found. In most cases it was showed that the group G0 was statistically different from both subgroups G30 and G60. Detailed results are presented in Table 2.

Table 2

Median values and the differences between groups in relation to the duration of a single training session in different domains of quality of life

Domain	Mann-Whitney						Descriptive statistics		
	Group	Group to compare	*U*	*z*	*p*	*r*	Group	Median	IQR
K-score	G0	vs. G30	955.0	-2.68	0.012	0.24	G0	127.5	14.5
		vs. G60	1607.0	-2.94	0.000	0.24	G30	132.0	20.5
		vs. G90	539.5	-1.29	0.203	0.12	G60	135.0	12.0
	G30	vs. G60	633.0	-0.50	0.604	0.06	G90	131.5	19.0
		vs. G90	179.0	-0.62	0.534	0.09			
	G60	vs. G90	311.5	-0.30	0.764	0.07			
Health	G0	vs. G30	1070.0	-2.05	0.040	0.18	G0	13.0	2.0
		vs. G60	1535.0	-3.29	0.000	0.27	G30	13.0	3.5
		vs. G90	468.5	-1.94	0.051	0.18	G60	14.0	3.0
	G30	vs. G60	625.5	-0.61	0.544	0.07	G90	13.5	3.0
		vs. G90	186.0	-0.45	0.654	0.09			
	G60	vs. G90	325.0	-0.70	0.944	0.06			
Social relationships and participation	G0	vs. G30	804.5	-3.55	0.000	0.32	G0	26.0	6.0
		vs. G60	1368.0	-3.96	0.000	0.33	G30	28.0	4.5
		vs. G90	449.5	-2.09	0.049	0.20	G60	28.0	4.0
	G30	vs. G60	636.5	-0.48	0.628	0.03	G90	28.0	10.3
		vs. G90	196.5	-0.17	0.865	0.03			
	G60	vs. G90	315.0	-0.24	0.809	0.10			
Psychological and emotional well-being	G0	vs. G30	934.5	-2.84	0.000	0.25	G0	16.0	4.0
		vs. G60	1797.0	-2.17	0.037	0.18	G30	18.0	4.0
		vs. G90	519.5	-1.49	0.144	0.14	G60	17.0	3.0
	G30	vs. G60	599.0	-0.89	0.371	0.09	G90	18.0	3.0
		vs. G90	181.5	-0.57	0.571	0.10			
	G60	vs. G90	324.5	-0.80	0.938	0.09			

K score – overall Older People's Quality of Life (OPQOL) scale score, G0 – group not engaged in a physical activity; G30 – group whose training lasted for 30 min; G60 – group whose training lasted for 60 min; G90 – group whose training lasted for 90 min; IQR – interquartile range.

Moreover, the difference between the groups divided in relation to the number of training sessions per week was analyzed. Applying the Kruskal-Wallis test, differences were found in the quality of life domains and the total score on the depression scale among the four studied groups. As in the previous comparison, statistically significant difference was determined in the K-score ($\chi^2 = 14.2$; $p < 0.01$), as well as in the following domains: health ($\chi^2 = 16.0$; $p < 0.01$), social relationships and participation ($\chi^2 = 23.2$; $p < 0.01$), psychological and emotional well-being ($\chi^2 = 10.3$; $p = 0.016$) and leisure, activities and religion ($\chi^2 = 11.0$; $p = 0.012$). No statistically significant differences were confirmed in other examined domains: life overall ($\chi^2 = 4.48$; $p = 0.214$), independence, control over life, freedom ($\chi^2 = 6.20$; $p = 0.102$), home and neighbourhood ($\chi^2 = 2,93$; $p = 0.403$), financial circumstances ($\chi^2 = 5.71$; $p = 0.127$).

Differences were detected among the four groups following the subsequent application of Mann-Whitney *U*-test. In most cases it was confirmed that the G0 group was statistically different from both G2 and G3 subgroups. Detailed results are shown in Table 3.

Furthermore, differences were noticed in the total GDS score (i.e. G-score). It was found that there were statistically significant differences between groups divided according to the duration of a single training ($\chi^2 = 47.3$, $p < 0.01$) and the number of training sessions per week ($\chi^2 = 46.3$, $p < 0.01$).

Differences were detected among the four groups following the subsequent application of Mann-Whitney *U*-test. In most cases it was confirmed that the G-score of the group of participants not engaged in a physical activity (G0) was statistically different from the G-score of all other groups. Detailed results are shown in Table 4.

Discussion

In this study, differences in the quality of life and the occurrence of depression in the elderly in relation to the duration of a single training session and frequency of physical activities per week were examined. Statistically significant differences were determined in both examined characteristics of physical activities practice.

Table 3

Median values and the differences between groups in relation to the number of training sessions per week in different domains of quality of life

Domain	Group	Mann-Whitney					Descriptive statistics		
		Group to compare	*U*	*z*	*p*	*r*	Group	Median	IQR
K-score	G0	vs. G2	1069.5	-3.30	0.000	0.29	G0	127.5	14.5
		vs. G3	989.0	-2.01	0.047	0.18	G2	138.0	22.0
		vs. G3+	1043.0	-1.93	0.053	0.17	G3	135.0	11.0
	G2	vs. G3	385.0	-1.24	0.214	0.16	G3+	132.0	14.0
		vs. G3+	382.5	-1.49	0.137	0.20			
	G3	vs. G3+	369.5	-1.43	0.886	0.18			
Health	G0	vs. G2	997.0	-3.72	0.000	0.32	G0	13.0	2.0
		vs. G3	1058.5	-1.61	0.112	0.14	G2	14.0	3.0
		vs. G3+	1018.0	-2.11	0.031	0.19	G3	13.0	4.0
	G2	vs. G3	359.0	-1.63	0.102	0.21	G3+	14.0	3.0
		vs. G3+	396.5	-1.31	0.189	0.18			
	G3	vs. G3+	359.0	-0.33	0.744	0.04			
Social relationships and participation	G0	vs. G2	1021.5	-3.50	0.000	0.31	G0	26.0	6.0
		vs. G3	792.5	-3.19	0.000	0.29	G2	29.0	5.0
		vs. G3+	808.0	-3.32	0.000	0.30	G3	28.0	4.0
	G2	vs. G3	460.5	-0.17	0.864	0.02	G3+	28.0	4.0
		vs. G3+	481.0	-0.13	0.900	0.02			
	G3	vs. G3+	369.0	-0.15	0.879	0.02			
Psychological and emotional well-being	G0	vs. G2	1181.0	-2.77	0.011	0.24	G0	16.0	4.0
		vs. G3	1000.0	-1.96	0.048	0.18	G2	18.0	3.0
		vs. G3+	1070.0	-1.80	0.073	0.16	G3	18.0	4.0
	G2	vs. G3	459.5	-0.19	0.851	0.02	G3+	17.0	3.0
		vs. G3+	449.0	-0.58	0.563	0.08			
	G3	vs. G3+	359.0	-0.32	0.746	0.04			
Leisure, activities and religion	G0	vs. G2	1269.0	-2.30	0.020	0.20	G0	9.5	4.0
		vs. G3	1259.5	-0.38	0.701	0.03	G2	11.0	3.0
		vs. G3+	1151.0	-1.31	0.195	0.12	G3	9.0	3.0
	G2	vs. G3	305.5	-2.39	0.017	0.30	G3+	10.0	3.8
		vs. G3+	238.0	-3.53	0.000	0.48			
	G3	vs. G3+	353.0	-0.42	0.671	0.05			

K score – overall Older People's Quality of Life (OPQOL) scale score; G0 – group not engaged in a physical activity;G2 – group who had 2 training sessions per week; G3 – group who had 3 training sessions per week; G3+ – group who had more than 3 training sessions per week; IQR – interquartile range.

Table 4

Median values and the differences between groups in relation to the duration of a single training and the number of training sessions per week according to overall depression score

Domain	Mann-Whitney						Description		
	Group	Group to compare	*U*	*z*	*p*	*r*	Group	Median	IQR
G-score – duration of a training session	G0	*vs*. G30	526.5	-5.15	0.000	0.46	G0	7.0	7.3
		vs. G60	1084.5	-5.16	0.000	0.43	G30	2.0	4.5
		vs. G90	275.5	-3.62	0.000	0.34	G60	3.0	4.0
	G30	*vs*. G60	566.5	-1.24	0.214	0.14	G90	1.0	7.0
		vs. G90	195.5	-0.20	0.842	0.03			
	G60	*vs*. G90	267.0	-1.07	0.283	0.14			
G-score – number of training sessions per week	G0	*vs*. G2	792.0	-4.73	0.000	0.41	G0	7.0	7.3
		vs. G3	557.0	-4.61	0.000	0.41	G2	3.0	6.0
		vs. G3+	537.5	-4.91	0.000	0.44	G3	2.0	4.0
	G2	*vs*. G3	467.5	-0.07	0.943	0.01	G3+	3.0	4.5
		vs. G3+	466.0	-0.34	0.736	0.04			
	G3	*vs*. G3+	371.0	-0.12	0.905	0.02			

G-score – overall GDS score; G0 – group not engaged in a physical activity; G30 – group whose training lasted for 30 min; G60 – group whose training lasted for 60 min; G90 – G90- group whose training lasted for 90 min; G2 – group who had 2 training sessions per week; G3 – group who had 3 training sessions per week; G3+ – group who had more than 3 training sessions per week; IQR – interquartile range.

Positive relation between physical activity and health status was examined in previous studies, especially in the population of elderly persons [8]. However, numerous studies has generally been focused on persons with various chronic conditions (cancer, cardiovascular diseases, diabetes, osteoporosis, neurodegenerative diseases). On the other hand, only few studies dealt with physically active healthy ageing population, that is a population of elderly engaged in physical activities with no diagnosed chronic or infectious disease [23]. Positive relationship between certain characteristics of physical activities (i.e. frequency, duration, level) and quality of life was confirmed in several population-based cross-sectional studies [23–25]. In addition, in a longidutinal study published by Choi et al. [13] positive correlation between the intensity of physical activity and quality of life of elderly women was found. Moreover, an improvement of different domains of quality of life is associated to participation in physical activity in both longitudinal and cross-sectional studies [15, 26]. Finally, it should be highlighted that low-to-moderate intensity of physical activities may have stronger associations with quality of life in comparison to vigorous intensity, especially when performed on daily basis [8].

In this study, regarding the first examined characteristic of a physical activity (i.e. duration of a single training session), differences in several domains of quality of life were noted. Statistically significant differences were observed between the G30 and G60 groups in regards to the G0 groups. In particular, above-mentioned differences were observed in the following domains: K-score, health, social relationships and participation and psychological and emotional well-being (Table 2). Moreover, participants from the G90 group differed significantly from participants from the G0 group in the domain of social relations only. This could be explained by an overload that excessive physical activity represents for the elderly in some ways and that shorter training sessions have greater benefit. The higher scores were noted in the aforementioned domains of quality of life in the group of those participants whose single training sessions had lasted for 30 to 60 minutes. Similar to this result, Capodagilo et al. [27] 2005 comes to the conclusion that a single training session of 30 minutes once during the week is optimal to improve muscle function and functional ability in people ranging from 65 to 75 years.

Regarding the second characteristic of a physical activity examined (i.e. frequency or a number of training sessions per week), statistically significant differences were found between two groups in several domains of quality of life. Specifically, the G2 group was statistically different in both K-score and in the domains of health, social relationships and participation, psychological and emotional well-being when compared to the G0, as well as in the domains of leisure, activities and religion when compared to all observed groups (Table 3). Additionally, the G3 group differed significantly from the G0 group in K-score and in the domains of social relationships and participation and psychological and emotional well-being. Finally, the G3+ group differed significantly in the domains of health and social relationships when compared to the G0 (Table 3). Namely, similar results were reported in other studies that examined the influence of a number of training sessions per week in the elderly. Thus, Holviala et al. [28] 2014, came to the conclusion that training session frequency of twice a week was also optimal for large improvements in maximal strength, walking time, and balance in elderly. In addition, Ferrari et al. [29] 2013, found that among older men twice a week strength and endurance combined training led to similar neuromuscular and cardiovascular adaptations as three times per week.

Similar to other research results [17, 18, 30], the outcome of this study is expected, given that a physical activity is associated with reduced levels of depression occurence in the elderly. Table 4 shows that there is a statistically significant difference in both characteristics examined between the three groups of participants engaged in a physical activity when compared to the group of participants not engaged in a physical activity. Namely, when it comes to the occurrence of depression, the differences were found in both examined characteristics of a physical activity. This finding is essentially encouraging because it shows that it does not matter for how long an individual training session lasts and how many training sessions there are per week. When it comes to the prevention of depression in the elderly, it is important to be engaged in a physical activity.

Summing up the above, our results confirm that there is an association between selected characteristics of physical activities (duration and frequency), on one hand, and several domains of quality of life, as well as a total quality of life, on the other hand. Moreover, these characteristics of physical activities are associated with the occurrence of depression in the elderly persons.

Several limitations should be noted within the framework of this study. The first limitation refers to the initial division of the entire sample. More precisely, the same participants from the G-PA group were divided twice for each observed characteristic. The second limitation refers to the inconsistency of the number of participants within groups. The next limitation refers to the type of a physical activity practiced which was not taken into account. Perhaps the future research should take into account the different participants in regards to each observed characteristic as well as equal distribution of the number of participants within each group. Also, differences related to the type of a physical activity should be examined further.

Conclusion

In order to promote more active ageing, it is important to pay attention to the age-related decline in physical activity of elderly persons, on one hand, and to the confirmed differences in quality of life and the occurrence of depression related to the certain characteristics of physical activity practices. The results of this study suggest that persons who participate in a physical activity for 60 minutes or twice a week have better quality of life than those with no physical activity. More precisely, the most evident differences are in the domains of health and psychological well-being, as well as in the domain of social relations and participation and leisure, activities and religion. When it comes to the occurrence of depression, it can be concluded that being engaged in a physical activity is sufficient and that differences are evident regardless of the duration of a single training session and the frequency of physical activities (number per week).

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The relationship between adiposity parameters and C-reactive protein values in overweight and obese women

Odnos između parametara gojaznosti i vrednosti C-reaktivnog proteina kod predgojaznih i gojaznih žena

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Abstract

Background/Aim. Overweight/obesity has become important health problem in developed countries. It may be related to a presence of low-grade inflammation in white adipose tissue. The aim of this study was to investigate the levels of inflammatory marker C-reactive protein (CRP) and its relation to anthropometric parameters in overweight and obese females. **Methods**. This study included 200 apparently healthy, overweight and obese women (18–45 years). Their standard and alternative anthropometric parameters [body mass index (BMI), percentage of fat (%F), waist circumference (WC), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR), body adiposity index (BAI)] were determined and correlated to serum CRP concentration. **Results.** Average CRP level was 5.56 ± 2.43 mg/L, and it significantly positively correlated to all investigated anthropometric parameters. There was significant difference between overweight and obese group in all investigated anthropometric parameters, as well as in CRP values. When investigated separately, according to BMI, values regarding obese females showed significant correlation between CRP and every investigated anthropometric parameter. In overweight subjects, no such correlation was recorded. In the obese group, all investigated parameters were significantly related to F. In overweight subjects, body weight (BW), BMI, WC and WHtR showed significant relation to F. **Conclusion.** The significant difference between the overweight and obese group in all parameters of central obesity was found as well as in the CRP levels. In the obese group, we found strong correlation between adiposity measured by fat percentage and parameters of central obesity, while in the overweight group WHR and BAI did not correlate to fat percentage. Our results confirmed that CRP is a valuable marker of metabolic risk in obese females, and BMI, although not so new, is still reliable parameter of adiposity.

Key words:
obesity; overweight; women; anthropometry; c-reactive protein.

Apstrakt

Uvod/Cilj. Prekomerna telesna masa i gojaznost postali su značajan zdravstveni problem u razvijenim zemljama, a mogu biti povezani sa prisustvom hronične inflamacije niskog intenziteta u belom masnom tkivu. Cilj rada bio je da se ispitaju nivoi markera inflamacije, C-reaktivnog proteina (CRP), i njegova povezanost sa standardnim antropometrijskim parametrima kod predgojaznih i gojaznih žena. **Metode.** Studijom je bilo obuhvaćeno 200 zdravih žena (18–45 god) kojima su određeni standardni i alternativni antropometrijski parametari [indeks telesne mase (BMI), procenat masti (%F), obim struka (WC), odnos obima struka i kukova (WHR), odnos obima struka i visine (WHtR), kao i indeks telesne masnoće (BAI)] koji su zatim korelisani sa koncentracijama CRP u serumu. **Rezultati.** Prosečna vrednost nivoa CRP u serumu u celoj grupi iznosila je 5,56 ± 2,43 mg/L i utvrđena je njegova značajna pozitivna korelacija sa svim ispitivanim antropometrijskim parametrima. Uočena je statistički značajna razlika između grupa predgojaznih i gojaznih žena u svim ispitivanim antropometrijskim parametrima, kao i u pogledu koncentracije CRP. U grupi gojaznih ispitanica utvrđena je značajna povezanost između CRP i svih antropometrijskih parametara, dok u predgojaznoj grupi nije zabeležena statistička značajnost. U grupi gojaznih, svi antropometrijski pokazatelji pokazali su značajnu korelaciju sa procentom telesne masti, a kod predgojaznih žena, korelacija je bila značajna samo za telesnu masu, BMI, WC i WHtR. **Zaključak.** Između predgojaznih i gojaznih ispitanica postoji značajna razlika u pogledu svih pokazatelja centralne gojaznosti, kao i u pogledu

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koncentacije CRP u serumu. U grupi gojaznih, pokazana je značajna korelacija između sadržaja masti, izraženog kao procenat masnoće, i svih pokazatelja visceralne distribucije masti, dok u grupi predgojaznih značajna povezanost nije urađena za WHR и BAI. Naši rezultati potvrđuju da CRP može predstavljati značajan marker metaboličkog rizika kod gojaznih žena, kao i da je BMI, iako spada u tradicionalne parametre, i dalje pouzdan pokazatelj sadržaja telesne masti.

Ključne reči:
gojaznost; telesna masa, prekomerna; žene; antropometrija; c-reaktivni protein.

Introduction

Overweight/obesity has become important health problem in developed countries. It may be related to presence of low-grade inflammation in white adipose tissue [1]. Precise mechanisms of chronic inflammation induction in obesity as well as the relation between obesity and inflammatory markers are yet to be explained [1, 2]. So far, the importance of C-reactive protein (CRP), as the most versatile inflammatory marker, is still in the spotlight.

CRP is acute-phase protein and inflammatory marker. Increased concentrations of CRP are present in serum after tissue injury, infection and inflammation [3]. Its synthesis takes place in liver, and is mostly regulated by interleukin-6 (IL-6). Numerous surveys classified CRP as important marker of inflammation, which may indicate early vascular lesions, and may be a predictor of cardiovascular events, even in apparently healthy population [4]. Most of the studies confirm that even levels below the accepted upper physiological limits (1 mg/dL) may indicate the increased risk of heart and cerebral stoke, peripheral atherosclerosis, and sudden death in both gender [1, 5, 6]. CRP outstands as independent risk factor, apparent from traditional risk factors such as increased total cholesterol, increased levels of glucose and homocystein, hypertension, age, high body mass index (BMI), smoking and physical inactivity [7].

In general, recent studies indicate that white adipose tissue produces numerous mediators, for example, cytokines tumor necrosis factor alpha (TNFα) and IL-6. In addition, in obese persons, white adipose tissue is infiltrated by macrophages with increased local production of proinflammatory mediators. These factors promote acute phase reaction and chronic inflammation in obese persons [4].

However, some authors proposed the existence of a subgroup of obese persons who are metabolically normal (without increased risks of heart diseases, type 2 diabetes, hypertension, stroke, gallbladder disease, cancers, etc.) [8]. They hypothesize that in this subpopulation obesity seems to be uncomplicated and is characterized by early onset, hyperplasticity of otherwise normal adipocytes, and peripheral type of fat distribution. The inflammation in these persons should be absent, and they supposed to have normal levels of inflammatory markers.

The aim of this study was is to investigate the levels of inflammatory marker CRP and its relation to standard anthropometric parameters [body mass (BM), BMI, percentage of fat (F), waist circumference (WC), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR)], as well as to alternative parameter, the body adiposity index (BAI), in population of apparently healthy overweight and obese females.

Methods

This study enrolled 103 overweight (BMI between 25 and 29.9 kg/m^2) and 97 obese (BMI ≥ 30 kg/m^2) females, nonsmokers, aged 18–45 years, without any comorbidities, and with regular menstrual cycles. Our study did not include persons with: clinically confirmed hypertension, glucose intolerance or diabetes, other endocrine or systemic inflammatory diseases, cardiovascular and/or cerebrovascular diseases, malignant diseases, pregnancy, and those with CRP values higher than 10 mg/L at the moment of investigation. None of them had previous history of stroke, transitory ischemic attack, angina pectoris, heart stroke, or congenital heart abnormality. At the moment of investigation, they did not take any medical drugs, supplements, oral contraceptives, or hormonal substitution drugs. Investigation was approved by the local Ethical Committee and was conducted in Department of Nutrition, Institute of Hygiene, Military Medical Academy, Belgrade, Serbia, during 2013 and 2014.

Standard anthropometric measurements were performed: body weight (BW), body height, WC and hip circumference [9], skinfold thickness, and followed parameters were calculated: BMI [kg/m^2], percentage of fat (%F) [10], waist-to-hip ratio (WHR), waist-to-height ratio (WHtR) [9], and BAI [hip circumference (cm)/height$^{1.5}$ (m) – 18] [11]. High sensitive CRP was measured in serum using enzymatic kits (Roche Diagnostics, Basel, Switzerland) on a Siemens autoanalyser (Dimension®, RxL Max, Siemens Dade Behring). Concentrations lower than 1 mg/L refer to low risk for cardiovascular diseases, values between 1 and 3 mg/L were in an average range, and values higher than 3 mg/L were considered as high risk [6]. WC less than 80 cm considered normal, between 80 and 87.9 cm referred to increased risk of metabolic complications, and ≥ 88 cm referred to substantially increased risk [9]; WHR ≥ 0.8 was also considered increased, as well as WHtR ≥ 0.62 [12].

Obtained data were statistically processed and presented as means ± SD; some of the data were presented as proportions (%). Difference between groups and correlation between chosen parameters and CRP values were analyzed by Student's *t*-test (χ^2 test for categories) and Spearman's rank correlation (r), respectively. Statistical significance was accepted at $p < 0.05$.

Results

Anthropometric parameters and serum concentrations of CRP in all 200 investigated subjects together are presented in Table 1.

Average CRP was 5.56 ± 2.43 mg/L, and significantly positively correlated to all investigated anthropometric parameters (BW: $r = 0.3329$, $p < 0.01$; BMI: 0.3567, $p < 0.01$;

Table 1

Anthropometric parameters and C-reactive protein (CRP) values of the study subjects

Parameters	$\bar{x} \pm SD$	min–max
Age (year)	31.12 ± 7.26	18–45
BW (kg)	87.99 ± 14.99	60.60–144.00
BMI (kg/m^2)	31.16 ± 5.08	25.04–51.24
%F	41.04 ± 4.98	28.70–56.00
WC (cm)	95.75 ± 12.98	70.00–148.00
WHR	0.85 ± 0.08	0.65–1.12
WHtR	0.57 ± 0.08	0.43–0.87
BAI	33.93 ± 4.99	23.06–50.40
CRP (mg/L)	5.56 ± 2.42	0.82–10.00

BW – body weight; BMI – body mass index; %F – fat percentage; WC – waist circumference; WHR – waist-to-hip ratio; WHtR – waist-to-height ratio; BAI – body adiposity index; CRP – C-reactive protein.
$\bar{x}$ – mean value; SD – standard deviation.

%F: 0.2589, $p < 0.01$; WC: 0.3645, $p < 0.01$; WHR: 0.2417, $p < 0.01$; WHtR: 0.3637, $p < 0.01$, and BAI: 0.2063, $p < 0.05$).

Statistical analysis showed the significant difference between the overweight and obese group for all investigated anthropometric parameters, except for the age as well as CRP values (Table 2).

Moreover, when the overweight and obese groups were presented separately, according to BMI, the different correlation between anthropometric parameters and CRP values were found. The results are presented in Table 3.

In both groups, there was no statistically significant correlation between CRP levels and age. In addition, in overweight subjects, no significant correlation was recorded between CRP and any anthropometric measurement or index whatsoever. On the other hand, in the obese group, every investigated anthropometric parameter was significantly correlated to CRP levels.

Percentage of fat measures body adiposity. In both groups, age was not significantly correlated to this parameter. In the overweight subjects, neither WHR nor BAI showed significant relation to %F, opposite to BW, BMI, WC and WHtR. In the obese group, all investigated parameters were significantly related to %F.

Table 2

Anthropometric parameters and C-reactive protein (CRP) values in overweight and obese groups of women

Parameter	Overweight	Obese	*p*
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	
Age (years)	31.55 ± 7.66	30.69 ± 7.60	n.s.
BW (kg)	77.82 ± 6.89	98.80 ± 13.67	< 0.000
BMI (kg/m^2)	27.49 ± 1.35	35.05 ± 4.67	< 0.000
%F	38.23 ± 3.26	44.03 ± 4.75	< 0.000
WC (cm)	88.13 ± 6.94	103.84 ± 13.03	< 0.000
WHR	0.83 ± 0.07	0.87 ± 0.08	0.0011
WHtR	0.52 ± 0.04	0.62 ± 0.08	< 0.000
BAI	30.86 ± 2.55	37.19 ± 4.89	< 0.000
CRP (mg/L)	4.95 ± 2.29	6.21 ± 2.41	0.0002

For abbreviations see under Table 1.

Table 3

Spearman's correlation coefficient (r) in overweight and obese women

Parameter	Correlation to CRP, (r; *p*)		Correlation to %F; (r; *p*)	
	overweight	obese	overweight	obese
Age	-0.1225; n.s.	-0.0525; n.s.	-0.1152; n.s.	0.0843; n.s.
BW	0.0625; n.s.	0.3101; < 0.01	0.2000; < 0.05	0.6077; < 0.01
BMI	0.1927; n.s.	0.3103; < 0.01	0.4664; < 0.01	0.6179; < 0.01
WC	0.1206; n.s.	0.3628; < 0.001	0.3679; < 0.01	0.6366; < 0.01
WHR	0.0935; n.s.	0.2831; < 0.001	0.1693; n.s.	0.4566; < 0.01
WHtR	0.1493; n.s.	0.3455; < 0.001	0.4081; < 0.01	0.6096; < 0.01
BAI	0.0749; n.s.	0.2806; < 0.01	0.0749; n.s.	0.3776; < 0.01
F	-0.064; n.s.	0.2757; < 0.001		
CRP			-0.064; n.s.	0.2757; < 0.001

n.s. – non significant.
For abbreviations see under Table 1.

According to BMI, all subjects were overweight or obese. However, fat distribution was different: in the overweight group, 10 (9.7%) women had normal values of WC, in 52 (50.5%) women the recorded values were in the range of increased risk of metabolic complications and in 41 (39.8%) women substantially increased risk values were recorded. In the obese group, there was only 1 person with normal WC, 5 with values suggesting increased risk, and 91 (93.8%) with substantially increased risk. These differences were statistically significant ($p < 0.05$). When risk is estimated according to WHR, there were 37 (35.9%) subjects in the overweight group with normal WHR, and 66 (64.1%) with increased WHR. In the obese group, normal WHR was recorded in 17 (17.5%) subjects and increased WHR in 80 (82.5%). In the overweight group, all subjects had normal WHtR, while in the obese group 33 (34%) of them had increased risk, i.e. WHtR ≥ 0.62 ($p < 0.05$).

In addition, 2 persons in the overweight group had CRP concentration in the range of low risk, 15 persons in the range of average, and 86 (83.5%) in the range of high risk of cardiovascular disease development. In the obese group, increased values of CRP were recorded in 93 (95.9%) subjects, with 4 persons in average range and no subject in low risk range, but these differences were not statistically significant.

Discussion

Obesity is extensively spread disease, which is, due to its metabolic effects, considered the most frequent risk factor for development of diabetes, hypertension and atherosclerosis. However, obesity itself, when defined according to body mass, is less powerful predictor comparing to central obesity, so recently anthropometric parameters have been adopted in order to achieve better discrimination of body adiposity and metabolic risk. The accuracy and usefulness of numerous parameters were analyzed, such as WC, WHR, WHtR [12], and BAI [11].

The important difference between men and women regarding body fat storage is considering the fact that women have more adipocytes at the start of their adult life (hyperplastic obesity), so they may accumulate more fat compared to men [13]. Later, process of fat accumulation is particularly rapid in subcutaneous abdominal area as the result of adipocyte hypertrophy. These observations served as foundations for suggestions that, especially in young women, obesity without comorbidities might be present. In our study, all women were apparently healthy and relatively young (18–45 years). However, the average levels of CRP were elevated in both groups, particularly in the obese one, and the difference between groups was statistically significant.

Despite the absence of metabolic diseases, according to our results, average WC of all investigated subjects was 95.55 ± 12.98 cm, and WHR 0.85 ± 0.08, suggesting high frequency of visceral obesity, and increased risk of metabolic complications. However, when observed as WHtR ratio, visceral obesity was less pronounced: mean value was 0.57 ± 0.08, which is bellow the accepted limit of increased risk (0.62). In the overweight group, we found 90% of women with increased or substantially increased values of WC, but all subjects in this group were in the normal range of WHtR. In the obese group, we found that almost every woman had increased WC, but only one-third was in the range of increased metabolic risk when categorized according to WHtR. WC was significantly related to increased risk of hypertension even in young (22–30 years) student population in Serbia [14].

To date, the investigations were directed to obese or morbid obese men and women. Studies performed on overweight persons are sparse. Considering the frequency of overweight persons in our population, and the possibility of prediction the cardiovascular risk regardless of age and actual health status, we wanted to investigate this problem in particular.

The major characteristic of our results is significant difference observed between overweight and obese subjects in almost all important features. Besides the anthropometric differences which were expected (BW, BMI, %F), in the overweight group we recorded significantly lower values of parameters that reflect the metabolic risk: WC, WHR, WHtR, as well as significantly lower values of inflammatory marker CRP. CRP is a strong predictor of future vascular events, and the value obtained from initial blood sample may be useful in prediction even after twenty years [6]. When analyzed several large studies conducted both in Europe and the USA, the same author found the strong relationship between CRP concentrations and future cardiovascular events, apart from other risk factors. Prospective random study from MONICA project has processed large sample of initially health middle aged population and indicated the same strong relationship [5]. Moreover, the results from this study demonstrated that obese persons showed two-fold higher concentrations of CRP compared to persons with normal BMI. The importance of central obesity is confirmed in numerous studies, which proved stronger correlation of WC and WHR to higher cardiovascular risk, compared to BMI [14]. The role of visceral obesity is particularly important in persons with normal BMI.

The results from the Women's Health Study which enrolled 39,876 middle-aged women showed that women with CRP levels higher than 75th percentile (> 0.59 mg/dL) also had higher BMI, WC and WHR. After adjusting for age, CRP demonstrated strong correlation with BMI and W, but less strong correlation with WHR [15]. The results of our investigation confirmed the strong correlation between CRP levels and parameters of central adiposity in obese women, but also with BMI. This association is absent in overweight females.

A meta-analysis of 10 studies with a total of 88,514 subjects concluded that discriminatory power of markers of central obesity, such as WHtR, regarding cardiovascular risk is better than BMI, but this difference is small, insignificant, and with no clinical relevance [12]. Our results confirmed that WHtR significantly correlated with overall fat percentage, both in the overweight and obese group, but the correlation between WHtR and CRP was present only in the obese group.

The same pattern is observed for BAI. In this study we also estimated BAI as a new parameter of body adiposity,

which is very simple for use, since it does not require weight measurement. It is calculated from body height and hip circumference [11] and can be used to reflect overall fat (visceral and subcutaneous) percentage in both sexes and in different ethnicity. Although in mentioned large study conducted by Bergman et al. [11], BAI was defined as a strong predictor of fat percentage, our result showed significant correlation between these two parameters only in obese women, and not in overweight ones. In general, in the obese group we found that all anthropometric parameters and indices (BW, BMI, WC, WHR, WHtR and BAI) were significantly correlated to %F and hence reflect the overall adiposity.

Central obesity is strongly related to metabolic risk and has been identified as a useful predictor of metabolic syndrome. The link that connects visceral obesity to its metabolic complications may be systemic insulin resistance, however, the influence of adipose tissue on insulin sensitivity is not clear enough. Chronic inflammation is common characteristic of metabolic syndrome, particularly related to insulin resistance. On the other hand, infection and inflammation are also related to insulin resistance, while visceral obesity is related to chronic low-grade inflammation. Hence, these observations may introduce inflammation as a possible mechanism of effects of obesity on insulin resistance.

The proposed mechanism of induction of inflammation in obesity is increased secretory activity in adipose tissue. Adipose tissue is the site of production of numerous secretory factors (adipokines), with pro- and anti-inflammatory effects [16–18]. Homeostasis is maintained due to the balance in secretion of different factors. This homeostasis may be impaired in presence of adipose tissue enlargement, resulting in dysregulation of adipokine production, and following local and/or systemic inflammatory reaction [19]. Excessive fat tissue produces proinflammatory factors, particularly TNFα and IL-6, but also generates acute-phase reactants such as plasminogen activator inhibitor-1 (PAI-1), haptoglobin, serum amyloid A, which are the major contributors of low-grade systemic inflammation [20]. Obese persons seem to have increased activation of kinases in adipose tissue, and subsequently increased expression of inflammatory cytokines [21]. Levels of inflammatory cytokines in obese men and women are higher than in non-obese ones, and might be related to insulin resistance [22]. TNFα and other pro-inflammatory cytokines can contribute to inhibition of insulin receptor stubstrate-1 by inducing serine phosphorylation. Enlarged adipose tissue in humans also leads to infiltration of inflammatory immune cells and macrophages with substantial loss of functional programming, which may promote inflammation [23, 24].

These inflammatory mediators then induce synthesis of acute-phase reactants in liver, among them CRP, which is induced largely by IL-6. This results in increased serum concentration of these molecules even in healthy obese persons [25], including children [26]. These observations are in agreement with our results.

In order to explore this suggested association, Pannacciulli et al. [3] investigated CRP levels in apparently healthy women of different age (18–60 years), and with different nutritional status (normal weight, overweight, and obese) in relation to body composition and fat distribution. They found that CRP concentrations are correlated to fat mass, regardless of age, but visceral adiposity (measured by WC) was stronger predictor of CRP compared to overall fat mass. In addition, another study performed in 119 young (20–40 years) and healthy obese adults of both sexes showed that fat mass and fat percentage were predictors of CRP levels in a group of females under 30 years, but body composition did not predict CRP neither in older females, nor in males of any age [27].

Conclusion

In population of apparently healthy relatively young women, we found significant difference between the overweight and obese group in all parameters of central obesity, as well as in CRP levels. However, we recorded elevated concentrations of CRP even in overweight women. CRP levels were positively correlated to BMI, WC, WHR, WHtR, BAI and in the obese group, regardless of age, but in the overweight group no correlation was recorded. Finally, in the obese group, we found strong correlation between adiposity measured by fat percentage and parameters of central obesity, while in the overweight group WHR and BAI did not correlate to fat percentage. Our results confirmed that CRP is a valuable marker of metabolic risk in obese females, and BMI, although not so new, is still a reliable parameter of adiposity.

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The influence of the position of the medial portal and of lower leg flexion on the length of the femoral tunnel in anatomic anterior cruciate ligament reconstruction – A cadaveric study

Uticaj pozicije medijalnog portala i stepena fleksije u zglobu kolena na dužinu femoralnog tunela prilikom anatomske rekonstrukcije prednje ukrštene veze – kadaverska studija

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Abstract

Background/Aim. The key to successful anterior cruciate ligament reconstruction lies in the proper positioning of the femoral tunnel within the anatomical footprint and in providing for an adequate length of this tunnel without perforation to the lateral cortex. The aim of this study was to determine the change in the length of the femoral tunnel drilled during anatomic anterior cruciate ligament (ACL) reconstruction, depending on: the position of the limb being operated on, the degree of knee flexion, as well as the angle between the drill and the medial aspect of the lateral condyle. **Methods.** This study was performed on 16 cadaveric knees (6 male and 10 female) of the average age of 83. After the subcutaneous tissue was dissected, the femoral insertion of the ACL was identified. Then, 18 tunnels were drilled through the center of the femoral insertion with the help of 2 mm thick Kirschner wires. This was performed in two stages. In the first phase the leg was positioned on an arthroscopic leg holder, while in the second phase the leg was positioned on the table. In each phase the knee was placed in three different flexion positions (110°, 120° and 130°) and for each position three tunnels were drilled (70°, 60° and 50°) in relation to the medial aspect of the lateral condyle. **Results.** The average length of the femoral tunnel drilled with the leg positioned on the operating table (36.6 ± 4.7 mm) was highly statistically significantly greater ($p = 0.000$) in comparison with the length of the femoral tunnel obtained by positioning the leg on a fixed arthroscopic leg holder (35.4±4.3 mm). The greatest lengths of the femoral tunnel were obtain with the leg flexed at 130º and the reamer positioned at 50º angle in relation to the medial aspect of the lateral condyle (43 mm on the operating table and 41 mm on a fixed leg holder), while the shortest tunnel (33 mm on the operating table and 31 mm on a fixed leg holder) was obtained with the lower leg flexed at 110º and the reamer positioned at a 70º angle. **Conclusion.** The optimal position of the leg on a fixed leg holder for obtaining a femoral tunnel of sufficient length requires lower leg flexion of 120º and the position of the medial portal which enables the positioning of the reamer at a 60º angle in relation to the medial aspect of the lateral condyle. With the leg positioned on the operating table, it becomes unnecessary to push the leg into flexion greater than 110º; rather a longer femoral tunnel is achieved by lateralization of the medial portal.

Key words:
anterior cruciate ligament reconstruction; cadaver; anatomy, regional.

Apstrakt

Uvod/Cilj. Ključ uspešne rekonstrukcije prednje ukrštene veze nealazi se u pravilnom pozicioniranju femoralnog tunela unutar anatomskog otiska i obezbeđivanju adekvatne dužine ovog tunela bez perforacije prema bočnom korteksu. Cilj ove studije bio je utvrđivanje promene u dužini femoralnog tunela bušenog tokom anatomske rekonstrukcije prednje ukrštene veze (ACL). **Metode.** Studija je rađena na 16 kadaverskih kolena (6 muških i 10 ženskih) prosečne starosti 83 godine. Nakon disekcije potkožnog tkiva i zglobne kapsule, odstranjena je prednja ukrštena veza, a po-

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tom je identifikovan femoralni pripoj prednje ukrštene veze. Potom je uz pomoć Kiršenovih igala, kroz centar femoralnog pripoja prednje ukrštene veze, bušeno 18 tunela u dve faze. U prvoj fazi noga je pozicionirana na artroskopskom držaču, dok je u drugoj fazi noga pozicionirana na operacionom stolu. U svakoj fazi koleno je postavljano u tri stepena fleksije (110°, 120° i 130°), pri čemu su u svakom stepenu fleksije bušena po tri tunela koja su sa unutrašnjom stranom spoljašnjeg kondila zaklapala uglove od 70°, 60° i 50°. **Rezultati.** Prosečna dužina femoralnog tunela, izbušenog dok je noga bila postavljena na operacionom stolu (36.6 ± 4.7 mm), bio je statistički značajno veća ($p = 0.000$) od dužine femoralnog tunela dobijene postavljanjem noge na fiksni držač noge (35.4 ± 4.3 mm). Najveće dužine femoralnog tunela dobiju one sa fleksijom noge od 130º i postavljanjem burgije pod uglom od 50º u odnosu na unutrašnju stranu spoljašnjeg kondila (43 mm na operacionom stolu i 41 mm na fiksnom držaču noge). **Zaključak.** Optimalan položaj noge na fiksnom držaču noge za dobijanje dovoljno dugačkog femoralnog tunela podrazumeva fleksiju potkolenice od 120º i poziciju medijalnog portala koja omogućava postavljanje rimera pod 60º u odnosu na unutrašnju stranu spoljašnjeg kondila. Postavljanjem noge na operacioni sto nepotrebno je prilikom bušenja tunela gurati nogu u fleksiju veću od 110º, već se dobijanje veće dužine femoralnog tunela može obezbediti lateralizacijom medijalnog portala.

Ključne reči:
ligament prednji, ukršteni, rekonstrukcija; leš; anatomija, regionalna.

Introduction

The key to successful anterior cruciate ligament (ACL) reconstruction lies in the proper positioning of the femoral tunnel within the anatomical footprint and in providing for an adequate length of this tunnel without perforation to the lateral cortex. Surgically speaking, it is necessary to choose the place of anatomic insertion on the medial aspect of the lateral condyle, and then it is necessary to drill a tunnel of adequate length across that spot without compromising the integrity of the posterior femoral cortex [1]. By decreasing the angle between the reamer and the medial aspect of the lateral condyle the length of the tunnel is increased, but the position of the tunnel is brought closer to the posterior cortex. The application of the suspensory device for fixation on the femur where the tunnel is shorter than 25 mm cannot provide adequate incorporation of the graft into the bone [2, 3], while perforation of the posterior femoral cortex during tunnel drilling, not only increases the duration of the procedure, but also prevents fixation and leads to a new, most frequently non-anatomical position of the femoral graft fixation.

Anatomically speaking, the ideal site of femoral insertion in single-bundle reconstruction would be the place where the superior and the middle third of the bifurcation ridge meet on the medial aspect of the lateral femoral condyle, described in detail by Ferretti et al. [4]. In order to locate this marker, as the place where two angulated surfaces meet, in addition to the surgeon's experience, i.e. his/her "skilled eye", an appropriate position of the lower limb is also necessary, providing for a sufficient level of flexion, so that the entire surface of the medial aspect of the lateral condyle is completely visible.

Although the site of femoral anatomic ACL insertion has been the topic of many studies [4–9], which have, in various ways, visually described the precise insertion site of the ACL and its bundles, this theoretical knowledge meets with numerous obstacles in practice, when a surgeon needs to choose, in the very confined space of the intercondylar notch, with 30° or 70° optics, the center of graft insertion on the medial aspect of the lateral femoral condyle. This segment of a seemingly simple task decides the fate of the reconstruction and makes the difference between successful and poorly performed reconstructions. Many colleagues believe that they are indeed performing an anatomic reconstruction of the ACL, but the position of the femoral insertion does not speak to that effect.

The purpose of this study was to determine the extent to which the position of the reamer, which is primarily dependent on the position of the medial portal, and the flexion of the lower leg influence the length of the femoral tunnel in anatomic ACL reconstruction.

Methods

This study was performed on 16 cadaveric knees (6 male and 10 female, 8 left and 8 right) of the average age of 83 ± 6 years. These knees displayed neither advanced degenerative changes nor bone damage.

Following precise dissection of the soft tissue structures, with preservation of the ligaments of the knee (with the exception of the ACL) the site of anatomical ACL insertion was identified on the medial aspect of the lateral femoral condyle. Then, 18 tunnels were drilled (into two phases) through the center of the femoral insertion with the help of 2 mm thick Kirschner wires.

In the first phase the leg was positioned on an arthroscopic leg holder while in the other phase the leg was positioned on the table. In each phase the knee was placed in three different flexion positions (110°, 120° and 130°) and for each position three tunnels were drilled (70°, 60° and 50°) in relation to the medial aspect of the lateral condyle (Figure 1). Lower leg flexion of 130º approximately matched maximal possible flexion on a fixed leg holder, while the angle between the reamer and the medial aspect of the lateral condyle of 70º coincided with the position of the reamer leaning against the medial femoral condyle.

The three flexion positions of the knee reflected the change stemming from the flexion of the lower leg during surgery (position of the tunnel in the sagittal plane). In the process of drilling each tunnel, the Kirschner wire, i.e. reamer, rested against the anterior margin of the proximal end of the tibia, i.e. against the anterior horn of the medial meni-

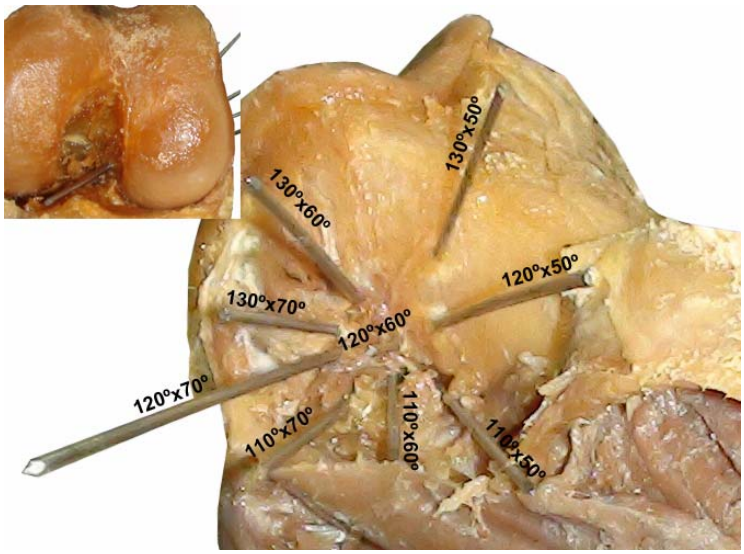

Fig. 1 – Point of exit of nine femoral tunnels. The first number represents the angle of knee flexion, while the second number represents the angle between the reamer and the internal surfaces of the lateral femoral condyle. The picture at the top left corner shows the entrance of the tunnel.

scus, so that lower leg flexion directly influenced the direction of the femoral tunnel. The three different angles formed by the reamer and the medial aspect of the lateral condyle reflected the position of the medial portal through which the reamer was inserted (position of the tunnel in the frontal plane). A wider angle denoted the medial position of the reamer, whereas a more acute angle indicated lateralization of the medial portal.

After drilling, each tunnel was dilated with a 5 mm reamer. The length of each tunnel was measured with the aid of a Kirschner wire and a caliper with the accuracy of 1 mm. The integrity of the posterior femoral cortex was checked for each tunnel.

All data were processed with the aid of the SPSS 11.0 program. The differences between the groups were tested with the Student's *t*-test for linked pairs. The statistical significance was set a ≤ 0.05.

Results

There was not a single case of posterior femoral cortex perforation in any of the 288 drilled tunnels. By positioning a leg on the operating table, the greatest femoral tunnel length (43.4 mm) was obtained with the leg flexion of 130º and the reamer positioned at an angle of 50º in relation to the medial aspect of the lateral condyle, while the shortest tunnel length (32.6 mm) was achieved when the lower leg was flexed at 110º angle, with the reamer positioned at an angle of 70º (Figure 2). This difference was highly statistically significant. A statistically significant difference was not found in the femoral tunnel length related to the degree of the lower leg flexion between any two neighboring lower leg flexion positions, within any of the three set reamer positions ($p > 0.05$ in all cases). These values were somewhat greater for greater degrees of flexion, however the noted differences did not prove

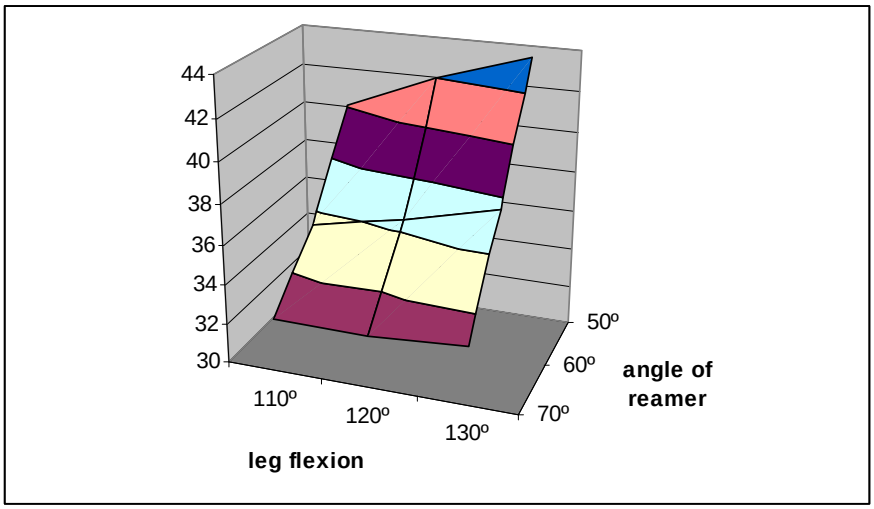

Fig. 2 – The dependence of the tunnel length (mm) on the degree of the lower leg flexion and the angle of the reamer when the leg is positioned on the operating table.

to be statistically significant. On the other hand, for the same degree of lower leg flexion, the decrease in the angle between the reamer and the medial aspect of the lateral condyle lead to a highly statistically significant increase in the length of the femoral tunnel ($p < 0.01$ in all of the cases). In other words, when the angle of the reamer was unchanged, the increase of lower leg flexion by 10º lead to an increase of the femoral tunnel length of less than 1 mm, while, when the flexion of the lower leg remained unchanged, the decrease in the angle of the reamer by 10º lead to an increase in the length of the femoral tunnel of more than 4 mm on average.

When the operated leg was positioned on a fixed leg holder, the greatest femoral tunnel length (41 mm) was achieved with the leg flexed at 130º angle and the reamer positioned at 50º angle in relation to the medial aspect of the lateral condyle, while the shortest tunnel (31 mm) was obtained when the lower leg was flexed at 110º angle with the reamer positioned at an angle of 70º (Figure 3). This difference is highly statistically significant ($p = 0.001$). The increase in the lower leg flexion from an angle of 110º to an angle of 120º lead to a statistically significant increase in the length of the femoral tunnel ($p = 0.001$) in all three set reamer positions, while an increase in the lower leg flexion from an angle of 120º to an angle of 130º did not lead to a statistically significant increase in the tunnel length in any of the cases ($p > 0.05$). On the other hand, for the same degree of lower leg flexion, the decrease in the angle between the reamer and the medial aspect of the lateral condyle lead to a highly statistically significant increase in the length of the femoral tunnel ($p < 0.01$ in all cases), with the exception of the position of the reamer at 50º angle with the lower leg flexed at 110º. When the leg was positioned on a fixed leg holder, if the angle of the reamer remained unchanged, the increase of the lower leg flexion from 110º angle to an angle of 120º lead to the increase of the femoral tunnel by more than 2 mm, while the increase in lower leg flexion from an angle of 120º to an angle of 130º lead to the increase of femoral tunnel length by less than 1 mm. When the degree of flexion of the lower leg remained unchanged, the decrease in the reamer angle from 70º to 60º lead to the increase of femoral tunnel length of 2 mm on average, while the decrease in the angle of the reamer from 60º to 50º lead to the increase in femoral tunnel length of up to 5 mm (for the position of lower leg flexion of 120º and 130º).

The average femoral tunnel length drilled with the leg positioned on the operating table (36.6 ± 4.7 mm) was highly statistically significant ($p = 0.000$) than the femoral tunnel length achieved with the leg positioned on the fixed leg holder (35.4 ± 4.3 mm).

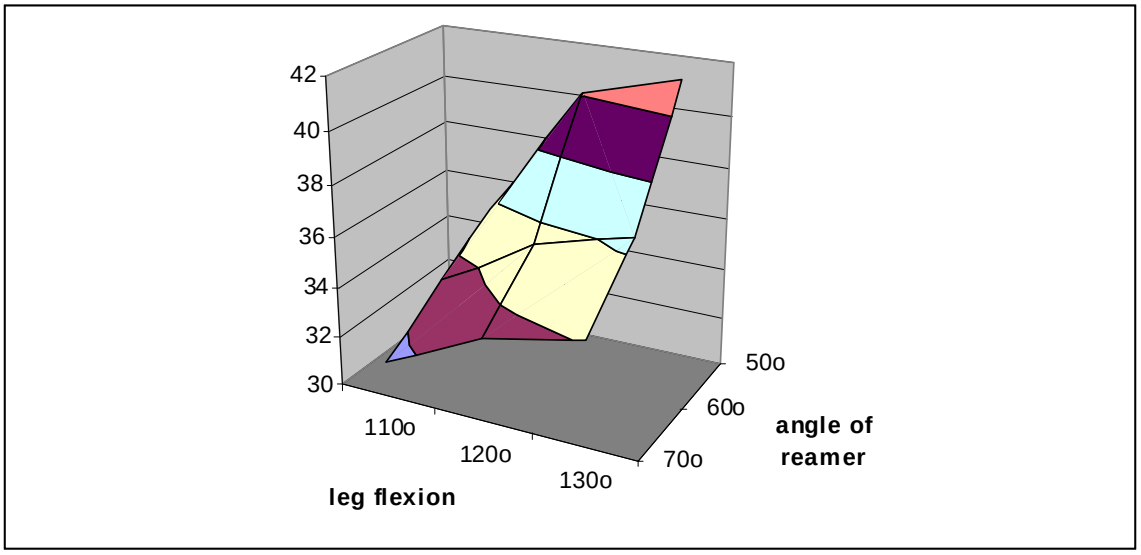

Fig. 3 – The dependence of the tunnel length on the degree of the lower leg flexion and the angle of the reamer when the leg is positioned on a fixed leg holder.

Discussion

The most important finding of the present study is that femoral tunnels drilled on the operating table are significantly longer than the femoral tunnels drilled on a fixed leg holder. Furthermore, the length of the femoral tunnels drilled with a leg positioned on the operating table is minimally dependent on the degree of the lower leg flexion, regardless of whether the lower leg is flexed at an angle of 110º, 120º or 130º. On the other hand, the decrease of the angle of the reamer by 10º results in a significant lengthening of the femoral tunnel. Although the position of the reamer set at an angle of 50º provides for the greatest length of the femoral tunnel (40 mm), the authors of the present study consider that the optimal position of the reamer angle is 60º, as none of the tunnels drilled at this angle was shorter than 31 mm regardless of the degree of flexion of the lower leg. Also, with the decrease of the angle of the reamer from 70º to 60°, the reamer is brought away from the cartilage of the medial condyle, which is often damaged during manipulation when the tunnel is dilated.

When the fixed leg holder is employed it is necessary to provide for lower leg flexion at an angle of 120º, since with lower leg flexion of 110º the frequency of the femoral tunnel

length of 30 mm or less is 25–55% (depending on the angle of the reamer). Further pushing of the lower leg into flexion greater than 120º does not result in a significant increase of the femoral tunnel length.

The breadth of the lateral femoral condyle at the level of the popliteal notch amounts to 27 mm, and at the level of the lateral epicondyle to 33.5 mm [10]. These results, obtained by a direct measurement on macerated thigh bones speak in favor of the fact that, by drilling the femoral tunnel perpendicularly to the medial aspect of the lateral condyle, a length of the tunnel which is rather greater than the breadth of the condyle at the level of the popliteal notch, can be achieved. Flexion of the lower leg at an angle of 120º enables the reamer to be positioned in the direction of the lateral epicondyle and, in that way, the tunnels can be made a little longer. Also, the positioning of the medial portal more laterally, i.e. a reduction of an angle between the reamer and the medial aspect of the lateral condyle provides for a longer femoral tunnel, primarily because the proximal opening of the femoral tunnel surpasses the domain of the epicondyle and in that way extends to the distal portion of the femoral diaphysis.

A part of the results of the present study completely support the results published by Steiner and Sencert [11]. Their femoral tunnel drilled with rigid reamers (32.5) with the lower leg flexed at 110° are in accordance with the results of the present study (32.6 mm) obtained by drilling the femoral tunnel with the leg positioned on a fixed leg holder, with an identical degree of the lower leg flexion and maximal medialization of the medial portal (angle of reamer amounting to 70º). In their study on 106 patients, whose femoral tunnels were drilled with rigid reamers through the anteromedial portal, Tompkins et al. [12] noted the length of the femoral tunnel of 37 mm, with the knee flexed at 134°. For the approximately same lower leg flexion position (130º) on a fixed leg holder, the authors of the present study obtained an approximately equivalent femoral tunnel length (36.4 mm), with the reamer positioned at 60º angle in relation to the medial aspect of the lateral condyle. This identical tunnel length (36.4 mm) was noted by Dong et al. [13] who also measured femoral tunnel lengths obtained with lower leg flexion of 130º–135º on cadaveric knees. Other authors who drilled the femoral tunnel with rigid reamers through the anteromedial portal also noted tunnel lengths supporting the lengths obtained in the present study by using the same means for drilling the tunnels [14].

In their study, which was a comparison of the length of the femoral tunnel drilled by the application of two different techniques (transportal and outside-in), Kim et al. [15] reported somewhat greater tunnel lengths achieved by the application of the outside-in technique, both for the anteromedial tunnel (38.9 : 34.8 mm) and for the posterolateral tunnel (39.3 : 32 mm). The results of the transportal technique speak in favor of the results of the present study obtained with the lower leg flexed at 110º and a reamer angle of 70º.

In their study, 20 out of 47 subjects, who had undergone anatomic ACL reconstruction, Hensler et al. [16] noted an approximately equal femoral tunnel length (31 ± 6 mm) to the ones achieved in the present study when the leg was positioned on the leg holder and the lower leg was flexed at 110º angle (reamer angle of 70º). However, the authors did not elaborate on the precise method of femoral tunnel drilling.

By using the anteromedial portal and flexing the lower leg at an angle of 120º, Lee et al. [17] measured an average femoral tunnel length of 34.4 mm on 52 subjects. However they did not specify the angle between the reamer and the medial aspect of the lateral condyle. This tunnel length is somewhat greater than the length achieved in the present study with identical lower leg flexion, but with the reamer angle set at 70º, and it is equally smaller than the tunnel length obtained with the reamer angle set at 60º.

It can be noted that many studies do not specify the angle between the reamer and the medial aspect of the lateral condyle. The authors of the present study believe that this is the result of the operating technique itself, which entails maximal medialization of the medial portal with the reamer leaning against the medial condyle during the tunnel drilling. In such a position, the reamer forms 70º angle with the medial aspect of the lateral condyle, preventing in this way some possible perforation of the posterior femoral cortex. When the medial portal is moved laterally, the angle between the reamer and the medial aspect of the lateral condyle is decreased to 60º or 50º, while the tunnel itself increases in length, ending above the lateral epicondyle in the region of the distal portion of the femoral diaphysis. At the same time, flexion of the lower leg of 110º or more, moves the proximal end of the femoral tunnel forward and prevents the perforation of the posterior cortex.

Clinical relevance

The use of the operating table, rather than the fixed leg holder, provides for a longer femoral tunnel, independently of the degree of lower leg flexion or the position of the reamer. When the medial portal is moved closer to the ligament of the patella, a more acute angle is achieved between the reamer and the medial aspect of the lateral condyle, which in turn provides for a longer femoral tunnel.

Study limitations

This study has two significant limitations. Although none of the knees displayed any degenerative changes, the average age of the subjects (83 years) is far above the average age of the patients who normally undergo ACL reconstruction. The other limitation relates to the diameter of the tunnel. Although the authors of this study have noted that no perforation of the posterior femoral cortex was registered in any of the cases, the fact that the tunnel was dilated only to the diameter of 5 mm must be taken into consideration.

Conclusion

The optimal position of the leg for obtaining a sufficiently long femoral tunnel, when placed on a fixed leg holder, requires a lower leg flexion of 120º and the position

of the medial portal which enables the positioning of the reamer at 60º angle in relation to the medial aspect of the lateral condyle. When the leg is positioned on the operating table it is unnecessary to push the leg into flexion greater than 110º when drilling the tunnel; a longer femoral tunnel can be achieved by lateralization of the medial portal.

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Sleep disturbances in restless legs syndrome

Poremećaj spavanja zbog sindroma nemirnih nogu

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Abstract

Background/Aim. Resteless legs syndrome (RLS) is chronical neurological disorder characterized by urge to move legs that is usually accompanied by unpleasant sensations in the lower extremities. Sleep disturbance is one of the main accompanying symptoms of RLS which exists in approximatelly 90% of patients. Impairment of sleep is related to daily sleepiness, depressive and anxiety disorders. The aim of this study was to detect frequency and characterisitics of sleep-related symptoms in patients with RLS, and its impairrment to daily sleepiness, fatique, anxiety and depression. **Methods.** We have examinated 94 patients with RLS. The diagnose of RLS was based on questionnaire with 4 specific questions according to the International Restless Legs Syndrome Study Group (IRLSSG) criteria updated in 2003. Severity of symptoms was astimated with IRLSSG Rating Scale, depression and anxiety with Hamilton Depression Rating Scale (HDRS) and Hamilton Anxiety Rating Scale (HARS) and sleepiness with Epworth Sleepiness scale (ESS). We astimated sleep characteristics and disturbances with specific questionnaire. **Results.** In our study 79.9% of patients had sleep-related symptoms. Average sleep duration was 6.50 ± 1.42 hours, with average frequency of awakening 2.34 ± 1.69 times per night. Average ESS score was 5.12 ± 4.08 (0–17). Patients with more severe symptoms had higher degree of sleepiness ($p = 0.005$). Patients with higher symptoms frequency, significantly more often had sleep disturbance ($p = 0.016$), tiredness and daily sleepiness ($p = 0.001$). Daily sleepiness (ESS) also significantly correlates with depression ($p < 0.05$) and anxiety ($p = 0.012$). **Conclusion.** Our results confirm that sleep disturbances are one of the key accompanying symptoms of RLS which cause daily sleepiness, tiredness, depression and anxiety. Therefore, their early recognition and appropriate treatment must be a priority in RLS patients.

Key words:
restless legs syndrome; sleep disorders; anxiety; depression; fatigue; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Sindrom nemirnih nogu (SNN) je hronična neurološka bolest koju karakteriše potreba za pomeranjem nogu udružena sa neprijatnim senzacijama u donjim ekstremitetima. Poremećaji spavanja spadaju u glavne prateće simptome SNN koji se javljaju kod oko 90% bolesnika. Poremećaji spavanja dovode do dnevne pospanosti, depresivnosti i anksioznosti. Cilj ove studije bio je da detektuje učestalost i karakteristike poremećaja spavanja kod bolesnika sa SNN, kao i njihov uticaj na dnevnu pospanost, umor, anksioznost i depresivnost. **Metode.** Ispitano je 94 bolesnika sa SNN. Dijagnoza SNN postavljena je na osnovu kriterijuma Internacionalne grupe za proučavanje sindroma nemirnih nogu (*International Restless Legs Syndrome Study Group* – IRLSSG) iz 2003. godine. Težina kliničke slike procenjena je pomoću IRLSSG *roling scale*, depresivnost i anksioznost Hamiltonovom skalom za procenu depresivnosti i anksioznosti, a pospanost Epworthovom skalom pospanosti. Posebnim upitnikom ocenjivali smo karakteristike sna i njegove poremećaje. **Rezultati.** U našoj studiji 79,9% bolesnika imalo je poremećaje spavanja. Prosečna dužina sna bila je 6,50±1,42 sati sa prosečnim buđenjem 2,34 ± 1,69 puta u toku noći. Prosečan skor na Epworthovoj skali pospanosti bio je 5,12 ± 4,08 (0–17). Bolesnici sa većom učestalošću tegoba imali su statistički značajno veći stepen pospanosti ($p = 0,005$). Bolesnici sa većom učestlošću tegoba imali su češće poremećaje spavanja ($p = 0,016$), umor i dnevnu pospanost ($p = 0,001$). Dnevna pospanost značajno je korelirala sa depresivnošću ($p < 0,05$) i anksioznošću ($p = 0,012$). **Zaključak.** Naši rezultati potvrđuju da su poremećaji spavanja ključni prateći simptomi SNN koji uzrokuju dnevnu pospanost, umor, depresivnost i anksioznost. Zato je njihovo rano prepoznavanje i adekvatno lečenje od velikog značaja.

Ključne reči:
sindrom nemirnih nogu; spavanje, poremećaji; anksioznost; depresija; zamor; ankete i upitnici.

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Introduction

Resteless legs syndrome (RLS) is a chronic neurological disorder characterized by urge to move legs which is usually accompanied by unpleasant sensation in the lower extremities. It begins or worsens during the periods of rest (during the evening and nighttime hours), and it is partially or totally relieved by movement [1]. Prevalence of RLS in general population of Caucasians is ranging from 3.2% to 23.5% [2], which we have confirmed in the population of Sombor (Serbia) with prevalence of 5.1% (95% CI 4.2–6.2) and after correction (age-adjusted by European standard population) 4.4% (95% CI 3.6–5.4) [3]. Sleep disturbance is one of the main accompanying symptoms of RLS which exists in approximatelly 90% of patients and conversely, in 20% patients with insomnia, it is due to RLS [4]. Impairment of sleep is related to daily sleepiness, depressive and anxiety disorders [2] and also to cardiovascular and cerebrovascular diseases [5], cognitive and short attention impairment, executive functions and verbal fluency [6].

The aim of this study was to detect frequency and characterisitics of sleep-related symptoms in patients with RLS, and its impairment to daily sleepiness, fatique, anxiety and depression.

Methods

In our study we examined 94 patients with RLS, which had previosly been detected in the study of prevalence of RLS and the results of which have already been published [3]. The diagnose of RLS was based on questionnaire with 4 specific questions, essential criteria for RLS, and 3 additional questions, supportive criteria, according to the International Restless Legs Syndrome Study Group (IRLSSG) criteria updated in 2003 [1]. All patients have been examined in the General Hospital in Sombor and Clinic of Neurology in Belgrade, where they completed questionnaire with clinical and demographic data. The questionnaire was specificaly created for this study. Severity of symptoms were detected with International rating IRLSSG Rating Scale [1], depression and anxiety with Hamilton Depression Rating Scale (HDRS) [7] and Hamilton Anxiety Rating Scale (HARS) [8], respectively, and sleepiness with Epworth Sleepiness Scale (ESS) [9]. We have detected sleep characteristics and disturbances with specific questionnaire about sleep latency, occurrence, duration and characteristics of symptoms at the time of falling asleep, quality and continuity of sleep, and frequency and number of awakenings during night.

Results

We investigated 94 patients with primary RLS whose clinically-demografic data is shown in Table 1. The most severely affected subjects were ones with significantly more frequent symptoms of RLS ($p = 0.001$). Amongst 94 patients only 11 previosly had diagnosis of RLS, and only 7 had adequate therapy with dopa-agonist. Some patients (28/94) had been treated with sedatives, antidepressants, hypnotics or its combination.

In our study 79.9% of patients had sleep-related symptoms, 56.4% had problems with sleep continuity, 45.7% of patients had RLS related problems during night in duration of more than one hour, and 11.7% of patients had these problems in duration of more than three hours. Average sleep duration was 6.50 ± 1.42 hours, with average frequency of awakening 2.34 ± 1.69 times per night. Symptoms, in the case of our patients, would mostly begin at the moment of falling asleep, between 22–24 h (78.7%), then about 18 h (12.8%), or before 18 h (8.5%). Average sleep latency was 62.0 ± 45.36 min. None of the patients was on dopa-agonist therapy, but 27.6% was treated with benzodiazepines, 4% with antidepressants and 7.9% with some of the combinations of benzodiazepines, antidepresssants and hypnotics, with poor effect on sleep-related symptoms.

Table 1

Clinical and demographic data of patients with restless legs syndrome (RLS)

Parameters	Values
Total number of patients	94
Gender, n	
female	67
male	27
Age in the moment of investigation (years) $\bar{x} \pm SD$	58.91 ± 13.42
Age in a moment of beginning of symptoms (years) $\bar{x} \pm SD$	44.3 ± 14.2
Frequency of RLS, %	
permanent	32.4
progressive	18.9
intermittent	48.6
> 1x a week	63.8
≤ 1x a week	36.2
Severity of symptoms (IRLSSGS), %	
mild	41.5
moderate	48.9
severe and very severe	9.6

IRLSSGRS – International Restless Legs Syndrome Study Group Rating Scale.
$\bar{x}$ – mean value; SD – standard deviation; n – number of patients; % – percentage of patients.

Average ESS score was 5.12 ± 4.08 (0–17). Totally 22.3% of patients had severe sleepiness and needed medical treatment and following-up, and 12.8% of patients had moderate sleepiness (score 7–8). No sleepiness during the day was found in 64.9% of patients (score 0–6), even though most of them had problems with sleep continuity.

We found a statistically significant correlation between severity of symptoms according to IRLSSG rating scale and sleepiness according to ESS – patients with more severe symptoms had higher degree of sleepiness ($p = 0.005$), but their duration had no influence on sleepiness. Frequency of symptoms also had significant influence on sleep disturbances, tiredness and daily sleepiness. Patients with higher frequency symptoms, acording to the statistics, significantly more often had sleep disturbances ($p = 0.016$), tiredness and daily sleepiness ($p = 0.001$) (Table 2). Daily sleepiness (ESS) also significantly correlated with depression (HDRS) ($p < 0.05$) and anxiety (HARS) ($p = 0.012$).

longer twice than in the REST study – 62.0 minutes. Our patients would wake-up two to three times per night, like in other investigations.

Severity of symptoms correlated with sleep disturbances, so subjects with moderate and severe disease had sleep duration less then 5 hours per night (50%), or even less then 3 hours per night (14%), and their sleep was less efficient because, in our study, symptoms were related to sleep initiation (22–24 h) in 78.7% or started during afternoon (18 h). Only 10.6% had symptoms after falling asleep (1–2 a.m.) [11]. Duration of sleep-related symptoms was, in the case of our patients, longer than one hour in 45.7%, and longer than three hours in 11.7%, with significant sleep impairment. Average sleep duration was 6.5 hours.

As a consequence of chronic sleep disturbances patients may feel irritabile, have a lack of initiative, memory disturbances, depression and anxiety [12]. This leads to social isolation and problems in daily activities [13]. These patients more

Table 2

Correlation of symptoms frequency, sleep disturbances and mood in patients with restless legs syndrome (RLX)

IRLSSGRS	Patients (n = 94) n (%)	Frequency of symptoms		*p*
		< 1x per week, (n = 34) n (%)	≥ 1x per week, (n = 60) n (%)	
Sleep disturbances				
no	17 (18.1)	11 (32.4)	6 (10.0)	
mild	14 (14.9)	7 (20.6)	7 (11.7)	0.016
moderate	18 (19.1)	4 (11.8)	14 (23.3)	
severe	30 (31.9)	10 (29.4)	20 (33.3)	
very severe	15 (16.0)	2 (5.9)	13 (21.7)	
Daily tiredness or sleepiness				
no	32 (34.0)	18 (52.9)	14 (23.3)	
mild	25(26.6)	11 (32.4)	14 (23.3)	
moderate	25 (26.6)	5 (14.7)	20 (33.3)	0.001
severe	12 (12.8)	0 (0.0)	12 (20.0)	
very severe	0 (0.0)	0 (0.0)	0 (0.0)	

IRLSSGRS – International Restless Legs Syndrome Study Group Rating Scale.
n (%) – number (percentage) of patients.

Discussion

Our results showed that more than 4/5 of our patients had some kind of sleep disturbances, and more than a half suffered from disruption of sleep continuity. Severity of symptoms correlated with their frequency, and also with sleep disturbances, tiredness and daily sleepiness. A degree of daily sleepiness positively correlated with depression and anxiety.

Sleep disturbance is one of the most important symptoms in RLS. Individuals with RLS are two to three times more likely to report these symptoms then non RLS subjects [2]. In the REST study [10], carried out in several European countries and in the United States of America, patients mostly had inability to fall asleep (48.1%), inability to stay asleep (39.2%), disturbed sleep (60.6%) and insufficient sleep (40.1%). Over two-thirds of the patients took 30 minutes or more to fall asleep, and 60% awoke three or more times per night. Similary, our study [3] showed disturbed sleep in 79.7% of patients, and 56.4% had difficulties in sleep maintaining, like in REST study [10]. Average sleep latency was

often have episodes of night smoking and eating than healthy population [14].

Daytime sleepiness is one of the expected consequences of sleep deprivation, but there are contradictory results in different studies. In some of them there was converging evidence that around 20–25% of subjects with untreated idiopathic RLS are likely to experience increased daytime sleepiness [15], and 32–42% excessive sleepiness [2]. Excessive sleepiness is two to three times higher than in healthy population [2]. For daytime sleepiness assessment we used ESS. Average score was 5.12 ± 4.08 (0–17). No sleepiness during the day was found in 64.9% of patients (score 0–6), even though they had problems with sleep continuity, 12.8% had moderate sleepiness and 22.3% suffered from severe sleepiness and they needed medical treatment and following-up. Daytime sleepiness statisticaly correlated with disease severity; according to IRLSSG – patients with more severe simptoms had a higher degree of daytime sleepiness. Duration of symptoms had no influence on sleepiness. Similar results were found in a study of Kim et al. [16] with also positive correlation between simp-

toms of severity according to IRLSSG Rating Scale and sleepiness according to ESS. In accordance with what was already mentioned above, there was a correlation of frequency of RLS symptoms and sleep disturbances, tiredness or sleepiness during the day estimated by IRLSSG Rating Scale. Sleep disturbances due to RLS were also significantly more common among RLS sufferers with frequent symptoms.

Sleep disorders can cause depression and anxiety [17]. Insomnia is independent factor for depression, but depression may be a consequence of other factors which disturb quality of life like social isolation and the chronic nature of the disease. Depression and anxiety appears at least twice as often in RLS patients than in healthy population [2, 18]. In our investigation daily sleepiness (ESS) significantly correlated with depression (HDRS) and anxiety (HARS), and conversely – patients with higher scores on HDRS and HARS had statisticaly significantly more often daily sleepiness.

Conclusion

Our results confirm that sleep disturbances are ones of the key accompanying symptoms of RLS which cause daily sleepiness, tiredness, depression and anxiety. Therefore, their early recognition and appropriate treatment must be a priority for RLS patients.

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SHORT COMMUNICATIONS

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Gastric stimulation to treat the type 2 diabetes: results on week 16

Gastrična stimulacija u terapiji dijabetesa tip 2: rezultat posle 16. nedelje od implantacije pejsmejkera

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Abstract

Background/Aim. Gastric contraction modulation (GCM) with the implanted DIAMOND device improves glycemic control and decreases weight. The main aim of this study was to evaluate the early efficacy of GCM using the DIAMOND (previously named TANTALUS) System in the improvement of glycemic control measured by changes in glycosylated hemoglobin (HbA1c). The effects of GCM on weight loss, body mass index (BMI), reduction of the waist circumference and metabolic parameters other than HbA1c were also evaluated. **Methods.** A total of 18 adult patients with type 2 diabetes were surgically treated at the Department for Minimally Invasive Upper Digestive Surgery, Clinic for Digestive Surgery in Belgrade, Serbia, using gastric pacemaker (DIAMOND System) from November 2014 to March 2016. Out of the total number of patients, 11 finished week 16 visit and were enroled in this prospective cohort study. **Results.** During the observed period, the average weight loss amounted to 8.05 kg ($p < 0.05$). The average difference between the baseline fasting glucose level and the level after 16 weeks period is 2.56 mmol/L. Similar findings were noted in fasting insulin levels, with an average decrease of 6.44 m/U/L after 16 weeks. The majority of patients experienced a decrease in HbA1c value: in 4 patients higher than 2%, and in 4 patients up to 2% ($p < 0.05$). Lower level of fasting insulin with simultaneous decrease in fasting glucose indicates improvement in insulin sensitivity on week 16 [homeostatic model assessment of insulin resistance (HOMA IR) average 5.25]. **Conclusion.** Gastric stimulation using the DIAMOND System for 16 weeks causes significant early improvement in glycemic control and insulin resistance. There is an additional positive effect on weight loss, body mass index (BMI) and reduction of the waist circumference as a main parameter of the metabolic syndrome.

Key words:
diabetes mellitus, type 2; obesity; stomach; electric stimulation therapy; treatment outcome; hemoglobin A, glucosylated; blood glucose.

Apstrakt

Uvod/Cilj. Stimulacija želuca [*gastric contraction modulation* (GCM)] implantiranim sistemom DIAMOND poboljšava glikomijsku kontrolu i smanjenje telesne mase. Primarni cilj studije bio je da se proceni inicijalna efikasnost stimulacije želuca upotrebom DIAMOND sistema (ranije poznatog kao TANTALUS) u cilju poboljšanja glikoregulacije merene promenom nivoa glikoziliranih hemoglobina (HbA1c). Sekundarni ciljevi bili su analiza sniženja telesne mase, indeksa telesne mase (BMI), obima struka i drugih metaboličkih parametara. **Metode.** U Odeljenju za minimalno invazivnu hirurgiju gornjeg digestivnog trakta Klinike za digestivnu hirurgiju u Beogradu, u periodu između novembra 2014. godine i marta 2016. godine, kod 18 bolesnika sa dijabetesom tipa 2, laparoskopski je postavljen gastrični pejsmejker (DIAMOND sistem). Od ukupnog broja bolesnika, 11 bolesnika je bilo praćeno najmanje 16 nedelja posle operacije i uključeno u prospektivnu kohortnu studiju. **Rezultati.** U toku perioda praćenja, prosečan gubitak telesne mase iznosio je 8.05 kg ($p < 0.05$). Prosečna razlika između inicijalne vrednosti glikemije i glikemije posle 16 nedelja od implantacije pejsmejkera iznosila je 2.56 mmol/L. Sličan rezultat dobijen je prilikom procene vrednosti insulina natašte; prosečno smanjenje vrednosti posle 16 nedelja iznosilo je 6.44 m/U/L. Kod većine bolesnika konstatovano je i smanjenje vrednosti HbA1c: kod 4 bolesnika veće od 2% i kod 4 osobe do 2% (prosečno 1.19%, $p < 0.05$). Niži nivo insulina natašte i istovremeno smanjenje visine glikemije natašte ukazalo je na poboljšanje insulinske senzitivnosti posle 16 nedelja od implantacije pejsmejkera [*homeostatic model assessment of insulin resistance* (HOMA IR) prosek 5.25]. **Zaključak.** Stimulacija želuca DIAMOND sistemom tokom 16 nedelja od implantacije, prouzrokuje statistički značajno poboljšanje glikoregulacije i insulinske rezistencije. Dodatan pozitivan terapijski efekat odnosi se na smanjenje telesne mase, BMI i smanjenje obima struka kao glavnog parametra metaboličkog sindroma.

Ključne reči:
dijabetes melitus, insulin-nezavisan; gojaznost; želudac; elektroterapija; lečenje, ishod; hemoglobin, glukozilovan; glikemija.

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Introduction

Type 2 diabetes mellitus already affects over 500 million people, out of which 60% are obese [1]. The upsurge in obesity and the concomitant rise in diabetes have imposed a substantial burden on public health. Total costs for diabetes treatment are expected to reach $192 billion by the year 2020 [2].

Significant and durable weight loss associated with a clinically beneficial improvement of metabolic control in patients with type 2 diabetes mellitus was reported after bariatric surgical procedures in recent publications [3, 4]. However, the surgical risk and significant physiological and anatomical changes associated with bariatric procedures as well as the number of bariatric surgeons are currently the main obstacles for wider use of this technique. It was found that overweight type 2 diabetics can benefit from a fully reversible minimally invasive surgical procedure which could improve their metabolic control and help them to lose weight.

Multiple clinical studies designed to evaluate the TANTALUS System for the treatment of obesity, type 2 diabetes and comorbid conditions, have been and are currently being conducted worldwide. As with most organs, electrical stimulation used in TANTALUS System serves to regulate many aspects of gastric function [5–7].

Gastric stimulation [gastric contraction modulation (GCM)] to treat the type 2 diabetes mellitus was, for the first time, performed at the Department for Minimally Invasive Upper Digestive Surgery, Clinic for Digestive Surgery in Belgrade, Serbia, in November 2014.

The main objectives of this study was to evaluate the early efficacy of GCM using the DIAMOND (previously named TANTALUS) System in the improvement of glycemic control measured by changes in glycolyzed hemoglobin (HbA1c). The effects of GCM on weight loss, body mass index (BMI), reduction of the weight circumference and metabolic parameters other than HbA1c were also evaluated.

Methods

Study design

A total of 18 adult patients with type 2 diabetes were surgically treated at the Department for Minimally Invasive Upper Digestive Surgery, Clinic for Digestive Surgery in Belgrade, Serbia, using gastric pacemaker (DIAMOND System) from November 2014 to March 2016. Out of the total number of patients, 11 finished week 16 visit and were enroled in this prospective cohort study.

Patients underwent baseline evaluation during which the stability of their glycemic parameters, medical treatment and medical condition were assessed. All patients gave informed consent prior to study enrolment. The study was reviewed and approved by the Clinical Center of Serbia Institutional Review Board. Patients fulfilling all inclusion/exclusion criteria were implanted. Inclusion criteria were as follows: male and female subjects aged between 18 and 70 years of age; body mass index (BMI) > 30 and < 45 kg/m^2; type 2 diabetes duration of at least 6 months; type 2 diabetic subjects treated with oral antidiabetic agents [sulfonylurea, metformin, thiazolindiedione (TZD) or dipeptidyl peptidase 4 (DPP-4) inhibitors]; stable antidiabetic medications for at least 3 months prior to enrolment, 6 months for TZD; the subject was under routine diabetes care of the investigator or another single physician that can supply a medical record for at least 6 months prior to enrolment; HbA1c ≥ 7.3% and ≤ 9.5 % on the first visit; stable HbA1c, defined as no significant change (variation ≤ 0.5%) between a historical value recorded in the subject's medical record within 3 months prior to enrolment and the HbA1c gathered on the first visit; fasting blood glucose >120 and < 350 mg/dL on the first visit; stable weight, defined as no significant weight change (variation less than 3%) within 3 months prior to enrolment as documented in the subject's medical record. For the subject treated with TZD, the criteria also included: stable weight within 6 months; if taking these medication, stable antihypertensive and lipid-lowering medication for at least 1 month prior to enrolment; able to provide voluntary informed consent.

Exclusion criteria were: insulin therapy in last 3 months; taking glucagon-like peptide-1 (GLP-1) agonists or taking them over the last 3 months before the enrolment; currently taking fibrates, nicotinamide and omega-3 fatty acids as antilipidemic treatment; subjects with an ejection fraction (EF) less than 35%, or otherwise, indicated for an implantable cardiac defibrilator (ICD); taking medications known to affect gastric motility such as narcotics (chronic use) and anticholinergics/antispasmodics; experiencing severe and progressing diabetic complications (i.e. retinopathy not stabilized, nephropathy with macroalbuminuria); prior wound healing problems due to *staphylococcus* or *candida*; diagnosed with an eating disorder such as bulimia or binge eating; obesity due to an endocrine disorder (e.g. Cushing disease); pregnant or lactating; diagnosed with impaired liver function (liver enzymes 3 times greater than normal); any prior bariatric surgery.

Surgical procedure

Implantation was typically performed under general anesthesia using a laparoscopic procedure. Two sets of bipolar stitch leads were placed in the antrum, one set in the anterior wall and the other set in the posterior wall. A third set was placed in the fundus (Figures 1 a and b). Endoscopy was performed during the implantation to ensure that the stitch electrodes are placed entirely within the muscle of the gastric wall without perforation of the gastric mucosa. The leads were tunneled and connected to the implantable pulse generator (IPG) (Figure 2). An abdominal subcutaneous pocket was created for the IPG and charge coil. The pocket should only be large enough to accommodate the IPG and charge coil before closing, proper electrode contact with the tissue was verified by measuring leads impedances using the programmer (placed in a sterile nylon sleeve) over the IPG. In the perioperative period the patient's blood glucose was monitored using a calibrated glucose meter to avoid any hypo or hyperglycemic events.

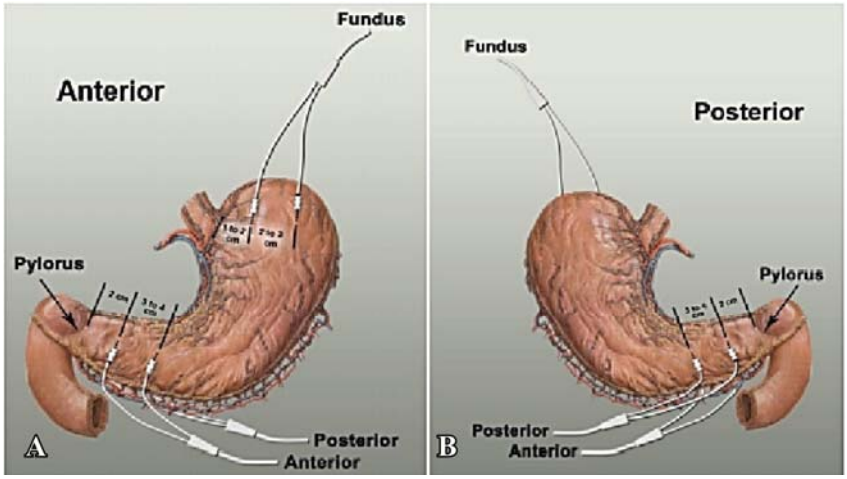

Fig. 1 – Lead placement: a) anterior and b) posterior view.

Fig. 2 – Implantable pulse generator (IPG).

The patients were released from the hospital with their diabetes treatment reinitiated and the device set so that no GCM signals are applied. It was stressed out that compliance (acceptance of the lifestyle advices and dietary regimen) is necessery to achieve therapeutic goals. Two weeks after the surgery, the IPG was turned on to continously delivered sygnal.

Antropometric (body mass, BMI, waist circumference) and laboratory data (fasting insulin, fasting blood glucose, HbA1c) were collected before the surgery, on week 2, week 8 and week 16 after the surgery.

Statistical analysis

Descriptive statistics for all efficacy and safety endpoints will include a number of subjects, mean, standard deviation, minimum and maximum for continuous variables.

Results

The study population included 11 patients who underwent DIAMOND System implantation and finished week 16 visit between November 2014 and March 2016. The study group consisted of 6 male and 5 female patients, with an average age of 52 years (range from 43 to 68 years). There were 2 males and 2 females in the group of 40–49 years, 1 male and 3 females in the group of 50–59 years and 3 males and no female in the group of 60–69 years.

During the observed period, the average weight loss amounted to 8.05 ± 5.09 kg (range 2 to 18 kg). Four patients lost more than 10 kg, 3 patients lost between 5–10 kg, 4 of them lost up to 5 kg. BMI correlated with the weight loss, and the difference between the baseline value and the value at the end of week 16 ranged from 1.1 to 4.5 kg/m^2, with an average reduction of 2.42 kg/m^2 (Table 1).

We also noted a waist circumference reduction in the majority of the patients. Reduction of more than 5 cm waist circumference was noted in 4 patients and up to 5 cm in 6 patients. One patient gained 3 cm in waist, without a change in weight.

Even though the 16 weeks period is considered to be relatively short, substantial metabolical effects could be detected. The average difference between the baseline fasting glucose level and the level after 16 weeks period is 2.56 ± 2.96 mmol/L (range 0.9 to 8.6 mmol/L). The decrease in fasting glucose level higher than 6 mmol/L was observed in 2 patients, between 3–6 mmol/L in other 2 and up to 3 mmol/L in 5 patients. We detected higher levels of fasting

Table 1

Change in anthropometric and laboratory data from the baseline until the end of week 16 visit

Parameters	Baseline ($\bar{x}$ ± SD)	Week 2 ($\bar{x}$ ± SD)	Week 8 ($\bar{x}$ ± SD)	Week 16 ($\bar{x}$ ± SD)	Difference Baseline-Week 16 ($\bar{x}$ ± SD)	Difference range (min/max)
Anthropometric						
weight (kg)	119.17 ± 21.93	113.78 ± 20.17	111.34 ± 18.38	111.13 ± 18.34	8.05 ± 5.09	(2/18)
BMI (kg/m^2)	39.35 ± 4.14	37.57 ± 4.57	36.95 ± 4.18	36.93 ± 3.94	2.42 ± 1.00	(1.1/4.5)
Waist circumference (cm)	126.27 ± 10.61	123.86 ± 11.77	122.36 ± 11.43	119.91 ± 9.76	6.36 ± 6.74	(-3/20)
Laboratory						
fasting glucose (mmol/L)	11.15 ± 2.74	9.22 ± 3.57	8.49 ± 3.41	8.59 ± 2.83	2.56 ± 2.96	(-0.90/8.6)
fasting insulin (mIU/L)	23.81 ± 11.76	19.95 ± 6.52	18.72 ± 9.50	17.37 ± 6.33	6.44 ± 11.06	(-5.06/35.49)
HbA1C (%)	8.74 ± 0.54	8.19 ± 1.13	7.35 ± 1.04	7.55 ± 1.22	1.19 ± 1.16	(-0.7/2.6)
HOMA IR	11.58 ± 5.76	7.86 ± 3.03	7.02 ± 4.08	6.33 ± 2.15	5.25 ± 5.94	(-1.11/20.47)

BMI – body mass index; HbA1C – glycosylated hemoglobin; HOMA-IR – insulin resistance index; $\bar{x}$ – mean; SD – standard deviation.

glucose in 2 patients. One of them experienced worse glycoregulation due to inability to conduct a dietary regimen between week 12 and 16. The other had an increase just in fasting glucose in week 16, without change in HbA1c value (Table 1).

Similar findings were noted in fasting insulin levels, with an average decrease of 6.44 ± 11.06 m/U/L after 16 weeks (range 5.06/35.49). Most of the patients had more or less pronounced reduction in fasting insulin level. One patient had an increase in fasting insulin level up to 0.2 m/U/L compared to the baseline value (Table 1).

The majority of patients experienced a decrease in HbA1c value: in 4 patients higher than 2%, and in 4 patients up to 2% (average 1.19 ± 1.16%, range -0.7% to 2.6%. In one patient HbA1c level raised by 0.7% (Table 1).

Lower level of fasting insulin with simultaneous decrease in fasting glucose indicates improvement in insulin sensitivity in week 16 (HOMA IR 5.25 ± 5.94, range – 1.11/20.47 (Table 1).

Discussion

Obesity and type 2 diabetes (diabesity) are global epidemic problem. Less than 50% patients accept lifestyle advice and achieve therapeutic goals [8]. In order to improve glucose control, patients committed to insulin treatment frequently experienced weight gain and hypoglycemia. Meal-mediated electrical GCM could be an alternative treatment for the patients with type 2 diabetes with poor glucose control on oral anti-diabetic drugs [7]. This therapy can be applied to patients with sufficient amount of endogenous insulin. Some researchers promote gastric pacing in patients with poor dietary compliance due to the fact that this system automatically delivers electrical impulses, when programmed to. This device can be implanted via laparoscopy, with very low perioperative risk. Gastric pacemaker stimulates afferent fibers of the vagal nerve which interacts with hypothalamic satiation centre. This pathway is responsible for insulin secretion and insulin sensitivity. The patients with electric GCM demonstrate an improvement in glucose and weight control [9]. Previous studies in obese patients suggested that blocking vagal efferent impulses with high-frequency and short pulse width, resulted in weight loss. It was suggested that such weight reduction is a consequence of delayed gastric emptying and inhibition of postprandial gastric contractions [10].

In our study, there was significant decrease in body weight (119.17 ± 21.93 kg *vs* 111.13 ± 18.34 kg; $p < 0.05$) and BMI (39.35 ± 4.14 kg/m^2 *vs* 36.93 ± 3.94 kg/m^2, $p < 0.05$), 16 weeks after DIAMOND System implantation. The average body weight reduction was 8.05 ± 5.09 kg and BMI reduction was 2.42 ± 1.00 kg/m^2. Satiety center in ventromedial nuclei and hunger center in ventrolateral nuclei in hypothalamus regulate food intake regarding an energy content, volume and duration of a single meal. Energy homeostasis is achieved via afferent nerve signals from oral and gastric mucosa with support of enteropeptides from gut mucosa. Thus, gut-brain axis plays a role in exchange of information between gut mucosa and food intake centers in the brain, through vagal nerve [11, 12]. Vagotomy reverses the obesity in animals with hypothalamic damage induced obesity. The same effect could be achieved with selective efferent vagal stimulation with gastric pacemaker without side effects on cardiovascular system. Long-term effects on weight loss are different in various studies, from 1.3% up to 40% of excessive weight loss (EWL) during two years of treatment. Ghrelin is very potent orexigenic hormone which stimulates gastrin release. Ghrelin also stimulates gastric acid secretion and has prokinetic effect on the small bowel. Gastric bypass decreases ghrelin secretion. Similar effect on ghrelin secretion has vagal nerv dissection as well as gastric pacemaker with electrodes positioned in the fundus of the stomach [13, 14].

Many studies demonstrated undesirable effect of antidiabetic drugs on body weight. Weight gain varied from 1.5 kg on oral antidiabetic drugs, up to 10 kg on insulin therapy. Weight reduction with DIAMOND System in obese type 2 diabetes mellitus patients is an added benefit to glucose control [10]. In our study, waist circumference decreased 6.36 ± 6.74 cm, from initially 126.27 ± 10.61 cm to 119.91 ± 9.76 cm at week 16 which indirectly indicates reduction of visceral fat. It was reported previously that the reduction in the amount of intraabdominal fat is associated with positive effect on metabolic parameters [15].

Fasting glucose decreased in average 2.56 ± 2.96 mmol/L (11.15 ± 2.74 mmol/L *vs* 8.59 ± 2.83 mmol/L; $p = 0.05$) and positive therapeutic effect regarding fasting glucose level was observed in 80% of patients. The other 20% had higher levels of fasting glucose. The one of them had deterioration of glycoregulation (the both, fasting glucose and HbA1c) due to uncompliance with dietary regimen during the last four weeks. The other had an increase in fasting glucose in week 16, but with change in HbA1c value, which could not be defined as a deterioration in glycoregulation. The exact mechanisam of glucose control with this device is not known but it was assumed that the regulation of blood glucose is partially weight loss independent. Gastric contractility reduces fasting and postprandial blood glucose, suppresses glucagon and increases GLP-1 release [16]. GLP-1 is an incretine, secreted by L-cells in the ileum and colon in response to food intake. GLP-1, as an anorexigene peptide, delayed gastric emptying and reduces the postprandial demand of insulin. These effects of GLP-1 are mediated via receptors on vagal nerves [17, 18]. GLP-1 could be clasiffied as neurohumoral agens with central action as neurotransmiter and with periferal action as hormon. With this activity, GLP-1 increase satiety in normal-weight subjects as well as in obese subjects, including patients with diabetes [19, 20]. Therapy with GLP-1 agonist, during 30 weeks decreases HbA1c by 0.8%, but 12 weeks after implantation of gastric pacemaker, HbA1c decreases 1% and remained at lower level for further 6 months [6]. Our results demonstrated that HbA1c decreases 1.19 ± 1.16% after 16 weeks (8.74 ± 0.54 *vs* 7.55 ± 1.22; $p < 0.05$). In about 40% of patients HbA1c decreases more than 2% and in further 40% patients decreases between 1 and 2%. In 1 patient there was no change in HbA1c level, and in 1 patient, HbA1c increases 0.7% and that was the patient uncompliant with dietary regimen between week 12 and 16.

A greater risk for serious hypoglycemia was found in therapeutical procedures using a combination of two or more oral antidiabetic drugs or treatment by insulin. Very low incidence of hypoglycemia was found in studies with DIAMOND System where strictly blood glucose control was achieved. Lowest level of hypoglycemia registered in patients with this device was 3.8 mmol/L and they had very mild symptoms of hypoglycemia [6, 7]. In our investigation, 3 out of 10 patients (30%), on gliclazide and metformin experienced hypoglycemia after 8 weeks of enrolement. Blood glucose during the day decreased to 4.1 mmol/L. Those patients had mild symptoms of hypoglycemia and according to the study protocol recommendation, dose of gliclazide was reduced or the drug was completely withdrown.

All of our patients have hyperinsulinism with insulin resistance at enrolment time (HOMA IR 11.81 ± 5.84). After 16 weeks, fasting insulin decreased to 6.44 ± 11.06 mIU/L (23.81 ± 11.76 *vs* 17.37 ± 6.33). Lower level of fasting insulin with simultaneous decrease in fasting glucose indicates improvement in insulin sensitivity on week 16 (HOMA IR 5.25 ± 5.94; 11.58 ± 5.76 *vs* 6.33 ± 2.15).

Due to a small sample size and short follow-up period we express the need for further evidence regarding this relatively new treatment option.

Conclusion

The overall conclusion was that in most of 11 patients the metabolic benefits followed the bariatric effect. On the other hand, a lack of compliance predicted poor metabolic bariatric outcome.

Gastric stimulation using the DIAMOND System for 16 weeks causes significant early improvement in glycemic control and insuline resistance. There is an additional positive effect on weight loss, body mass index and reduction of the waist circumference as a main parameter of the metabolic syndrome. In order to achieve therapeutic goals (positive metabolic, as well as bariatric effects) it is crucial to have good adherence and persistance to the lifestyle advice and dietary regimen. However, due to a relatively small number of patients and short follow-up period we express the need for further evidence regarding this treatment option.

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The efficacy of moxifloxacin in patients with bacterial keratitis

Efikasnost primene moksifloksacina kod bolesnika sa bakterijskim keratitisom

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Abstract

Background/Aim. Keratitis is a sociomedical problem of moderately developed countries. including Serbia, too. The incidence of bacterial keratitis in the world is about 20% in relation to all keratitis, but its frequency in Serbia is still not known. Bearing in mind the complications in the front segment of the eye after bacterial keratitis (ulcer, neovascularization, fibrosis) and decline in visual acuity, it was necessary to assess the efficacy of local therapy by moxifloxacin which will shorten the healing time and reduce complications. The aim of the study was to analyze the efficiency of shortening the treatment of bacterial keratitis with moxifloxacin. **Methods**. The study was designed as prospective, randomized, double-blind study. The study included 30 patients with diagnosed keratitis and locally applied antibiotic moxifloxacin, and 60 patients in a control group, with locally applied artificial tears. All participants were subjected to complete clinical ophthalmologic analysis (2015/16), for a period of 1–15 days after the application of therapies (healing time of corneal pathology). The following was determined in all patients: degree of hyperemia, degree of epithelial defect, level of corneal sensitivity, level of tear secretion and tear quality, degree of conjunctival secretion, degree of neovascularisation and corneal scarring, degree of visual acuity, score of subjective symptomatology and correlation of ophthalmological findings and subjective symptoms. **Results**. There was a statistically significant difference in times of therapeutic efficacy/clinical response between the study group patients who received moxifloxacin and the control group patients who received arteficial tears. **Conclusion**. Local application of moxifloxacin had therapeutic effect (total benefit) both in terms of the effective shortening of the healing time and also the reduction of the complications of bacterial keratitis, without unwanted effects.

Key words:
keratitis; moxifloxacin; bacterial infections; treatment outcome.

Apstrakt

Uvod/Cilj. Keratitisi su sociomedicinski problem srednje razvijenih zemalja u koje spada i Srbija. U odnosu na sve keratitise, učestalost bakterijskih keratitisa u svetu je oko 20%, a u Srbiji je još nepoznate učestalosti. Imajući u vidu komplikacije na prednjem segmentu oka nakon bakterijskog keratitisa (ulkus, neovaskularizacija, fibroza) i pad oštrine vida, bilo je potrebno proceniti efikasnost lokalne terapije moksifloksacinom, koja će skratiti vreme izlečenja i redukovati komplikacije. Cilj rada bio je analiza efikasnosti lečenja bakterijskih keratitisa moksifloksacinom. **Metode**. Studija je osmišljena kao prospektivna, randomizovana, dvostruko slepa klinička studija. Ona je obuhvatila 30 bolesnika sa dijagnostikovanim keratitisom i lokalno aplikovanim antibiotikom moksifloksacinom i 60 ispitanika kontrolne grupe, sa lokalno aplikovanim veštačkim suzama. Ispitanici su podvrgnuti kompletnoj oftalmološkoj analizi tokom 2015/16. godine u planiranom periodu od 1. do 15. dana od primene terapija (vreme sanacije kornealne patologije). Kod svih ispitanika određivan je stepen hiperemije, težina kornealnog defekta, kornealni senzitivitet, količina suza i kvalitet suznog filma, konjunktivalna sekrecija, neovaskularizacija i ožiljne formacije rožnjače, funkcionalna oštrina vida, skor subjektivne simptomatologije i korelacije oftalmološkog kliničkog nalaza i subjektivnih simptoma. **Rezultati**. Utvrđena je statistički značajna razlika u pogledu terapijske efikasnosti/kliničkog odgovora između studijske grupe, koja je primala moksifloksacin i kontrolne grupe, koja je primala placebo. **Zaključak**. Lokalna primena moksifloksacina je ostvarila terapijsku efektivnost (ukupan terapijski benefit), kako u domenu efikasnog skraćenja vremena izlečenja, tako i u redukciji komplikacija keratitisa, bez neželjenih dejstava.

Ključne reči:
keratitis; moksifloksacin; infekcija, bakterijska; lečenje, ishod.

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Introduction

Keratitis is an acute or chronic inflammation of the cornea which is medically significant, clinically clearly rated as sufficiently specific and important in diagnosis and therapy. The incidence of bacterial keratitis in the world is about 20% in relation to all keratitis [1]. Bacterial keratitis, after the herpetic keratitis transactions, is most common in the developed world [30,000 cases per year in the USA (8 : 100,000)] [2, 3]. Frequency of incidence in Serbia is still unknown.

The clinical picture of bacterial keratitis is characterized by relatively non-specific symptoms [1].

Bacterial keratitis is characterized by generally good clinical prognosis and often is monocular [4]. With regard to innervation the cornea is one of the most sensitive tissues, innervated by the sensitive nerves. Epithelization of cornea defect shows not only control "eye score", but also neurotrophic rehabilitation in patients with bacterial keratitis/ulcer [4, 5].

Moxifloxacin is an antibiotic belonging to the 4th generation of fluoroquinolones and an antibacterial agent in the local treatment of bacterial keratitis and/or conjunctivitis. It covers a spectrum of gram positive, gram negative, anaerobic and atypical microorganisms inhibiting topoisomerase II - DNA gyrase and topoisomerase-IV. Both enzymes are necessary for DNA replication, repair and recombination of bacterial microorganisms [6].

Moxifloxacin has excellent penetration through the cornea and conjunctiva, destroys pathogens quickly and efficiently, and it is characterized by low level of development of potential adverse effects as well as by low level of resistance. It is well tolerated clinically when applied locally and its effect is parallel to "ocular discomfort", caused by application of artificial tears, or placebo [6–9].

The aim of this study was to investigate whether the local application of moxifloxacin in patients with bacterial keratitis can effectively accelerate corneal epithelialization, or soft cure with a reduction of complications, in comparison with local placebo treatment (artificial tears).

Methods

The study was designed as a prospective, randomized, double-blind, clinical study. The study involved patients with acute bacterial keratitis diagnosed according to clinical protocols in the period 2015/16, and treated at the Clinic of Ophthalmology in the Clinical Center "Kragujevac", Kragujevac, Serbia. The study group included 30 hospitalized patients, aged 7–70 years (average age 44.2 ± 15.8 years). The control group included twice as many respondents, 60 of them (average age 40.8 ± 13.6 years) with bacterial keratitis, treated with topical application of artificial tears (hydroxypropyl methylcellulose). Out of the total number of respondents in the study group, there were 17 (56.7%) females and 13 (43.3%) males. In the control group there were 29 (48.3%) females and 31 (51.7%) males. The respondents were subjected to a study of control/analysis with a daily control pattern from 1 to 15 days (the visit/rounds on 8–10 h) after application of the local therapies. Epithelialization defect of cornea showed neuro-quality of sensation to touch-pain, a threshold of tactile sensitivity and dynamics of the recovery of corneal innervation (estenziometar by Cochet-Bonnet). Length was gradually decreased until the first reactions of patients, avoided spontaneous blinking and/or subjectivity. Sensitivity decreases from the center to the upper-temporal, nasal and lower regions (a phenomenon of "failure sensitivity" and the existence of bilateral asymmetry, depending on pathological changes and/or approximation of pathological values) [10].

We analyzed the intensity of fluoresccin (Fl)-staining defects of the cornea (epithelium/stroma, larger lesions, equal or smaller than 2 mm, of the slit-lamp (evolution of keratitis in ulcer) with standardized gradation: Fl +++, Fl ++, Fl +, Fl – [11–19].

Schirmer's test-1 (without local anesthesia), was used for estimating amount of tears („soaked paper" less, normal 10 mm/5 min, or more), and break-up time of tear film [tear break-up time (TBUT)] test, which determined the required quality of tear film (destabilization/stabilization of film), and appearance of the first cracks in tear film (time: less, normally for 10 sec, or more) [20–22].

The presence/absence and occurrence/evolution of the eye-hyperemia (conjunctival, ciliary, mixed), presence/absence of macro-characteristics and degree of conjunctival secretion, the presence/absence and type of pannus (superficial, deep, mixed), presence/absence, type and degree of scar-formation (nubecula, macula, leucom) were analyzed on the slit-lamp [23–25]. We analyzed the visual acuity (Snellen optotype) from causal minus (fall), through the normal/unchanged, until normal/enhanced (increased) functional visual acuity (with and/or without correction).

Analyses of subjective symptoms with elimination of pain/burning, foreign body sensation and reduction of epiphora, blepharospasm and photophobia (trias irritative symptoms), i.e. discomfort (answers to questions from the authentic questionnaires with the score-system: 0 - no, 1 - discreetly, 2 - expressed, 3 - intense) were done [26–28].

On the basis of composite to monocular clinical findings/analysis in controls by day, bacterial keratitis was categorized as cured or uncured (dihotona binary variable), and the final outcome was assessed 15 days after the beginning of the local therapy with moxifloxacin or artifical tears, as healed or not healed (progressive phase of active lesions, regression and healing). All parameters were analyzed before starting drug application, or artificial tears as well as 3, 6, 9, 12 and 15 days from the beginning of treatments.

To calculate the sample size, the software package G-Power 3.1.7. was used - studies on power (1-β) = 0.8, α = 0.05 for the Student's *t*-test. For statistical analysis authors used SPSS (Statistical Package Social Sciences) program 18.0 for Windows. The differences of parameters through the study period of 15 days were analyzed by the Friedman's test, and the differences between the study and control group of patients were analized by the Mann Whitney test. The correlation between some variables were tested by bivariate correlation test, Pearson's and Spearman's coefficients.

Procedure of the study was conducted in accordance with the ethical standards of Committee for Experiments on

Humans (Helsinki Declaration) and with consent of the competent Ethic Committee in the Clinical Center „Kragujevac".

Results

The study analyzed the degree of hyperemia determined as mild, moderate or severe hyperemia. Before the therapy in the study group 7 (23.3%) patients had mild hyperemia, 11 (36.7%) patients had moderate and 12 (40%) patients had severe hyperemia. In the control group, 15 (25%) patients had mild, 18 (30%) patients had moderate and 27 (45%) patients had severe hyperemia. There was a statistically significant reduction in the degree of hyperemia in the group of patients treated with the antibiotic during the observation period of 15 days ($p < 0.001$). In contrast, it was shown that there was a statistically significant increase in the degree of hyperemia in the control group ($p < 0.001$).

Using mutual comparison of the values of hyperemia in the study and control group a significant progressive decrease in the degree of hyperemia in the study group, and an increase in hyperemia in the control group starting from the 6th day ($p < 0.001$) were found (Table 1).

The study also analyzed the degree of damage of the cornea, determined as mild (< 2 mm), moderate (2 mm) and severe defects (> 2 mm). In the study group, 8 (26.7%) patients had mild degree of corneal damage, 7 (23.3%) patients had moderate and 15 (50%) patients had high degree of damage of the cornea. In the control group, 17 (28.3%) patients had mild degree of corneal damage, 17 (28.3%) patients had moderate and 26 (43.3%) patients had high degree of corneal damage. There was a statistically significant reduction in the degree of the cornea damage in the study group ($p < 0.001$). In contrast, it was shown that there is a statistically significant increase in the degree of corneal damage in the control group of patients ($p < 0.001$). Using mutual comparison of the corneal defect degree in the study group and control group it was found that 3 days after the treatment, there was statistically significant difference in the severity of defects between these two groups of patients starting from the 6th day ($p < 0.001$) (Table 1).

The degree of sensitivity preservation was represented as anesthesia, hypoesthesia and maintained sensitivity. Before the treatment in the study group, 2 (6.7%) patients had anesthesia, 3 (10%) patients had hypoesthesia, and 15 (83.3%) patients had maintained sensitivity. In the control group of patients, 2 (3.3%) patients had anesthesia, 5 (8.3%) patients had hypoesthesia, and 53 (88.4%) patients had maintained sensitivity. By processing the obtained data, there was found a statistically significant increase in the degree of sensitivity preservation in the study group of patients ($p < 0.001$). In contrast, statistically significant reduction was shown in the degree of preservation of sensitivity in the control group ($p < 0.001$). Fall of corneal sensitivity had a progressive character.

Using mutual comparison of the value of sensitivity preservation in the study and control group the significant differences was found throughout all study period ($p < 0.001$) (Table 1).

The degree of tear secretion was analyzed by Schirmer-test, and the results displayed as reduced amount of tears, a normal amount of tears and increased amount of tears. Before the use of the antibiotic in the study group, 3 (10%) patients had a reduced amount of tears, 9 (30%) patients had normal and 18 (60%) patients had increased amount of tears. In the control group, 3 (5%) patients had reduced amount of tears, 26 (43.3%) patients had normal and 31 (51.7%) patients had increased amount of tears. Processing the obtained data, there was statistically significant reduction in the amount of tears in the study group ($p < 0.001$). In contrast, it was shown that there was a statistically significant increase in the secretion of tears in the control group ($p < 0.001$). Using mutual comparison of the value of the quantity of tears it was found a statistically significant difference in the secretion of tears between the two groups ($p = 0.009$) (Table 1).

The quality of the tear film (TBUT test) was analyzed and classified as bad quality, poor quality and good quality. Before the use of the antibiotic in the study group, 8 (26.7%) patients had bad quality, 20 (66.7%) patients had poor quality and 2 (6.6%) patients had good quality of the tear film. In the control group of patients prior to the treatment, 16 (26.7%) patients had bad quality, 35 (58.3%) patients had poor quality and 9 (15%) patients had good quality of the tear film. By processing the data obtained it was found a statistically significant improvement in the quality of tear film in the study group ($p < 0.001$). It was shown a statistically significant reduction in the quality of the tear film in the control group ($p < 0.001$). Using mutual comparison of the quality of the tear film between the two groups of patients statistically significant difference was found starting from the 3rd day of the treatment ($p = 0.033$) (Table 1).

The degree of conjunctival secretion was determined as poor, moderate and abundant secretion. Before the use of the antibiotic in the study group, 2 (6.7%) patients had no conjunctival secretion, 8 (26.7%) patients had scant secretion, 15 (50%) patients had moderate and 5 (16,6%) patients abundant secretion. In the control group, 5 (8.3%) patients had no secretion, 19 (31.6%) patients had meager secretion, 10 (16.7%) patients had moderate and 26 (43.4 %) patients had abundant secretion. By processing the data obtained a statistically significant reduction in the degree of conjunctival secretion was found in the group of patients treated with the antibiotic ($p < 0.001$). In contrast, a statistically significant increase in the degree of conjunctival secretion was shown in patients treated with artificial tears ($p < 0.001$). Mutual comparison of the value of conjunctival secretion showed the significant differences in the severity of conjunctival secretion between the two groups of patients starting from the 3rd day of the treatment ($p = 0.012$) (Table 1).

The study analyzed the presence or absence, and the degree of corneal neovascularization (determined as surface, deep and mixed). Before the use of antibiotics in the study group, 10 (33.3%), patients were without elements of neovascularization, 14 (46.7%) patients had surface, 4 (13.3%) patients had deep and 2 (6.7%) patients had mixed corneal neovascularization. In the control group, 22 (36.7%) patients had no neovascularization, 30 (50%) patients had superficial,

Table 1

Changes of the analyzed parameters (clinical and prognostic factors) in the study group of subjects during the period of 15 days

Parameter	Days					
	1	3	6	9	12	15
Hyperemia, degree						
study group	2.01 ± 0.63	1.88 ± 0.63	1.38 ± 0.50	0.72 ± 0.49	0.45 ± 0.42	0.32 ± 0.53
control group	1.95 ± 0.88	1.93 ± 0.67	1.80 ± 0.66	2.21 ± 0.77	2.41 ± 0.55	2.66 ± 0.61
Corneal defect, degree						
study group	2.05 ± 0.82	1.94 ± 0.78	1.44 ± 0.52	0.56 ± 0.47	0.28 ± 0.55	0.17 ± 0.44
control group	1.97 ± 0.83	1.98 ± 0.63	1.98 ± 0.63	2.30 ± 0.71	2.44 ± 0.66	2.29 ± 0.79
Preservation of corneal sensitivity, degree						
study group	2.96 ± 0.3	2.94 ± 0.10	2.98 ± 0.15	2.90 ± 0.25	2.74 ± 0.44	3.02 ± 0.19
control group	2.99 ± 0.40	2.5 ± 0.57	2.42 ± 0.58	2.39 ± 0.61	2.32 ± 0.54	2.1 ± 0.60
Tears secretion (Schirmer test), mm						
study group	2.45 ± 0.64	2.10 ± 0.34	1.89 ± 0.19	1.80 ± 0.43	1.78 ± 0.30	1.72 ± 0.27
control group	2.42 ± 0.51	2.30 ± 0.39	2.40 ± 0.58	2.61 ± 0.56	2.68 ± 0.57	2.78 ± 0.44
Quality of tear film (tBUT test), sec						
study group	1.78 ± 0.45	2.18 ± 0,39	2.37 ± 0.62	2.70 ± 0. 41	2.85 ± 0.33	2.98 ± 0.31
control group	1.80 ± 0.59	1.76 ± 0.47	1.43 ± 0.51	1.38 ± 0.55	1.24 ± 0.49	1.19 ± 0.41
Conjuctival secretion, degree						
study group	2.10 ± 0.42	1.72 ± 0.77	1.25 ± 0.68	0.90 ± 0.54	0.43 ± 0.56	0.31 ± 0.32
control group	1.99 ± 0.44	2.35 ± 0.53	2.42 ± 0.61	2.54 ± 0.48	2.68 ± 0.71	2.75 ± 0.37
Neovascularisation of cornea, degree						
study group	0.77 ± 0.67	0.50 ± 0.63	0.36 ± 0.55	0.24 ± 0.49	0.15 ± 0.5	0.1 ± 0.48
control group	0.81 ± 0.62	0.99 ± 0.69	1.21 ± 0.88	1.68 ± 0.56	2.02 ± 0.33	2.18 ± 0.74
Scar complications of cornea, degree						
study group	0.64 ± 0.68	0.60 ± 0.51	0.39 ± 0.47	0.28 ± 0.53	0.20 ± 0.52	0.13 ± 0.33
control group	0.62 ± 0.52	0.79 ± 0.76	1.20 ± 0.53	1.64 ± 0.66	2.10 ± 0.82	2.19 ± 0.89
Functional visual activity, degree						
study group	1.35 ± 0.41	1.75 ± 0.59	2.08 ± 0.66	2.28 ± 0.36	2.73 ± 0.47	2.94 ± 0.46
control group	1.56 ± 0.55	1.52 ± 0.41	1.24 ± 0.48	1.20 ± 0.48	1.17 ± 0.34	1.10 ± 0.37

tBUT – tear breakup time.

5 (8.3%) patients had deep and 3 (5%) patients had mixed neovascularization. After processing the obtained data it was found that there was a statistically significant reduction in the degree of neovascularization in the group of patients treated with the antibiotic ($p < 0.001$). In contrast, it was shown a statistically significant increase in the degree of neovascularization in patients treated with artificial tears ($p < 0.001$). Using mutual comparison of the corneal neovascularization values in the study and the control group, significant differences were found in the severity of these complications between the two groups of patients starting from the 3rd day of the treatment ($p = 0.025$) (Table 1).

The study analyzed the presence or absence (0) scar formations and change (determined as nubecula, macula and leucom). Before the use of the antibiotic in the study group, 15 (50%) of patients had no scars change, 11 (36.7%) patients had nubecula, 3 (10%) patients had macula, and 1 (3.3%) patient had leucom. In the control group, 35 (58.3%) patients were without scars change, 19 (31.6%) patients had nubecula, 3 (5%) patients had macula and 3 (5.1%) patients had leucom. By processing the obtained data a statistically significant severity reduction in scars changes was found in the group of patients treated with the antibiotic ($p < 0.001$). In contrast, a statistically significant increase in severity of scar changes was shown in the control group ($p < 0.001$). Using mutual comparison of scars weight changes severity in the study and control group, a significant difference between them was found in prevalence of scar changes starting from the 3rd day of the study period ($p = 0.029$) (Table 1).

We studied the functional visual acuity (determined as poor/reduced from 0.01–0.1, less 0.2–0.5 and good/improved 0.6–1.0). Before the use of the antibiotic in the study group, 23 (76.7%) patients had poor visual acuity, 6 (20%) patients had lower and 1 (3.3%) patient had good visual acuity. In the control group of patients, 31 (51.7%) patients had poor visual acuity, 24 (40%) patients had lower and 5 (8.3%) patients had good visual acuity. By processing the obtained data there was a statistically significant improvement found in visual acuity in patients treated with the antibiotic ($p < 0.001$). There was a statistically significant decrease in functional visual acuity found in patients treated with artificial tears ($p < 0.001$). Using mutual comparison of visual acuity in the

study group and the control group, a statistically significant difference was found in the functional visual acuity between the two groups of patients ($p < 0.001$) (Table 1).

The study analyzed the subjective symptomatology testing the presence of the pain/tingling feeling (0-absent/none, 1-discreet, 2-expressed, 3-intensive), the presence of foreign body feeling (0-absent/none, 1-discreet, 2-expressed, 3-intensive) and the presence of irritative trias (blepharospasm, epiphora, photophobia: 0-absent/none, 1-discreet, 2-expressed, 3-intensive). The results were then interpreted in relation to the score of subjective symptoms (0-9). Scores were analyzed before and after the therapy. Statistically significant reduction in the severity of subjective symptoms was found 15 days after the antibiotic treatment ($p < 0.001$). The average value score before administration of the antibiotics was 7.7 ± 0.73 and after the therapy 1.66 ± 0.82. In contrast, in the control group there was no statistically significant changes in subjective symptoms after application of artificial tears (7.52 ± 1.07 vs. 7.37 ± 0.59) ($p = 0.357$). Using mutual comparison of the scores in the study and control group, significant differences in the severity of subjective symptoms were found ($p < 0.001$) (Figure 1).

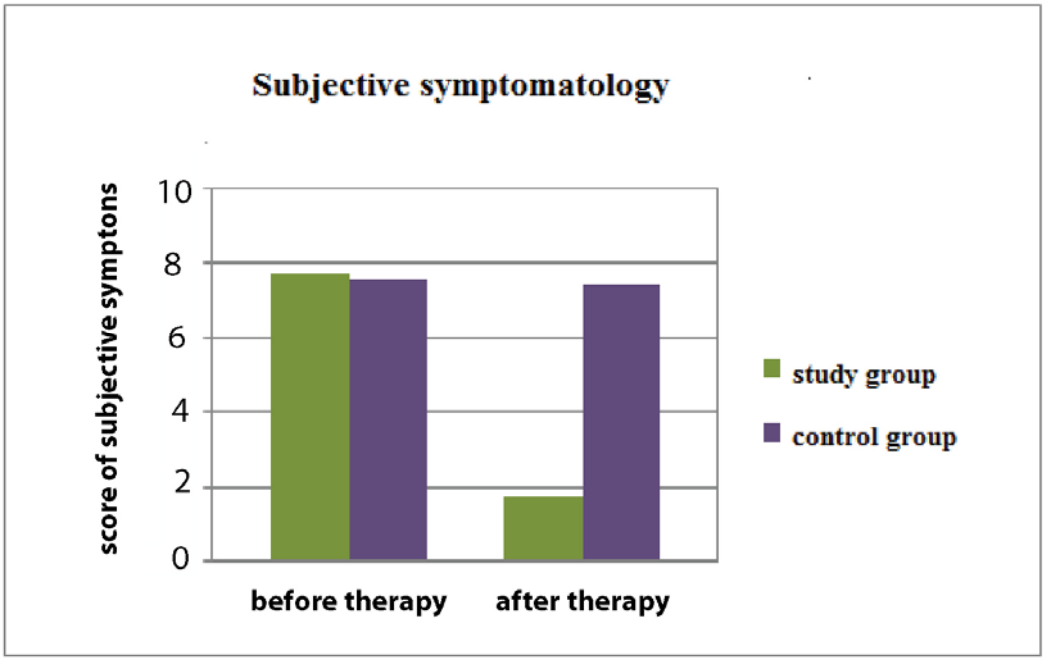

Fig. 1 – Subjective eye-symptoms (score) in the study and control group before and after therapy. (Study group – treated with local application of moxifloxacin; Control group – treated with arteficial tears).

We also analyzed the relation between the objective ophthalmologic findings and subjective symptoms. There was a statistically significant correlation between differences of the subjective symptoms score before and after the treatment and the degree of corneal sensitivity ($r = 0.365$, $p = 0.038$), the level of secretion of tears ($r = 0.510$, $p = 0.042$), the quality of the tear film ($r = 0.587$, $p = 0.037$), the degree of neovascularization ($r = 0.916$, $p = 0.009$), the level of scars complications ($r = 0.688$, $p = 0.033$), the degree of preservation of functional visual acuity ($r = 0.748$, $p = 0.025$). There was no statistically significant correlation between subjective symptoms score differences before and after the treatment and the degree of hyperemia ($r = 0.155$, $p = 0.180$), the degree of corneal defect ($r = 0.181$, $p = 0.243$), and the severity of conjunctival secretion ($r = 0.490$, $p = 0.069$).

Discussion

Modern clinical studies confirm that the 4th generation of fluoroquinolones have advantage over the previous generations against gram positive bacteria [29]. For example, moxifloxacin compared with non-fluoroquinolones antibiotics "kills" faster *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Haemophilus influenzae* [30].

The leading multicenter studies use moxifloxacin as fast and effective treatment of bacterial keratitis/ulcer, as we used in our study [31–33].

Many studies suggest different procedures available for purchase, the regime of antibiotic applications and different concentrations of pharmacological preparations for remediation of bacterial inflammation - keratitis [39, 40]. Respecting the principles of good clinical practice in the treatment of bacterial keratoconjunctivitis, there is a need to find new and/or alternative therapies which should bring overall benefits than current therapeutic options could do, including part-time as local steroid and/or combination of treatments [34, 35]. In our study we did not have any needs to use a cortisteroid for the treatment. It is encouraging to note that the early effects achieved with moxifloxacin "*in vitro*/*in vivo*" are therapeutic solution which shortens the time for healing treatment of bacterial keratitis, keratoconjunctivitis, and corneal ulcers [36–39]. Clinical studies now suggest different methods of application of moxifloxacin (e.g. subconjunctival injections) in patients with severe clinical forms of bacterial keratitis/ulcer [40], which was

not indicated for any of our patients. On the other hand, a certain pharmacological effects of fluoroquinolones on nervous tissue which belongs to moxifloxacin, could compromise the expected benefit in the treatment of the corneal inflammation. Recently it has been discovered that moxifloxacin inhibits fibroblast to myofibroblast differentiation, i.e. produces antifibrotic effect improving corneal wound healing [41].

Fluoroquinolones have an improved bactericidal effect against gram positive microorganisms, and reduced risk of phototoxicity and adverse events in relation to the previous quinolone generations [42–44]. Moxifloxacin resistance develops slowly [45]. Its adverse reactions were generally mild, at rate similar to placebo (artificial tears) (transient ocular discomfort – mild discomfort of 2.9%) [43, 44, 46].

Conclusion

Moxifloxacin had significant therapeutic effect (total benefit) both in terms of the effective shortening time of healing (epithelization corneal defects real fast) and reduction of complications of bacterial keratitis, without unwanted effects.

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GENERAL REVIEW

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Metabolic surgery and obesity related comorbidities

Metabolička hirurgija i komorbiditeti gojaznosti

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Ključne reči:
hirurgija, bariatrijska; gojaznost; komorbiditet; jetra, masna infiltracija; apneja u snu, sindromi; hipertenzija; neoplazme.

Introduction

Extreme obesity is associated with a lot of comorbidities like cardiovascular and cerebrovascular diseases, type 2 diabetes, gallstones, gastroesophageal reflux disease, malignancies, obstructive sleep apnea syndrome (OSAS), fatty liver disease, infertility, osteoarthritis, etc. These comorbidities significantly reduce quality of life and longevity. Extreme obesity shortens life expec-tancy by 8.9 years compared to the average life expectancy of 75 years. Middle aged with body mass index (BMI) 32 kg/m^2, compared to people with a BMI 24 kg/m^2 have 3 years less life expectancy [1].

Peripheral type of obesity is associated with peripheral venous stasis or thrombosis and degenerative diseases of the joints. Central or visceral obesity is accompanied by metabolic disorders and diseases arising from increased intra-abdominal pressure. Visceral obesity is often accompanied by insulin resistance with consequent disturbance of glucose control and type 2 diabetes, hepatic steatosis, non alcoholic fatty liver disease (NAFLD), hypertension, OSAS and polycystic ovary syndrome (PCOS) in women. The insulin resistance and chronic low-grade inflammation are the underlying mechanisms of most of comorbidities or perhaps, it is more proper to call them the complications of obesity [2–4].

Very early surgery attempt in order to control clinically severe obesity started in 1954. In 1991 the National Institutes of Health (NIH) Consensus Statement on Gastrointestinal Surgery for Severe Obesity officially designates surgery as a treatment of choice for patients with severe obesity. The introduction of laparoscopy in bariatric surgery increased the popularity of bariatric surgery among patients with morbid obesity. The desired effect of this therapeutic approach was excessive weight loss and significant reduction of comorbidities, primary the metabolic ones. This is the reason for increasing use of the term metabolic surgery instead of bariatric surgery, but the both terms are still in use equally [5, 6].

Diabetes remission after bariatric surgery

The risk of developing type 2 diabetes (T2DM) is exactly proportional to the increase of BMI and differs by gender. Specifically, women with BMI > 35kg/m^2 have a risk of diabetes 93.2%. That means that 9 out of 10 women with such BMI are likely to suffer from diabetes. In male population, the risk of diabetes in this BMI category is 42.1% [7]. A close connection between the risk of diabetes development and ethnicity is proven, regardless of BMI, so that the International Diabetes Federation (IDF) and World Health Organization (WHO) recommended specific reference values for the stages of obesity to different ethnic groups. The complexity of the epidemic of obesity and diabetes arises from many factors involved in both metabolic disorders, ranging from genetic factors, age of the mother, the microbiota in the intestine and epigenetic factors that affect fetus and newborn in the first few weeks of life. Insulin resistance is associated with impaired lipid oxidation in mitochondria, accumulation of lipid metabolites and inactivation of the insulin signal. Moderate weight loss in obese patients reduces the risk of diabetes by about 50%, with a favorable effect on the lipid status [8, 9]. Results of recently published Look AHEAD

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study [10] demonstrated significant improvement in glucose control in the group of obese patients with T2DM with lifestyle changes that included diet therapy and physical activity. During the study, body weight decreased by 8.6 % after one year in comparison with initial weight and reduction in body weight was sustainable for the next 4 years. Alternative treatment for obese patients with T2DM and poor glucose control with oral medications and with sufficient insulin secretion is electrical gastric stimulation. The gastric pacemaker is implanted laparoscopically into the abdominal cavity. Excessive weight loss (EWL) with this procedure is between 1.3% up to 40% during two years, with consequent significant improvement in glucose control measured by fasting glucose and glycosilated haemoglobin (HbA1c) [11]. In the mid-nineties, in the last century, Pories et al. [12] suggested a theory that type 2 diabetes is surgical disease, based on their surgical experience. The reason for this brave theory was the excellent results in diabetes management that were achieved after bariatric procedures [12].

Complete remission of T2DM after bariatric surgery was described in 78.1% of patients and improvement of diabetes in 86.6% patients and this percentages depend of type of surgery [13]. Data from National Database for the American Society for Metabolic and Bariatric Surgery (ASMBS) demonstrated that 12-month diabetes remission rates for BPD-DS (bilio-pancreatic diversion with duodenal switch) was 74%, for Roux-en-Y gastric bypass (RYGB) 62%, for sleeve gastrectomy (SG) 52% and 28% for adjustable gastric banding (AGB). Long-term diabetes remission rates after bariatric surgery have been reported in many observational studies like Swedish Obese Subjects Study (SOS study) [14]. The SOS study showed improvement in 30% of glucose control, lipids, blood pressure and decrease in mortality rate after bariatric surgery in comparison with 8.6 % in patients who introduced life style changes. Also, in 70% of patients, diabetes remission persisted for the next 3 years after bariatric surgery, and in about one third of patients for the next 10 years. STAMPEDE study [15], which compared 3 years of intensive medical treatment of type 2 diabetes obese patients who underwent bariatric surgery with patients on intensive medical therapy alone, demonstrated similar results. The patients with surgical treatment experienced better quality of life, more body weight loss and need less glucose lowering medications than non-surgical group. The sugested explanations for the observed changes may be: low-calorie diet (400–800 kcal/day in the first month after bariatric surgery) improves insulin sensitivity and β cell function; changes in gastrointestinal hormones secretion - glucagon-like-peptide-1 (GLP-1), glucose-dependent insulinotropic peptide (GIP), peptide-YY (P-YY), oxyntomodulin (OXM) and ghrelin; β-cell function improvement; improvement in insulin sensitivity changes in enterohepatic recirculation of bile acids and gut microbiota changes [16–18]. Diabetes remission score (DRS) can be used in order to predict the postoperative diabetes remission and select a type of surgical procedure. This score includes the age of the patient, BMI, duration of diabetes, micro or macrovascular complications, insulin use and C peptide level. Lower DRS indicates better chance for diabetes remission [19]. Very early application of bariatric surgery in the course of T2DM was recently suggested on the basis of the facts that the best diabetes remission rates were accomplished among the patients with recent onset of T2DM and among the patients with initial macro- and microvascular complications. Contrary to these, it was shown that end-stage chronic vascular complications could be worsened after bariatric surgery [20]. Accumulated experience gave evidence for the role of metabolic/bariatric surgery in the treatment of diabetes in obese patients. Consensus guideline that define the position of metabolic surgery in algorhythm for treatment of T2DM was developed during the 2nd Diabetes Sugery Summit in 2015, where 48 international clinicians and diabetes organizations concluded that metabolic surgery should be recommended for T2DM patients with BMI > 40 kg/m^2 as well as for those with BMI 35–39.9 kg/m^2 in whom previous therapy with changes of their lifestyle and medical treatment failed. In comparison with the European guidelines for bariatric surgery the only difference refers for the category of BMI 30.0–34.9 kg/m^2 (for Asians 2.5 kg/m^2 less) which now becames the indication for metabolic surgery if previous medical treatment of diabetes failed [21].

Non-alcoholic fatty liver disease and obesity

Non-alcoholic fatty liver disease is a progressive liver disease that starts with steatosis, continues with fibrosis and ultimately ends in cirrhosis. Underlying mechanism in NAFLD is insulin resistance and strong correlation was found between intrahepatic triglyceride deposition and BMI, waist circumference, alanine aminotransferase (ALT) and aspartate minotransferase (AST) levels, insulin level and blood pressure. NAFLD is a hepatic component of metabolic syndrome and is in close association with visceral obesity and T2DM. Dietary habits with high energetic meals with low ingestion of dietary fibers and sedentary lifestyle dramatically increase incidence of NAFLD. It was sugested that in the future NAFLD will be the main indication for liver transplantation [22]. In obese persons with decreased physical activity, low level of irisin could be a connection between insulin resistance and fatty liver. Irisin works as a signal transmitter between muscles and fat tissue by activation of mitochondria and expression of uncopling protein 1 (UCP-1) [23].

Bariatric surgery has beneficial effects on insulin resistance, lipid profile, inflammation and adipokines involved in the pathophysiology of NAFLD, and has positive effect on histological and biochemical parameters of NAFLD. A significant drop in blood transaminase levels was demonstrated by meta-analysis of studies for different methods of bariatric surgery (RYGB, SG and AGB). Improvement in insulin resistance and liver lipid content after RYGB occurred before a significant body weight loss, probably due to weight reduction independent mechanisms [24, 25].

It was described that patients with non alcoholic steato hepatitis (NASH), who under-went bariatric surgery, have a greater risk of death compared with patients without NASH during a follow-up of 10.2 years after bariatric surgery. There are 32 genes which may help to identify patients with NASH with potential shorter survival time after bariatric surgery [26, 27].

Obstructive "sleep apnea" syndrome and bariatric procedures

In obese people high intraabdominal pressure causes hypoventilation, interruptions of breathing during the sleep OSAS, pseudotumor of brain (idiopathic intracranial hypertension), gastroesophageal reflux disease (GERD) and urinary incontinence. The shortening of sleep (on average less than 7 hours a day), reduced energy expenditure, the use of drugs for the treatment of mental illnesses, autoimmune and chronic inflammatory diseases contribute to increase in body weight [28, 29]. Possible mechanisms for interaction between the "sleep apnea" and obesity are increased sympathetic activity due to interrupted sleep, reduced consumption of glucose in the brain, and increased levels of cortisol and growth hormone. These mechanisms impair neuroendocrine control of appetite which results in a further increase in body weight. OSAS increases 4 to 6-fold risk of death early in the morning [30]. Criteria for OSAS are apnea-hypopnea index (AHI) ≥ 15 during one hour or ≥ 5 and ≤ 14 during one hour with information of sleepiness during the day, mood changes, ischemic heart disease, hypertension or previously stroke. Weight reduction significantly decreases number of apnea episodes [31, 32].

Previous studies demonstrated that weight reduction after bariatric surgery significantly improves OSAS in approximately 75% of patients. Moderate to severe OSAS persists in 25% patients after RYGB. Predictive factor for the persistent OSAS were EWL after surgery less than 60%, preoperative AHI ≥ 30/hr, hypertension and patients with ≥ 50 years of age [33].

The systemic analyses of 22 related articles demonstrated that the combined restrictive-malabsorptive procedures like R-en-Y gastric bypass and biliopancreatic diversion with duo-denal switch are more efficient in all aspects of OSAS than pure restrictive procedures like sleeve gastrectomy or adjustable gastric banding. OSAS improvement was associated with decrease of the neck circumference after bariatric surgery. Also, after bariatric surgery the quality of sleep was better and rapid eye movement (REM) phase was longer [34].

Hypertension after bariatric surgery

High blood pressure in obesity may results from direct compression of fat tissue on kidney, increased pressure in kidney blood vessels and increased intrathoracic pressure [35]. Obese women with idiopathic intracranial hypertension, which is common finding in the extreme obesity, have increased intrathoracic pressure [28]. High intrathoracic pressure compromises blood flow, decreases the amount of blood from left ventricle to aorta and activates renin-angiotensin-aldosterone system which leads to blood vessels constriction and water retention in the body. Increased pressure in the renal veins can lead to glomerulopathy with following proteinuria [35].

Inflammatory cytokines from adipocytes, even in reference range have an impact on endo-thelial function. Obesity affects the morphology and function of the heart muscle which is associated with coronary heart disease and sudden cardiac death. The accumulation of body fat between muscle fibers and degeneration of myocytes (adipositas cordis) through lipotoxicity leads to cardiomyopathy. Heart failure in obesity is a result of the left ventricle hypertrophy, diastolic dysfunction, increased blood volume and increased ejection fraction [36]. Blood pressure is normalized in 60% of patients after RYGB. There is a difference in hypertension remission rate between the RYGB, SG and AGB. After RYGB, 63.6% of patients have hypertension remission, and after AGB, remission occurred in 34.8% of patients. The lowest remission rate of 14.3% was in the group of patients after sleeve gastrectomy [36]. One year after biliopancreatic diversion almost half of the patients experienced remission of hypertension and further 10% also become normotensive in the next 3 years. Some authors demonstrated that antihypertensive drugs were discontinued within the first year of bariatric operations in 58% of patients after RYGB and in 54% of patients after gastric banding. In the meantime, antihypertensive drugs were stopped only in 13% of patients after life style changes. In another investigation favourable effect of biliopancreatic diversion (BPD) on blood pressure was proven. After BPD, blood pressure was normalized and antihypertensive drugs were discontinued in 85% of patients, at least two years after operation [36–39]. Possible epigenetic mechanism for hypertension remission after bariatric/metabolic surgery, independent of age and sex is hypometylation of CpG sites six months after RYGB. CpG sites (cg00875989, cg09134341) were metylated in obese patients before the surgical treatment and were associated with hypertension [40].

Female reproductive function after bariatric surgery

Obesity has impact on almost every aspect of female reproductive system. The most common endocrine disorder in obese women is polycystic ovary syndrome (PCOS). PCOS is associated with metabolic diseases like diabetes, hypertension, hyperlipidemia and metabolic syndrome. Almost 60% women with PCOS are obese [41]. The exact pathogenetic mechanism for obesity in PCOS is still unclear. Some investigations described the differences in neuro-peptide Y and ghrelin levels between the obese women with PCOS in comparison with the obese women without PCOS [42].

On the other hand, it is known that visceral obesity is associated with low levels of sex hormone-binding globulin (SHBG) and elevated free estrogen levels. The lower level of SHBG results in elevated total testosterone level. Elevated free estrogen level through hypothalamo-pituitary-gonadal axis leads to suppression of gonadotropin relasing hormone (GnRH) secretion. Menstrual irregularity, anovulatory menstrual cycles and polycystic ovary syndrome may appear as a result of potentiation of negative feedback with GnRH suppression. Risk for anovulation increases with higher BMI [43].

Weight reduction following bariatric surgery is the most efficient method for PCOS management with improvement in a few endocrine aspects. In a retrospective study that analyses the effect of bariatric surgery on PCOS symptoms, it was for demonstrated that 82% of patients have improved

menstrual irregularity, hirsutism was improved in one third of patients and 77.8% of patients with diabetes achieved complete diabetes remission. Pregnancy occurred in all pregnancy desiring patients within 3 years after bariatric surgery. In meta-analysis it was confirmed that PCOS prevalence decreased from 45.6% to 6.8% ,12 months after bariatric surgery [44, 45]. Abnormal eating habits due to high level of allopregnenolone is one of the common disturbances in PCOS. Six months after RYGB, total testosterone, SHBG and progesterone decreased while estrogen levels increased. Also, restoration of preovulatory peak of follicle-stimulating hormone (FSH) and (LH) occured, FSH/LH relation became normal, fertile capability increased and overeating dis-appear due to decreased allopregnenolone synthesis [46].

Positive effect of bariatric surgery is a lower risk of macrosomia while negative effects are higher risk for maternal anemia and low birth-weight of newborns [47]. Bariatric surgery should be considered in preconception period in women with BMI $\geq$ 35 kg/m^2 with comorbidities or in women with BMI $\geq$ 40 kg/m^2 in order to prevent delivery maternal and fetal complications among these high risk pregnancies [48]. AGB is a minimaly invasive method with the lowest rate of complications, with relatively good effect on weight reduction and excessive vomiting in pregnancy. Retrospective studies confirmed a lack of differences between pregnancy outcomes in pregnancies that occurred within a first year after AGB application and those that happened more than 12 months after AGB application [49–50]. Bariatric surgery decreased risk for hypertensive disorders in pregnancy from 15% to 3%. Pharmacoeconomic studies based on meta-analyses, carried out by seven insurance companies, indicated that bariatric surgery could reduce neonatal costs associated with hypertension, preeclampsia and eclampsia [51, 52]. Pregnancy is not recommended at least one year after bariatric surgery due to possible nutritional deficiency in a period of the greatest weight loss. However, study of 489 women who became pregnant after bariatric surgery demonstrated lack of difference in pregnancy outcome, maternal complications and newborn outcomes between the women who concieved within one year after bariatric surgery and those who became pregnant more than one year after surgery [53].

Obesity related malignancies after bariatric surgery

World Cancer Research Fund (WCRF) and American Institute for Cancer Research (AICR) published the studies that indicated the relation between fat mass accumulation and esophageal adenocarcinoma, carcinoma of pancreas, large bowel, breast, kidney, gallbladder, and en-dometrial carcinoma. Several studies indicate a link between obesity and prostate cancer, ovarian cancer and non-Hodgkin lymphoma [54]. It was shown that the increase in BMI of 5 kg/m^2 increases relative risk (RR) to 1.5 ($p < 0.001$) for esophageal cancer in both sexes as well as endometrial and gallbladder carcinoma in female; increases RR to 1.3 for thyroid cancer in male and kidney cancer in female and increases RR to 1.2 for large bowel cancer in male. RR for melanoma and rectal carcinoma in male is higher than 1.2, as well as for postmenopausal breast cancer, thyroid and pancreatic cancer in females. In both sexes obesity was associated with higher risk for lymphoma and leukemia [55].

There are multiple links between obesity and malignancy with different mediators like growth factors, hormones, cytokines, and inflammation factors. Factors which are released during inflammation as tumor necrosis factor (TNF) alpha and interleukin (IL)-6 impair cell membrane facilitating low-density lipoprotein (LDL) deposition in blood vessels wall and accelerating the atherosclerosis. Proinflammatory cytokines become dominant in comparison to anti-inflammatory cytokines in obese subjects. This phenomenon is responsible for incapability for recognition and elimination of malignant cells and also, allows the unimpeded development of neoplasms of bowel, pancreas, breast, liver, endometrium and prostate [56, 57].

Weight reduction after bariatric surgery lowers symptoms of GERD and the risk for esophageal carcinoma. Bariatric surgery is highly recommendable treatment for GERD in obese patients. Improvement of symptoms depends on a type of bariatric procedures. RYGB is the most potent bariatric procedure in relation to symptoms of GERD. However, there is not enough literature data on the course of Barrett's disease after bariatric surgery [58]. Some of bariatric procedures like SG have undesirable effects on esophagogastric motility due to increased intragastric pressure. Esophageal dysmotility and symptoms of gastroesophageal reflux were frequent after SG [59]. In order to prevent worsening of GERD after SG, some authors recommended gastropexy to the preaortic fascia as a possible antireflux technique in combination with SG [60].

Recently, it has been suggested that bariatric/metabolic surgery reduces cancer risk from 40% to 50%. An analysis in large tertiary bariatric surgery center that retrospectively included 2943 patients with no history of cancer at the time of RYGB, indicated that 48 months after performed surgery patients that developed organ cancer achieved less weight loss, so that authors concluded that greater weight loss after metabolic surgery may be associated with lower organ cancer risk [61]. Systematic review and meta-analysis of four studies revealed that bariatric surgery was associated with significantly lower colorectal cancer incidence (RR = 0.73) when compared with obese non-operated individuals. Authors concluded that bariatric surgery was associated with a 27% of lower colorectal carcinoma risk [62]. Risk for malignincies after bariatric procedures decreased more in female than in male population [56]. Among the key results from the Swedish Obese Subjects (SOS) trial, a prospective controlled intervention study of bariatric surgery, a decreased incidence of cancer was found (women: adjusted hazard ratio 0.58, $p = 0.008$; men: n.s.) [63].

Conclusion

After few decades of experience in surgical treatment of obesity, bariatric or metabolic surgery becomes powerful approach to management of excessive body weight as well as different obesity related comorbidities which significantly

reduce quality of life and longevity among obese patients. High potential impact and cost effectiveness of some bariatric operations on metabolic comorbidities classify surgical treatment of obesity at the very high position and confirm that the term metabolic is more appropriate than bariatric surgery. Further investigations and meta-analyses are necessary for defining long-term effects and possible side effects of bariatric/metabolic surgery.

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Pathological lying and tasks of psychological assessment

Ciljevi psihološkog testiranja u proceni patološkog laganja

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Introduction

According to Webster's dictionary [1], the definition of the word lying has double content: a) a speaker claims something that he/she knows or believes to be untrue with intent to deceive, b) to create a false image or a wrong impression. It is essential that the speaker knows that the content is false and intends to deceive someone. Thus, people with delusional disorder cannot be considered pathological liars even though they do not tell the truth.

Lying is human behavior that can have its own normal and pathological forms of appearance. In daily life, people lie most often about their true feelings, incomes, achievements, sex life, and age [2]. If we have a look at everyday lies we can see the extent to which people use untruths in their communication considering them neither immoral nor subjecting such statements to moral judgement.

„Normal“ and pathological lying

There are individual differences among people in terms of frequency of lies, a degree of lies and objective they want to achieve. The motivation for lying is a result of complex mutual influences of conscious and unconscious contents. A lie is often generated from multiple sources [3]: lying to avoid punishment, to preserve a sense of autonomy (“Others do not need to know everything") and identity ("I do not love myself as I am, I will become someone else who is more attractive"), then, lying as an act of aggression with intention to inflict damage to others, as a way of fulfilling the desire (people make up what they want to happen), or as a way of getting a sense of power over partner, doctor, etc. Sometimes lies are told out of self-deception in order to boost and protect the suppression of self, as in the therapeutic process to prevent access to personal content related to the sense of personal weakness and failure. Lying may be motivated by the need to manipulate the behavior of others but also to help others. Lying is often motivated by the need for establishing self-esteem ("I'm not worth anything, so I have to lie to feel worthy or lovable").

Lying can be thought of as a defensive psychological strategy to protect a person from emotionally intense or traumatic events. If the events exceed the capacity of the person to deal with reality, they can use lies as a kind of fantasy and self-deception which falsifies reality, no matter if that reality is an everyday stress or some major life traumas. People who have experienced posttraumatic stress disorder (PTSD) due to exposure to the war, pathological lying has the function of protecting their psychological integrity [4]. This view emphasizes the role of lying in intrapsychic regulation, which is as important as the motivation directed towards external objectives – the person’s need to be socially accepted and wanted.

When we face a patient who tells untruth, the question is: where is the line between "normal lying" (it is common to all people if they are exposed to certain circumstances) and "pathological lying"? Pathological lying is compulsive and impulsive, pervasive behavior of individuals (persistent and stable as a personality trait); its goal is not to achieve material gain and sometimes it has self-defeating quality (e.g. people lie even when it is harmful or dangerous for them, even in the judicial process) [5]. Lying becomes pathological if it interferes with normal development or is destructive to the quality of life and environment of the person involved [5].

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Classification of pathological lying

There are many divisions of lies with respect to various criteria. The classification of lies that are available in the literature are not comprehensive, because they do not include all categories and are not consistent because they are not based on a single criterion of division.

Pathological lying and Pseudologia Fantastica are not recognized as an entity in psychiatric classification systems. Only Munchausen Syndrome is classified as F68.1, while other subtypes of lying related to false identities or false accusations are omitted. In the discussions, it is often asked whether pathological lying is a symptom or syndrome; whether it should be coded on Axis I or in the category of personality disorders it is usually associated with. In any case, the lack of a code in the classification systems prevents detection and monitoring of the incidence of this phenomenon.

Pathological lying has its subtypes [3]: 1) Pseudologia Fantastica (PF); 2) liars by habit (they lie superficially, easily, often recklessly, it is easy to recognize their lies, they are often suggestible and have some neuropsychological abnormalities like learning difficulties or marginal intelligence); 3) lying and impulse control disorder (associated with gambling, kleptomania and compulsive shopping, when lying has a function of protecting obsessive needs a person is ashamed of, or has a need to conceal them in front of their environment); 4) people who live their lives by lying: impostors (during their life they change their identity, name, profession, origin in order to present themselves as important people) and confidence artists who change their identities in order to achieve material gain, 5) Munchausen syndrome – people who simulate disease, identify themselves as patients to get attention and care. Munchausen syndrome by Proxy (MSBP) caregiver fabricates and reports constantly new symptoms of diseases in those who are in their care, for example, mother in her child, a nurse who induces health problems in a patient who has been entrusted to her.

Among the categories of pathological lying PF is the most extreme form of pathological lying, which is a mixture of facts and fantasy. The person likes to lie and at the same time they experience pleasure in the process and are excited by the possibility to be discovered. The researches on lie detector tests have showed that people with PF experience stress while they are telling lies, which means that they are aware of their falsity [6]. Despite defective morality, it is possible that they have a certain feeling of guilt, and therefore, they convince themselves and others that their claims are true and keep their circle of lies strong by rigid attitudes.

Mythomania or pathological lying was first clearly entitled and described in 1891 by a German psychiatrist Anton Delbrueck. The first authors who contributed to this subject in English were Healy and Healy [7]. Their research conducted on a group of 1,000 juvenile offenders showed that the prevalence of pathological lying in juvenile offenders is about 1%, which means that it is even smaller in non-forensic population. One of the few studies of pathological lying on a sample of 72 cases finds that pathological lying begins with adolescence and lasts throughout the lifetime of a person [8]. The intelligence is an average or slightly lower and 40% of the respondents has a central nervous system dysfunction such as epilepsy, abnormal EEG findings, brain trauma or a central nervous system infection. About 30% of exposed cases had a chaotic primary family and a history of mental illness among close relatives. People diagnosed with personality disorders are those who lie the most: antisocial, histrionic, borderline and narcissistic personality (Cluster B), and obsessive-compulsive personality disorder (Cluster C Personality Disorder) [3].

The question is whether the PF is a symptom or syndrome, if it is sufficiently stable, consistent and predominant to be recognized as a basic psychopathological entity in a classification and whether it would be placed on the Axis I or Axis II. It is believed that pathological lying can be a primary and secondary according to whether it occurs in a person that cannot be diagnosed with other psychiatric diagnosis or in a person who has the symptoms of other psychiatric disorders [7]. However, our clinical experience tells us that it is difficult to assume that pathological lying can be isolated symptom without existence of other problems in the personality functioning, regardless of whether or not they meet the criteria for diagnostic classification of comorbidity.

Pathological lying of PF type can be identified on the base of the following criteria [8]: the stories are not so unbelievable and they are usually based on truth; a tendency to make stories is permanent / stable; the stories do not have a purpose of obtaining some material profit and have a quality of the grandiose self; a patient always presents themselves as the central figure – a hero or victim; they differ from delusions because the person knows that the stories are untrue.

People with pathological lying are inclined to structure their stories around the army and the police which, as the institutions, are bearers of social power. Presenting themselves as people who are influential in these organizations they give themselves special importance. People who are misled by their stories can treat pathological liars in a different way: they can disclose some professional secrets to them or give them more responsible professional and social tasks, or involve them in some social or political action. The available literature mentions eight cases of pathological lying that contain stories related to the army, paramilitary organizations or espionage wherein the person can represent themselves as a hero or a victim of the military system [9].

The objectives of psychological testing

According to clinical experiences, people do not come for a treatment for pathological lying because they consider it ego-syntonic and feel no need to correct it. They see psychologists or psychiatrists because of other psychological symptoms or life problems which sometimes can be the consequences of their lying. Initially, false stories are more difficult to identify because the patients gradually introduce them as false fragments and then intensify and enrich them by extending communications in order to attract the attention of the examiner and conceal the real problems in their lives. Sometimes therapists tend to dismiss immediately or distan-

ce themselves quickly from the patient who is dishonest because they consider honesty a prerequisite for therapeutic work or assume that this is a manipulation where the patient wants to stay in a psychiatric ward for some other purpose. In those situations, it is common to get suitable information from people who know the patient (to perform so-called heteroamnesis), but these patients often avoid bringing their relatives, partly because they do not want their story to be uncovered, and partly because they need time to gain confidence in the therapist who would not reject and condemn them because of their lies. They make up stories easily and without any signs of discomfort, over time they complicate and generalize the increasing number of situations. They are eloquent and able to remember clearly what they said, but if the therapist confront discrepancies in their story, they quickly justify them with new lies. During the confrontation they do not recognize the lie and continue to lie without showing any signs of shame and remorse. Longer and more serious work with the patient raises the question of differential diagnosis of this disorder and how much a psychologist using psychological tests can contribute to proper detection of the disorder.

Differential diagnosis

The first aim of psychological testing is primarily the differential diagnosis: differentiation between pathological lying (or the specific subtype PF) and confabulations, delusional beliefs and simulation.

a) Pathological lying v.s. Confabulation: Unlike confabulations, in lying there is persistent quality of a story and there is no deficit of memory. Confabulations are the expression of unconscious feeling of a person that he/she has some gaps in their memory which must be compensated with an imaginary experience; the patient believes in confabulations but they have no basis in actual facts to corroborate them. Patients may be preoccupied with their confabulations which can arise in organic amnesia so the search for organic causes of mnestic deficit must be performed by objective methods.

The task of testing related to this dilemma: Is there a neuropsychological deficit especially problems in long-term memory and/or amnesia? Here neuropsychological tests, the Wechsler Memory Scale (WMS) [10] and the Wechsler Intelligence Scale (WAIS IV) [11] are used.

b) Pathological lying v.s. delusional beliefs: in pathological lying, when a person is confronted with the facts, he/she is able to recognize their own stories as false. People with delusional disorder are not able to do that and when confronted with the truth they even become hostile; they have a strong need to defend their beliefs and keeps them encapsulated in relation to the real arguments. In pathological lying the person changes story elements and combines them with elements of reality, and in delusions the story is always the same and usually has a simpler structure.

The task of testing: Is the person psychotic? Here the following tests are used: intelligence tests, the questionnaires that really perform well in detection of psychosis (Personality Assessment Inventory – PAI [12], Minnesota Multiphasic Personality Inventary – MMPI-2 [13]), the drawing projective test and the Rorschach method [14]. The Rorschach technique is particularly useful for estimating how well a person tests reality. Cluster mediation indicators just allow comparisons to what extent the respondent sees the reality the same way as most people, whether he/she does that in an idiosyncratic manner or creates perceptual distortion. One of the central diagnostic questions [15] is whether people with pseudologia have the reality test preserved or they believe in their lies as "wishful psychosis". Individual differences may be interesting and may show how the respondent is positioned on the reality testing dimension. Generally, we do not expect to find perceptual distortions or pathological errors of judgment in people with pseudologia but it is possible that they are characterized by idiosyncratic opinion that set them apart from the majority of people, as well as by a tendency towards arbitrary interpretation of reality.

c) Pathological lying v.s. Simulation: in simulation, a person has a clear material gain obtained from the stories he/she makes up but stops telling them when there is no tangible benefit. In pseudologia, profit is psychological and lying often distorts the social status of people and contributes to their self-defeating behaviour.

The task of testing: Does a person have a tertiary gain by lying? Are there test indicators of simulation? A battery of tests is used and the indicators of validity and simulation in test materials are particularly valuable (validity scales of the PAI and the MMPI-2 questionnaires). The psychopathy and personality disorders scales used in questionnaires also contain items that indicate the behavior correlated with lying [16]. The PAI inventory scales should be carefully followed: problems with identity (BOR-I), Antisocial Behaviors (ANT-A), Egocentricity (ANT-E), Stimulus - Seeking (ANT-S).

Monosymptomatic scales relating to psychopathy (e.g. Psychopathy Checklist- revised, PCL-R) [17] often contain items related to the lying or Machiavellian orientation. However, a respondent easily recognizes such issues and denies socially unacceptable behaviours. Such questions are less conspicuous in the multidimensional questionnaires and if it is a Likert-type scale responses then there is a possibility of gradation which is more acceptable for the respondent.

Intelligence and neuropsychological assessment

The height and structure of a respondent's intelligence is the second task that can be tackled by the psychological testing as well as whether there is a specific neuropsychological profile of a person who pathologically lies. While the application of modern technology enables us to record the brain functioning, it is known that neuropsychological deficits observed by testing do not have to correspond with computed tomography (CT) or magnetic resonance imaging (MRI) of the brain. A test of general intelligence and the difference between verbal and nonverbal skills can be in the first place of importance. There are no major researches that will show the level of general intelligence of people who lie; a study on a sample of 72 cases of

pathological lying has shown the intelligence levels between average and slightly lower, while verbal IQ was higher than non-verbal [8] one. In a recent meta-study [9] of 25 subjects, 19 had an average general intelligence score and there was no difference between verbal and nonverbal skills. It is logical that more complexly produced story requires a certain scope of general education, good attention, memory and the ability to combine the elements of the story. However, we do not have a sufficiently large group of respondents to verify that empirically and harmonize the method by always applying the same form of intelligence test. Only if we tested a sufficiently large group of patients, we could talk more reliably about whether there was a similarity in the general intelligence level and in the individual abilities structure of the group of people who pathologically lie.

The old dilemma of the psychology of individual differences is to which extent lying is hereditary-innate and how much it is a result of family and other social influences. Using neuroimaging people who lie were found to have significantly larger white matter volumes and slightly smaller gray matter volumes in the prefrontal brain structures compared to the groups of anti-social and normal subjects [18]. The prefrontal cortex is a brain area responsible for the process of remorse, learning of moral behavior and moral decisions. The larger white matter enables greater network of the prefrontal cortex, the faster flow of information which is connected to better verbal skills and a greater readiness to lie, while the reduction in gray matter represents lesser moral restraint and greater disinhibition when a person is telling a lie. An alternative hypothesis is that an increase in prefrontal white matter does not lead to greater functional efficiency but to disinhibition and increased lying [19]. Although there have been some earlier reports that in a group of people who lie even 40% of the respondents have a dysfunction of the central nervous system [9], new technologies enable us to recognize better the structure and function of the brain that may be correlated with the tendency of people to lie [20].

These recent findings raise the question whether there is a specific neuropsychological finding in a group of people who pathologically lie and what expected interrelationship (configuration) of individual cognitive abilities would be like. Neuropsychological tests that should be used taking into consideration the observed brain regions are: the Wisconsin Card Sorting Test (WCST), the Phonemic and Category Fluency Tests, Stroop test, Trail Making Test B (TMT B) [21].

Assessment of comorbidity with personality disorders

The third objective of testing may be to establish whether the person also meets, apart from pathological lying, personality disorder criteria. Conclusion on comorbidity is important for medication and psychotherapy process because sometimes by treating another disorder we indirectly reduce the person's need to lie pathologically. Also, the presence of severe character pathology significantly complicates the therapeutic changes. For this purpose we use projective methods and self-descriptive questionnaires especially these which have the power to differentiate categories of personality disorder (Revised NEO Personality inventary – NEO PI –R [22], Million Clinical Miltiaxial Inventary – MCMI III [23], and PAI).

Determining the position of the trait Honesty (Falsity) in the modern theories of personality

The fourth task of testing may be positioning of lying in some personality model that is dominant now. Honesty (falsity as well as the phenomenon on the opposite pole) can be treated as a personality trait that is related and correlated to other well-placed personality factors and aspects. It is particularly intriguing how this personality trait would be positioned in the five-factor model of personality which is operationalized excellently through the NEO PI-R personality questionnaire. The theoretical hypothesis is that the tendency towards pathological lying correlates to the highest degree with aspects of the Agreeableness domain: (A1) Trust, (A2) Straightforwardness, and (A3) Altruism. The relation with other aspects is also probable: (N5) Impulsiveness, (E3) Assertiveness, (E5) Excitement Seeking, (O5) Openness to ideas.

Another model that might be a good frame of reference is the Honesty-Humilty- Emotionality-Extraversion-Agreeableness-Conscientrouness-Openess to expirience HEXACO model [24] in which the domain honesty-humility is set as the sixth factor of personality. This factor is based on evolutionarily developed altruism and initially it was named Honesty. It begs the question whether the tendency towards lying is a separate bipolar line or an extreme pole on some of the existing traits within these models (e.g. Honesty). It is also questionable whether mendacity (including pathological lying as extremization of this trait) is a trait of a lower order compared to honesty, altruism, manipulative or some other already operationalized trait within these models. These questions can only be answered by some research conducted on larger samples and factor analysis of the scores.

Understanding the role of lying in the dynamics of personality

The fifth task of testing is to recognize the role/motive of lying in a dynamic personality, in the family system or social environment. Although the diagnostic interview is the crucial here, we can get some useful information by using some of the family therapy questionnaires or those intended to assess the development and differentiation of identity. Namely, if we looked for the dynamic concepts in relation to lying, we would apply methods designed to measure ego and superego deficits, the degree of identity development, self esteem concept and it would be challenging to see what defense mechanisms are mainly used by people who have a problem of pathological lying. Monosymptomatic scale, individual subscales of the questionnaire and application of projective methods can help with examining the correlations between lying and these dynamic concepts. Thus, in the Rorschach method we have scales used to evaluate ego maturity and differentiation (e.g. Mutuality of Autonomy Scale [25]). It would also be interesting to examine the interpersonal style of the person who pathologically lie, either through relationships of the Dominance (DOM) and

Warmth (WRM) scale on the PAI inventory or by applying some of the questionnaires that test specifically the social tactics of the subjects. Thus obtained data are important for assessing both the potential for psychotherapeutic work and the choice of the therapy type.

Research papers on groups about the problem of pathological lying are old and rare; these are usually case studies [26, 27] or in recent times, the results of neuroimaging studies.

Conclusion

The research base for this entity is very small because it is not recognized as a separate diagnostic category; we do not have operationalized criteria for its recognition, so there is no data on the prevalence in the general or psychiatric population. The rarity of the disorder and lack of agreement on the methodology that researcher will apply prevent clustering of the sample and results, including the integration of the findings of different researchers. We do not have consistent data on the amount and structure of intellectual abilities of these individuals, whether they express specific neuropsychological deficits, how they are positioned in today's dominant theoretical personality models or in relation to other psychiatric categories (particularly personality disorders). The use of modern neuro/psychological tests can give us the answers to numerous researches or diagnostic dilemmas appearing during the work with individuals who have a problem of pathological lying. The existence of an agreement among psychologists as to which tests will be administered in this category of patients and the development of an Internet database in one research center would allow the accumulation of results which could help us to better understand the phenomenon of pathological lying and to treat it more successfully.

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CASE REPORTS

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The cognitive impairment illustrated in drawings used in gaining insight and motivation in alcoholism treatment

Upotreba ilustracije kognitivnog oštećenja na crtežima za povećanje uvida i motivacije u lečenju alkoholizma

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Abstract

Introduction. The neuropsychological impairment such as different severity of executive deficit and mnestic disturbance among chronic alcoholics is frequent, but usually not explored. **Case report**. Two clinical vignettes of alcoholics who were not simultaneously treated in day hospital are presented, and they illustrated similar visuospatial impairments in free association drawings of a house made by. In this paper, the focus was on drawing not only as diagnostic tool, but also as a means of a confrontation within the group. Qualitative analysis of drawings and protocols from the group analysis were applied in the integrative day program. After confrontation, one alcoholic increased the insight into his cognitive impairment and strengthened motivation for early abstinence and treatment maintenance. He continued drawing houses as a theme of the following sessions till his obvious visuospatial impairments were repaired. Thus, he spontaneously trained his executive abilities and got group support for their improvement. **Conclusion**. Drawing could be a simple means of illustration cognitive impairments and the group analysis of drawings may serve as useful adjuvant method of strengthening insight and motivation for abstinence and treatment maintenance with documented cognitive recovery during abstinence.

Key words:
alcoholism; cognition; cognition disorders; art therapy; psychiatric status rating scales; self-evaluation programs; treatment outcome.

Apstrakt

Uvod. Kod hroničnih alkoholičara često postoji neuropsihološko oštećenje u vidu deficita izvršnih funkcija i mnestičkih poremećaja različitog intenziteta, mada se rutinski ne ispituju. **Prikaz bolesnika**. Prikazane su dve kliničke vinjete alkoholičara koji nisu bili istovremeno lečeni u dnevnoj bolnici, ali su ilustrovali slična vizuo-spacijalna oštećenja na crtežima kuće, nastalim prema slobodnim asocijacijama. U ovom radu fokus je bio na crtežu kao sredstvu za konfrontiranje u grupi, a ne samo kao dijagnostičkom sredstvu. U okviru integrativnog dnevnog lečenja alkoholičara primenjeni su kvalitativna analiza crteža i protokoli grupne analize crteža. Kod jednog alkoholičara, nakon konfrontiranja sa vizuo-spaciajalnim oštećenjem došlo je do povećanog uvida i jačanja motivacije za dalju apstinenciju i lečenje. Na sledećim sesijama on je samoinicijativno ponovo crtao kuću dok nije reparirao svoje oštećenje. Tako je spontano trenirao svoje izvršne sposobnosti i dobio grupnu podršku za njihovo poboljšanje. **Zaključak**. Crtež i grupna analiza crteža mogu biti korisna pomoćna metoda i jednostavno sredstvo za registrovanje i upotrebu kognitivnih oštećenja u cilju jačanja početnog uvida i motivacije za održavanje apstinencije kod alkoholičara, kao i za dokumentovanu ekspresiju njihovog oporavka.

Ključne reči:
alkoholizam; mentalni procesi; saznanje, poremećaji; lečenje umetnošću; psihijatrijski status, testovi; samoprocena, programi; lečenje, ishod.

Introduction

Alcoholism is often associated with mild to severe cognitive impairment of varying intensity while remaining underestimated [1]. The relationship between neuropsychological and emotional functioning may influence negatively alcoholics' compliance and diminish therapy efficacy [2]. The evaluation of cognitive deficits is of great importance for optimizing patient treatment. The question about period of cognitive recovery after detoxication from alcohol is stil open, but some data suggest that an average length of one year is needed [3]. There was evidence that recovery of cognitive functions could be facilitated by applying relevant tasks early in the treatment [4].

The challenge how to maintain abstinence and increase motivation in tretments that are easily applied, need more speci-

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fic procedures [5]. Besides cognitive behavioral therapy and combination of treatments, novel treatment strategies for improving treatment response are needed [6]. However, the specific psychotherapeutic treatment programs among small percentage of alcoholics are applied [7]. The reserach of therapeutic intervention and exploration of artistic production of alcoholics is still understudied. An important role of self-expression by the creative process of drawing in gaining insight is observed. Thus, intrapsychic content becomes visible and can be analysed and included into integrative treatment [8].

We presented two cases of verified clinical observations of the cognitive impairment expressed in drawings of the alcoholics with the focus on illustrations as a means of confrontation during group analysis and gaining insight as well as enhancing motivation for alcohol treatment.

Case report

The both cases were primary, right-handed, male alcoholics, who entered a two-month day integrative treatment after completion of the three week in-patient detoxification. It was the first alcohol treatment for each of them. They were admitted in the different periods and did not meet during therapy. The diagnose of alcohol dependence was established according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria [9]. There were no history of any other mental disorder, head injuries, convulsions, and use of psychoactive substances except tobacco smoking. Routine laboratory analyses showed normal range and there were no prominent medical, nor neurological disturbances detected on out-patient hospital admission.

The alcoholics were visiting the day hospital homogeneous group, focusing on alcoholism, which was meeting three times a week. Also, individual psychotherapy, family therapy and the occupational therapy were applied. After they had been informed about integrative treatment procedures, the patients gave written consent to participata in the art group therapy and use of their drawings for the analysis. Once a week, they took part in the art therapy. It was an open, heterogeneous group of patients. The group work consisted of free associations drawing followed by exibition with discussion of all drawings for 90 minutes [10]. For each session, the qualitative analysis of drawings and therapy protocols were made.

Case 1

A thirty-one year old male, graduated from high school and unemployed, was living with his parents after divorce while his young son stayed with his ex-wife. He had early onset of drinking at the age of 15. Having been verbally and physically agressive to his parents while drunk, he was admitted to the closed ward for three-weeks hospitalisation. He had never had psychiatric treatment earlier. After detoxication, his score on the Mini-Mental State Examination (MMSE) was 29. Despite the alcohol dependence was diagnosed, he was ambivalent to continue treatment in day hospital. He was aware that clinical examination did not show any medical and neurological disturbances and realised that he had recovered without consequences of alcohol abuse. He thought that he was not an alcoholic, but his parents persuaded him to enter day hospital treatment. During the homogenous group sessions discussing alcoholism he minimised his alcohol problems and rationalised his aggresive behaviour and alcohol use by marital disfunctons and his divorce. But, unexpectedly, his first drawing engaged the majority of the patients during group analysis of drawings to confront him with his illustrated impairment. It was a free association drawing (Figure 1a).

When he described his drawing, a therapist asked him to look at it again and put in some corrections if he had wished. But he said that everything was right. After that, most members of the group were surprised that he could not recognize his mistakes. One by one, they showed him and explained that the roof was drawn without parallel edges. Also, they showed that the stairs and path on the drawing had to be narrower at the door and wider from the house to the position of the observer. He was confused and ashamed that he was not able to recognize his mistakes, especially because other alcoholics, neurotic and psychotic patients corrected him. The therapist explained to him that alcohol could damage brain in that way, but by maintaining the alcohol abstinence give a chance for brain recovery. On the next group session, he accepted that he was an alcoholic and that he had brain impairment due to alcohol. He decided to draw another house on the follow-up art therapy and each week he made efforts to improve his abilities for drawing houses. At the beginning, he used a ruler to draw the parallel edges of the roof. When he was discharged, he got his group support for his obvious improvement and illustrated recovery (Figure 1b). He had maintained alcohol abstinence and follow-up treatment once a week for the next year.

Case 2

A thirty-six years old man diagnosed with alcohol dependence, divorced, living alone, had a 9 year old child. He started drinking at the age of 19 and drank more than 30 drinks a week after divorce over the previous 5 years. He had problems on the job due to alcohol, and after he stopped drinking the tremor was noticed and alcohol withdrawal intensified. He was admitted as an emergency in a close ward. After detoxification, he stayed for three-week in-patient standard treatment for alcoholics with only benzodiazepines, hypnotics and vitamins during hospitalization and disulfiram before he was discharge. The Mini Mental Scale Examination (MMSE) score was 28 and he was without presence of medical and neurological disorders. The laboratory findings on discharge were inside the normal range and he decided to enter day treatment only because his colleagues told him that he could lose his job because of his alcohol abuse. His resistance to accept that he was alcohol dependent was obvious after detoxification. Usually, he was drawing the sea and the sky, without objects in a three- dimensional perspective. But, once he drew a free association house, too (Figure 2).

After exibition, during the group discussion, other patients asked him why he drew the stairs upside-down and a crooked right edge of the roof, but he was confused and con-

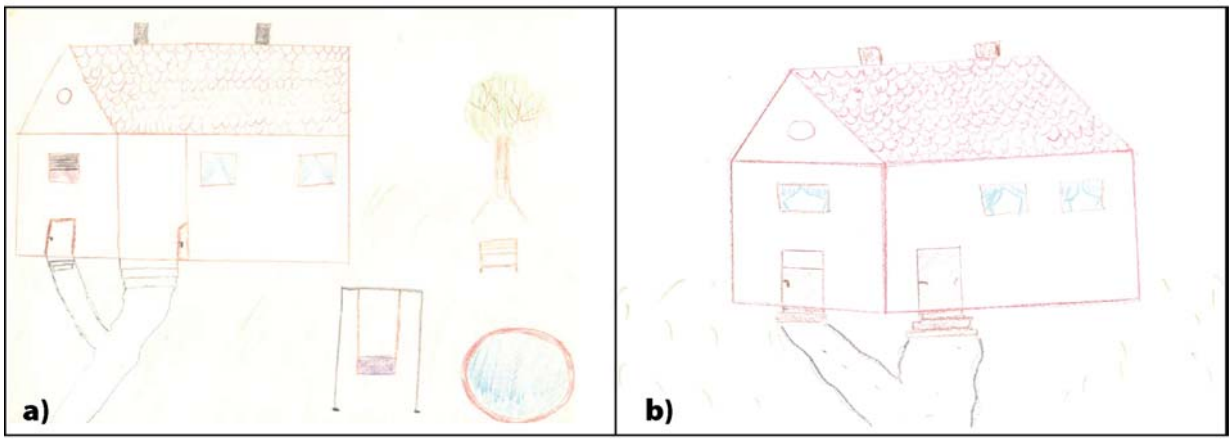

Fig. 1 –The first patient's free association drawing: a) at the beginning of the treatment; b) at discharge showing obvious improvement.

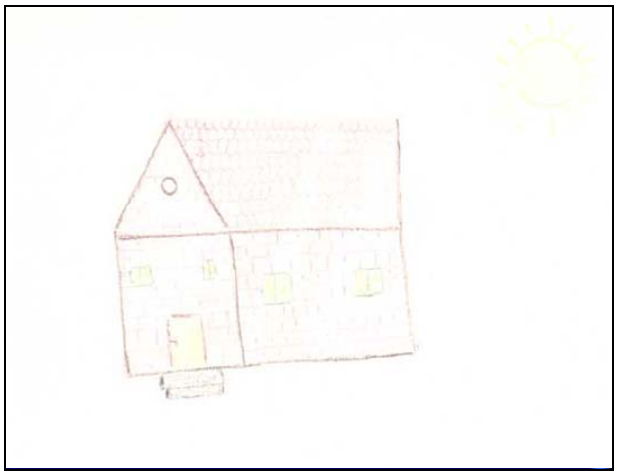

Fig. 2 – The second patient's free association drawing at the beginning of the treatment.

fessed that he did not see any wrong lines. After further discussion he was angry, but remained silent. On the following sessions he only used watercolours and painted the sheet evenly. After discharge, he continued the treatment just until the next month.

Discussion

The both alkoholics spontaneously drew the same free association motif in different periods in day patient hospital and they did not even met. The house as a motif is a universal symbol from filogenetic to ontogenetic human development. It is a frequent theme or a part of a theme in patients' drawings. The patients with dementia were not included, so our clinical experience revealed that among adult heterogenous patients with neurotic and stress related disorders, affective disorders and psychosis in day hospital the visuo- spatial cognitive impairments were not illustrated on the drawings, except among some alcoholics and some chronic psychotics.

Comprenhensive clinical assessments indicated moderate impairment across multiple cognitive functions: intelligence quotient, attention, verbal fluency/language, working memory, problem solving/executive functions, verbal learning and memory, visual learning, visual memory and visuo-spatial abilities during the first year of abstinence from alcohol [3]. In both cases the MMSE score indicated no cognitive impairment, but showed similar distorted perspective illustrated in Figure 1a and Figure 2, which may suggest that there could be visuo-spatial impairements related to alcohol abuse. In clinical researches and practice there are more sophisticated neuroimaging functional techiques and neuropsychological tests in diagnosing cognitive impairments of alcoholics. But our experience showed that alcoholics frequently deny it, or they are not able to understand an importance of these findings. The recent literature data suggest that cognitive training can facilitate brain recovery [11].

The focus of this paper was more on gaining insight and strenghtening motivation for alcohol abstinence because it is a key point in the treatment. Eearly intervention and motivational enhancement is a key goal of alcoholism treatment, because clinical data showed that after in-patient treatment only 30–40% of alcoholics remain abstinents [12]. The alcoholics exhibited poor performance across the domains of attentional control and executive function related to years of alcohol consumption. Therefor the need for therapeutic strategies to target these enduring neurocognitive deficits in improving the treatment of alcohol dependence are recognised [13]. The both cases were confused and ashamed during group analysis of drawings when their houses

were exibited. The first time they were confronted with obvious material proof of their brain disfunction and they had no more arguments that there were no consequences of alcohol abuse and that they were not alcoholics. The confrontation of alcoholic with other alcoholic during psychotherapy is of great importance for gaining insight. But, in this paper, even the psychotic members of the group analysis recognized distorted lines and perspectives in presented alcoholics drawings, what upset and make feel ashamed our subjects.

The limitations of the presented clinical expierience related to diagnostic and therapeutic use of cognitive impairments in alcoholics expressed in their drawings, are that they were spontaneously illustrated and series of more cases are needed to confirm frequent impairments among alcoholics. It would be useful to compare these impairments with standardised objective neuriopsychological and neuroimaging findings. Even if it was only an adjunctive therapy as a part of the the day integrative program, the group analysis of drawings gave possibility to intensified the therapeutic process itself.

Conclusion

Drawing could be a simple means of illustration of neuropsychological impairment among alcoholics. The group analysis of drawings may serve as a useful adjuvant method for gaining an insight and increasing the motivation for abstinence and treatment maintenance. The drawings could provide simple cognitive training and documented cognitive recovery during abstinence.

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Left ventricular noncompaction in a patient presenting with a left ventricular failure

Nekompaktna leva komora kao uzrok srčane slabosti

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Abstract

Introduction. Left ventricular noncompaction (LVNC) is a congenital disorder characterised by prominent trabeculations in the left ventricular myocardium. This heart condition very often goes completely undetected, or is mistaken for hypertrophic cardiomyopathy or coronary disease. **Case report.** A middle-aged female with a positive family history of coronary disease was admitted with chest pain, electrocardiography (ECG) changes in the area of the inferolateral wall and elevation in cardiac specific enzymes. Initially, she was suspected of having acute coronary syndrome. However, in the left ventricular apex, especially alongside the lateral and inferior walls, cardiac ultrasound visualised hypertrabeculation with multiple trabeculae projecting inside the left ventricular cavity. A short-axis view of the heart above the papillary muscles revealed the presence of two layers of the myocardium: a compacted homogeneous layer adjacent to the epicardium and a spongy layer with trabeculae and sinusoids under the endocardium. The thickness ratio between the two layers was 2.2:1. The same abnormalities were corroborated by multislice computed tomography (MSCT) of the heart. **Conclusion.** Left ventricular noncompaction is a rare, usually hereditary cardiomyopathy, which should be considered as a possibility in patients with myocardial hypertrophy. It is very often mistaken for coronary disease owing to ECG changes and elevated cardiac specific enzymes associated with myocardial hypertrophy and heart failure.

Key words:
ventricular dysfunction, left; heart failure; isolated noncompaction of the ventricular myocardium; coronary vasospasm; echocardiography; tomography; diagnosis, differential.

Apstrakt

Uvod. Nekompaktna leva komora (NKLK) je urođena bolest koju karakteriše izrazita trabekularizacija miokarda leve komore. Vrlo često se promene na srcu potpuno previde ili se zamene za hipertrofiju miokarda ili koronarnu bolest. **Prikaz bolesnika.** Bolesnica srednjih godina, sa porodičnim opterećenjem za koronarnu bolest, primljena je zbog bolova u grudima, promena u elektrokardiogramu (EKG-u) u zoni inferolateralnog zida i porasta kardiospecifičnih enzima. U prvom trenutku posumnjalo se na akutni koronarni sindrom. Međutim, na ultrazvučnom pregledu srca, u vrhu leve komore, posebno duž lateralnog i donjeg zida, viđena je hipertrabekularizacija sa brojnim resicama koje su prominirale u šupljinu leve komore. Poprečni presek srca iznad papilarnih mišića ukazao je na postojanje dva sloja miokarda: uz epikard je bio prisutan kompaktan i homogen sloj, a ispod endokarda rastresist sloj sa trabekulama i sinusoidima. Odnos debljine ova dva sloja iznosio je 2,2:1. Iste promene potvrđene su na multislajsnoj kompjute-rizovanoj tomografiji (MSCT) srca. **Zaključak.** Nekompaktna leva komora je retka, najčešće nasledna kardiomiopatija, na koju treba misliti kod bolesnika sa hipertrofijom miokarda. Vrlo često se zamenjuje za koronarnu bolest zbog promena u EKG-u i porasta kardiospecifičnih enzima koji su posledica hipertrofije miokarda i srčane slabosti.

Ključne reči:
srce, disfunkcije leve komore; srce, insuficijencija; miokard, komorni, izolovana nonkompakcija; aa. coronariae, spazam; ehokardiografija; tomografija; dijagnoza, diferencijalna.

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Introduction

Left ventricular noncompaction (LVNC) is a congenital disorder characterised by prominent trabeculations in the left ventricular myocardium. The disease is believed to appear during intrauterine development as a result of arrested morphogenesis of a compacted myocardium, the consequence of which is the formation of an excessively thick myocardium consisting of two layers: a thinner homogeneous layer under the epicardium and a prominently spongy and non-compacted layer with multiple trabeculations and recesses under the endocardium [1, 2]. It can develop either as an isolated disorder, or in association with congenital anomalies of the left or right ventricular outflow tract [3]. Actual prevalence of this disease is still unknown. However, in patients with heart failure, it appears in approximately 3–4% of the cases [4].

Case report

A 50-year-old female patient was admitted to our institution with signs of acute coronary syndrome. On the day of admittance, she felt pain in the left side of her chest and her left shoulder, accompanied by shortness of breath, coughing and diaphoresis. For the past month she had been experiencing extreme fatigue, even after slight exertion. The patient saw a physician at the primary health centre and was found to have high arterial pressure (160/100 mmHg), while electrocardiogram (ECG) showed negative T waves in leads D2, D3, aVF, V3-V6. Urgent blood tests revealed elevation in cardiac specific enzymes: troponin I 0.44 ng/mL (< 0.14). Dual antiplatelet therapy was prescribed: aspirin 300 mg and clopidogrel 300 mg and the patient was referred to hospital for treatment with suspicion of non-ST elevation myocardial infarction.

Anamnesis revealed that the patient had suffered from left-sided sciatica for two weeks, that she used non-steroidal antirheumatics, muscle relaxants and corticosteroids. Five years earlier, she had deep venous thrombosis of both lower limbs and had used anticoagulation therapy for 6 months. She cited that 10 years earlier she had underwent hysterectomy and cholecystectomy; she had had two childbirths and several miscarriages. The patient is a non-smoker, normotensive and has a family history of coronary artery disease.

Upon hospitalisation, her respiratory sounds were normal, with inspiratory crackles basally. Heart activity was rhythmic, the sounds distinct, with no murmurs, frequency (f) 88/min, blood pressure – 130/75 mmHg.

The ECG registered sinus rhythm, a normogram, a 1 mm downsloping ST segment depression with negative T waves in D_2, D_3, aVF, V_3-V_6 (Figure 1). Chest radiography revealed interstitial and perivascular edema basally, with hilar enlargement.

Blood test results were: erythrocyte sedimentation rate (ESR) – 7 mm/h, C-reactive protein (CRP) – 3.5 mg/mL, blood count: white blood cells (WBC) – 12.0 10^9/L dominated by granulocytes (10.7%), haemoglobin (Hgb) – 154 g/L, red blood cells (RBC) – 4.92 10^{12}/L, Platelets (Pt) – 330 10^9/L. The test showed increased concentration of enzymes associated with myocardial necrosis: creatine kinase (CK) – 485 U/L (< 200), CK_{MB} – 35 ng/mL (< 25), troponin I – 0.44 ng/mL (< 0.14), aspartate aminotransferases (AST) – 50 U/L, alanine transaminase (ALT) – 83 U/L, LDH – 269 U/L, as well as elevated levels of the brain natriuretic protein (BNP) – 361 pg/mL (< 256). Other blood and urine test values were in their normal reference ranges, except for an increased concentration of amyloids 16.5 pmol/L (< 6.8), while serum and urine protein electrophoresis and immunoelectrophoresis were normal.

Echocardiography showed: left atrium was dilated – 4.7 cm. The size of the left ventricle was in normal reference values: end systolic diameter (ESD) – 3.23 cm, endodiastolic diameter (EDD) – 4.73 cm. Concentric left ventricular hypertrophy was registered, with ventricular walls of 1.5 cm and a highly hyperechoic myocardium. Myocardial contractility was preserved, the ejection fraction (EF) was 58.2% according to the Teichholz formula and 56% calculated by Simpson's rule, Fractional shortening (FS) was 29.8%. Left ventricular mass was increased – 293 g or 161 gm^2 (Cube). In the left ventricular apex, especially alongside the lateral and inferior walls, ultrasound revealed hypertrabeculation with multiple trabeculae projecting inside the left ventricular cav-

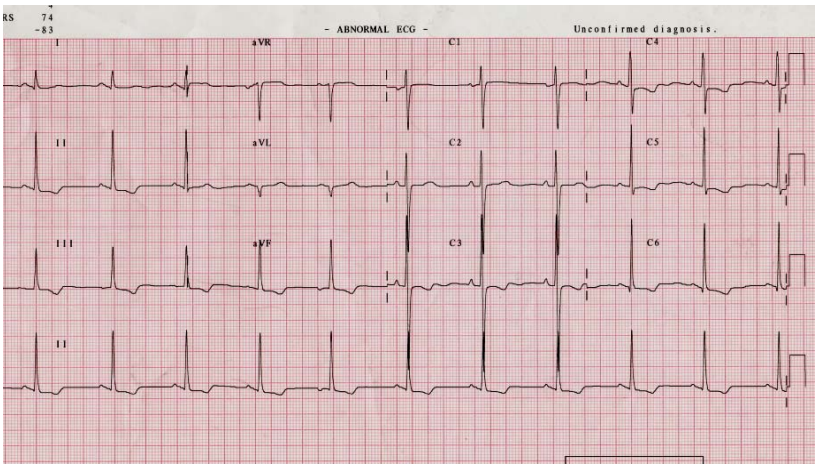

Fig. 1 – Electrocardiography showing ST segment depression of 1 mm with negative T waves in the leads D_2, D_3, aVF, V_3–V_6.

ity. Colour Doppler echocardiography showed that blood filled intertrabecular recesses in the lateral wall and the left ventricular apex (Figure 2).

A short-axis view of the heart above the papillary muscles revealed the presence of two layers of the myocardium: a compacted homogeneous layer adjacent to the epicardium and a spongy layer with trabeculae and sinusoids under the endocardium. The thickness ratio between the two layers was 2.2:1 (Figure 3).

The type of blood flow registered above the mitral valve was typical of impaired relaxation, with the E/A ratio of 0.6 and mitral regurgitation grade 1+. The size of the right ventricle was within the upper reference limit (2.8 cm), with tricuspid regurgitation grade 1+. The systolic pressure in the right ventricle was increased – 48 mmHg. A pericardial effusion of 0.4 cm was detected around the entire heart.

Holter monitoring registered a sinus rhythm, with a short episode of absolute arrhythmia, which lasted for 6 seconds at the frequency of 153/min. There were 163 atrial extrasystoles, which were isolated, rarely paired, as well as 4 single ventricular extrasystoles.

On the multislice computed tomography (MSCT) of the heart with angiography: blood vessel imaging showed normal results (Figure 4).

Since magnetic resonance imaging of the heart was not available in our institution, we conducted an MSCT test, which included multiple long- and short-axis slices that revealed concentric myocardial hypertrophy with prominent trabeculations and deep recesses above the papillary muscles, especially in the area of the apex and the anterolateral wall (Figure 5).

On the basis of the anamnesis, the clinical picture and the undertaken examinations, we concluded that the patient suffered from noncompaction cardiomyopathy presenting as heart failure (New York Heart Association – NYHA II/III). The initial diagnosis of acute coronary syndrome was abandoned. Laboratory analyses registered only slight elevations of cardiac-specific enzymes, which were explained by myocardial hypertrophy and heart failure, considering that coronary blood vessels were unobstructed. Owing to suspected transient ischaemic attack, we performed an MSCT of the endocranium, which returned

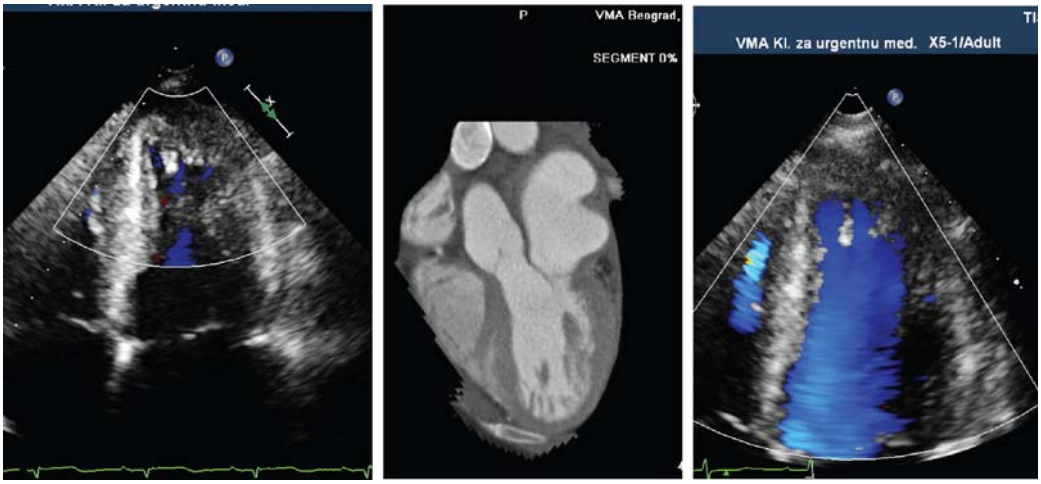

Fig. 2 – Echocardiography and multislice computed tomography of the heart – apical 4-chamber view showing prominent trabeculations of the left ventricular apex and lateral wall, with colour Doppler evidence of deep intertrabecular recesses filled with blood.

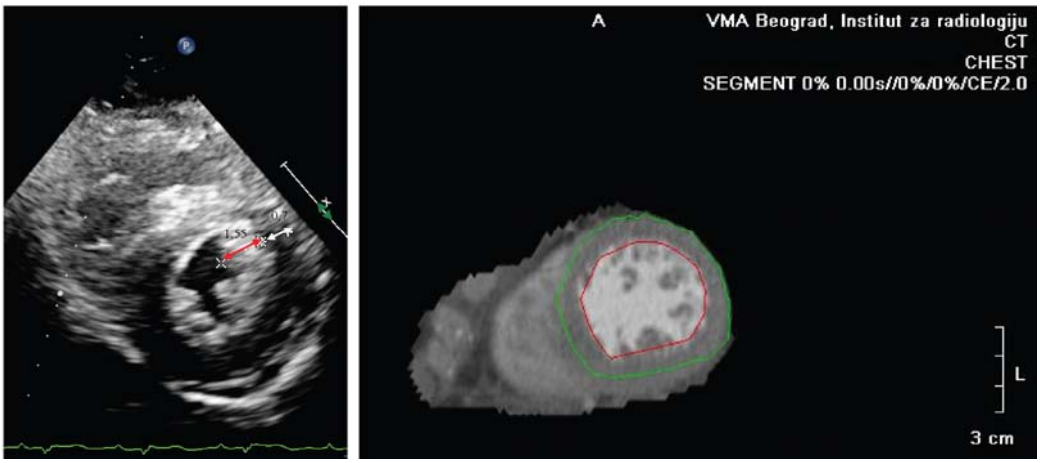

Fig. 3 – Echocardiography: short-axis view of the left ventricle at end systole, and multislice computed tomography of the heart: the left ventricular myocardium consists of 2 layers – the thick non-compacted layer and the thin compacted layer, their thickness ratio being 2.2:1.

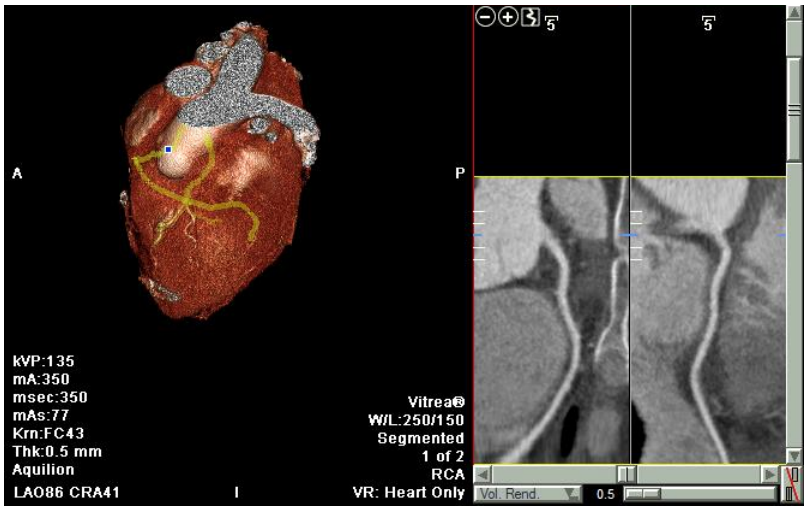

Fig. 4 – Multislice computed tomography coronarography: all coronary blood vessels are free of plaque and show no signs of stenosis.

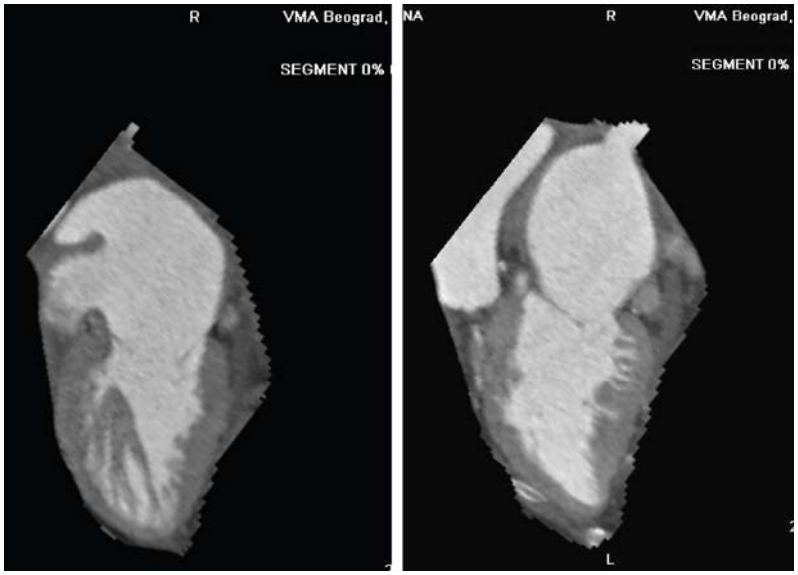

Fig. 5 – Multislice computed tomography of the heart with left ventricular noncompaction. Multiple trabeculations and recesses between them are visible apically, on lateral and inferior walls.

normal results. The patient was initially treated with dual antiplatelet therapy until the coronarography examination, and then an oral anticoagulant was introduced in the therapy, in addition to cardiotonics and diuretics, due to verified paroxysmal atrial fibrillation.

Discussion

Left ventricular noncompaction is a congenital disorder characterised by prominent trabeculations in the left ventricular myocardium [1, 2]. The European Society of Cardiology (Working Group on Myocardial and Pericardial Diseases) included this defect in the group of unclassified cardiomyopathies, along with Takotsubo cardiomyopathy, whereas the American Heart Association classifies it as a primary congenital cardiomyopathy [5, 6].

The disorder appears in isolated form, but it may also be inherited, usually through autosomal dominant inheritance. In many families, the disorder is found in the gene responsible for the synthesis of beta-myoglobin heavy chains. Furthermore, in some cases the mutation is found in the genes G4.5, P121L, E101K, which are responsible for the synthesis of cytoskeletal proteins: beta-myosin, alpha-actin and troponin [7, 8].

Noncompaction of the left ventricular myocardium is sometimes seen as part of congenital anomalies of the left or right ventricular outflow tract, such as: pulmonary artery atresia, bicuspid aortic valve, congenitally corrected transposition and Ebstein's anomaly. Moreover, this disorder is occasionally detected in conjunction with ventricular septal defect (VSD), or in association with neuromuscular diseases [3].

The disease is believed to appear during intrauterine development as a result of arrested morphogenesis of a compacted myocardium. Namely, the heart muscle is markedly heterogeneous during the embryonic stage, with many sinusoid blood vessels interwoven with myofibril bundles, which provide blood supply to the myocardium. Towards the end of the intrauterine development, the myofibrils are condensed

and the left ventricular myocardium becomes compacted. As a result of disrupted embryonic development, the myocardium becomes excessively thick and consists of two layers: a homogeneous layer, located under the epicardium, and a distinctly spongy and non-compacted layer, located under the endocardium, characterised by multiple trabeculations and recesses. The intertrabecular recesses open into the left ventricular cavity and are filled with blood, with no communication with epicardial blood vessels [9, 10].

Actual prevalence of this disease is still unknown. However, in patients with heart failure, it appears in approx. 3–4% of the cases [11].

Some patients are asymptomatic and their diagnosis is established fortuitously, usually based on echocardiography. Other patients develop three types of symptoms: heart failure, atrial and ventricular arrhythmias, thromboembolic complications.

The disorder usually manifests itself as heart failure in 79% of cases (dyspnoea, fatigue after slight exertion, cough and swelling). Chronic atrial fibrillation is the most common type of arrhythmia, while other registered abnormalities include left of right bundle branch block, fascicular block or ventricular tachycardia. In the left ventricular cavity, thrombi often form between trabeculae, which leads to thromboembolic complications. The clinical presentation usually appears in adults, but it is not rare to see children or even infants suffering from heart failure resulting from left ventricular noncompaction [4].

Electrocardiogram (ECG) reveals left ventricular hypertrophy, as well as various rhythm and conduction disturbances [12].

Echocardiography is the method of choice in diagnosing LVNC. Other applicable methods include magnetic resonance imaging, multi-slice scanner, left ventricular ventriculography and genetic testing.

In 2001, Jenni et al. [13] proposed 3 criteria for echocardiographic diagnosis of this disorder: considerable thickening of the left ventricular wall, which consists of two layers – thinner compacted layer underneath the epicardium, and thickened layer under the endocardium, with multiple trabeculae and deep recesses. The ratio of non-compacted to compacted myocardium is higher than 2:1 at end systole, at parasternal short-axis view; deep intertrabecular recesses, in which blood flow is visible in colour Doppler echocardiography; pronounced meshwork of trabeculae in the left ventricular apical area and the mid segments of the inferior and lateral walls.

For LVNC diagnosis to be established, all three of the above criteria need to be fulfilled. In addition to these manifestations, there is also the hypokinesia of the affected left ventricular segments [13].

Stöllberger et al. [14] proposed somewhat different criteria that involve hypertrabeculation: the presence of more than three trabeculations at the left ventricular apex, above the papillary muscles, all of which are visible in a single image plane, and intertrabecular spaces are perfused with blood from the left ventricular cavity, which is visible in colour Doppler imaging.

Aside from these, other manifestations that may be detected include diffusely reduced left ventricular systolic function, diastolic dysfunction, thrombi in the left ventricular cavity and structural anomalies of the papillary muscle, which is also trabeculated.

In terms of the differential diagnosis, one of the considerations is dilated cardiomyopathy, which occasionally also exhibits two myocardial layers, one of which is non-compacted. The ratio between the two layers is also higher than 2:1; however, the main difference is that dilated cardiomyopathy is characterised by myocardial wall thinning. The differential diagnosis should also take into consideration apical hypertrophic cardiomyopathy, various forms of infiltrative cardiomyopathies, heart anomalies associated with arterial hypertension, as well as the hypereosinophilic syndrome [15].

Magnetic resonance imaging and the MSCT scanner produce a somewhat different image to that of echocardiography. Trabeculations and recesses are detected much more frequently, even in healthy people, especially in patients who suffer from any type of myocardial hypertrophy, and they are located at the left ventricular apex, anterior or lateral walls. The only difference is that the ratio of non-compacted to compacted myocardium is greater than 2.3:1 in diastole, visible in three cross-section views; hence, this is the basic criterion for diagnosing LVNC using magnetic resonance imaging [16, 17].

Treatment is symptomatic and involves the therapy of heart failure and rhythm disorders. Focus should be on anticoagulation therapy, which is compulsory for patients with EF < 40% and/or absolute arrhythmia. In the final stage, heart transplantation or implantation of a cardioverter-defibrillator are recommended [18–24].

Until recently, LVNC was associated with poor prognosis and it was believed that most patients died as a result of complications. A long-term follow-up of 34 patients with an average age of 42 years, published in 2000, showed that heart failure manifested in 53%, ventricular rhythm disorders in 44% (cardioverter-defibrilators were implanted in 12% of them), while thromboembolic complications occurred in 24% of the patients. Over the follow-up period of 44 months, one third of the patients died – half of them by sudden death, whereas 12% underwent heart transplantation. They were all patients with serious health problems [20].

Following the publication of this study, physicians started paying more attention to similar patients who had much milder clinical presentation, or were entirely asymptomatic and identified by chance. In 2005, Murphy et al. [21] presented a study in which 45 patients of an average age of 37 years were followed up over an average period of 4 years. Survival rate was 97%, while thromboembolic events occurred in only 2 patients. Lofiego et al. [4] divided 65 patients with LVNC into two groups: symptomatic patients (48) had poor prognosis – 31% of them died or underwent heart transplantation over the average follow-up period of 46 months. Asymptomatic patients, who were usually family members of the affected patients, had no complications [22, 23].

Conclusion

Left ventricular noncompaction is a rare, usually hereditary cardiomyopathy, which should be considered as a possibility in patients with myocardial hypertrophy. It is very often mistaken for coronary disease owing to ECG changes and elevated cardiac-specific enzymes associated with myocardial hypertrophy and heart failure. The disorder is usually detected fortuitously and patients with LVNC have a normal life expectancy, while about a third of them present with symptoms of heart failure, arrhythmias or thromboembolic events, with a very poor prognosis.

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I N M E M O R I A M

prof. dr sc. med. MIHAILO ĐUKNIĆ
General-major u penziji
(1927–2017)

Dana 30.12.2017. godine završio se jedan ispunjen život vredan svakog poštovanja. Nemoguće je da se samoređanjem biografskih podataka jedan životni vek verno prikaže, ali sa njima nepoznatom čitaocu, činimo makar malo vidljivijim svo pregnuće, vrednost i uspeh jednog čoveka, hirurga, profesora i generala kakav je bio Mihailo Đuknić. Jer, iznimno je mali broj ljudi koji uspeju da svoj život oplemene na način da se ostvare i kao suprug i roditelj, učitelj, profesionalni i stručni autoritet, ugledni član društva, cenjeni prijatelj, kolega i komandant.

Mihailo Đuknić je rođen u Beogradu 1927. godine, ali sa porodičnim poreklom i nasleđem Belanovice, kraja u Srbiji koji vekovima predstavlja trajno izvorište ratnika i naučnika. Medicinski fakultet u Beogradu završio je 1951. godine sa prosečnom ocenom višom od devet. Po završetku lekarskog staža u sanitetskoj oficirskoj školi postavljen je za načelnika saniteta gardijskog puka u Sarajevu. Kao načelnik sanitetskog odreda Jugoslovenske narodne armije (JNA) u sastavu misije Ujedinjenih nacija boravi šest meseci na Sinaju tokom Arapsko-izraelskog rata. Najverovatnije je da se tokom boravka u Egiptu i „zarazio virusom" koji će opredeliti čitav njegov dalji profesionalni život, a to će biti hirurgija, veština neprekidnog davanja svojih godina, godinama života operisanih. Specijalizaciju iz opšte hirurgije završava u periodu između 1958. i 1962. godine sa odličnim uspehom, radeći i učeći od velikana vojne hirurgije, prof. dr Izidora Pape. Od tada je stalno zaposlen u Klinici za hirurške bolesti Vojnomedicinske akademije (VMA) u Beogradu, najpre kao kao asistent, a kasnije docent i profesor hirurgije.

Usavršavao se u poznatim klinikama u Sjedinjenih američkih država, Sovjetskom Savezu, Francuskoj, Engleskoj i Čehoslovačkoj. Po povratku iz Londona 1965. godine, prvi je u zemlji uradio operaciju suženja na karotidnoj arteriji i među prvima je operisao aneurizme abdominalne aorte. Nakon boravka u najpoznatijoj američkoj vojnoj bolnici Volter Rid, posvetio se unapređivanju vaskularne hirurgije, posebno zbrinjavanju povreda krvnih sudova. U Klinici za ratnu hirurgiju u Lenjingradu upoznaje se sa osobinama dejstva savremenog oružja i projektila velike početne brzine, kao i karakteristikama udruženih hemijskih i radijacionih povreda i načinima njihovog zbrinjavanja.

Doktorsku disertaciju iz ratne hirurgije „Udružena hemijska povreda" odbranio je 1977. godine. Kao autor i koautor objavio je više od 200 naučnih i stručnih članaka iz opšte, vaskularne i ratne hirurgije. Učestvovao je u pisanju niza poglavlja u knjigama i enciklopedijama.

Bio je načelnik Klinike za hirurške bolesti VMA, glavni hirurg JNA i načelnik VMA (1989–1992) u činu general-majora u koji je unapređen 1987. godine.

Tokom profesionalnog veka bio je redovni profesor hirurgije u VMA, redovni član Medicinske akademije Srpskog lekarskog društva (SLD), član Međunarodnog udruženja digestivnih hirurga i Internacionalnog udruženja za kardiovaskularnu hirurgiju. Više puta je biran u Predsedništvo hirurga Jugoslavije i u upravni odbor Hirurške sekcije. U periodu od maja 1980. do maja 1981. godine bio je i predsednik predsedništva Hirurške sekcije SLD-a.

Nosilac je najviše vojne nagrade „22. decembar".

Kao četvrti hirurg u svetu 1988. godine dobio je prestižnu američku nagradu za ratnu hirurgiju „De Bakey".

Za počasnog doktora medicinskih nauka VMA u Lenjingradu izabran je 1988. godine.

Nakon penzionisanja 1994. godine, znajući da uspešni ljudi retko sede i čekaju da se stvari dese, nastavlja da radi kao potpredsednik Jugoslovenskog crvenog krsta i Crvenog krsta Srbije, nastavljajući da čini ono što je najbolje znao: da pomaže ljudima u nevolji.

Ako ima istine u tome da je sreća harmonija između čoveka i načina na koji živi svoj život, da do uspeha dolaze oni kojima ništa nije nemoguće, jer imaju volju da pokušaju i ako se znanje množi deljenjem, kao lekari, hirurzi i pripadnici Vojske Srbije ponosni smo na svog profesora i generala.

Neka mu je večna slava i hvala!

pukovnik, doc. dr Zoran Kostić
Klinika za opštu hirurgiju VMA

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

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

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

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

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

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