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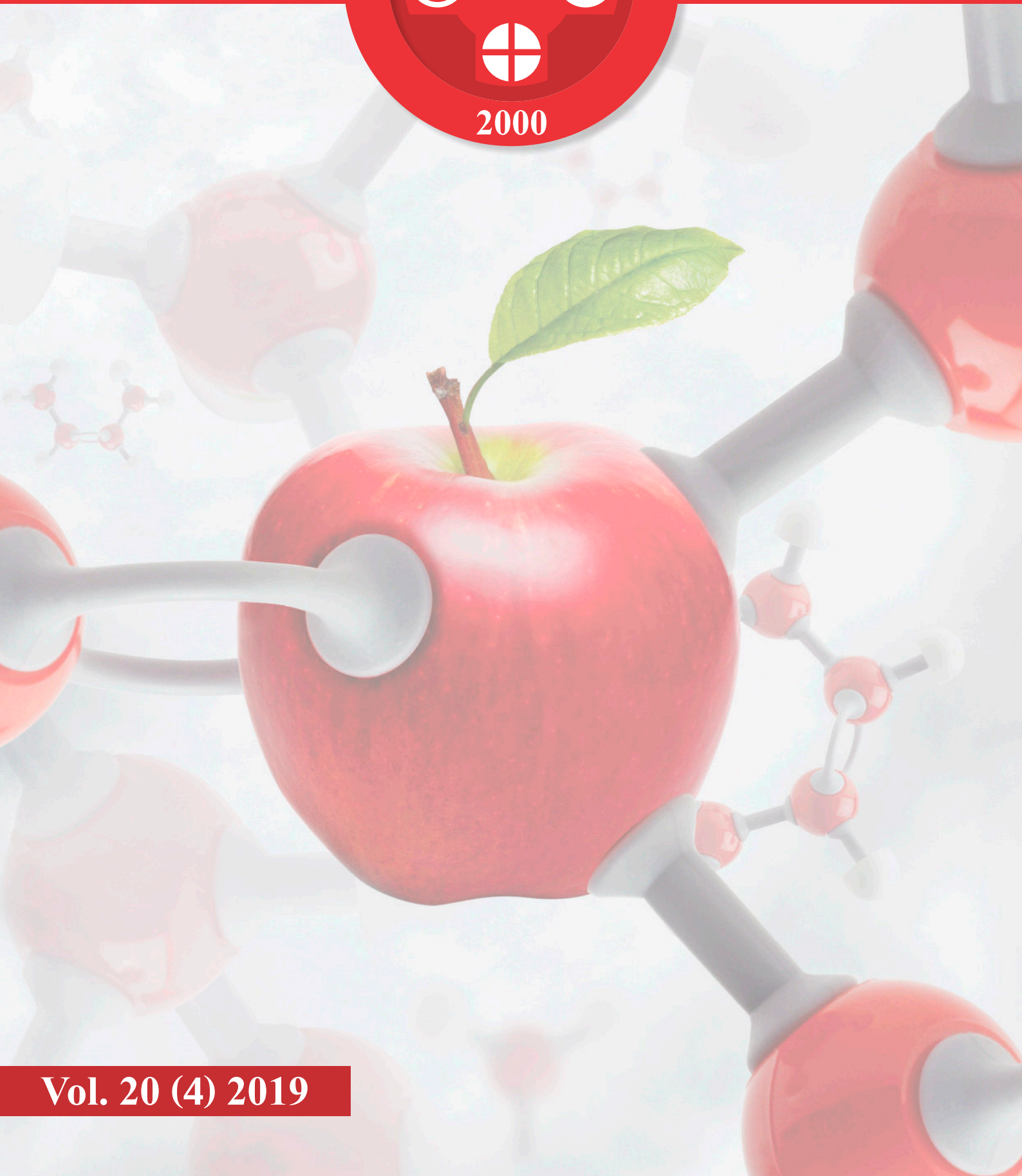

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PREEKLAMPSIJA – FAKTORI PREDVIĐANJA I PRAĆENJA

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ABSTRACT

Preeclampsia is one of the leading causes of maternal and perinatal morbidity and mortality, usually characterized by hypertension and proteinuria. Despite high incidence of preeclampsia the pathophysiological basis of preeclampsia is still not clear and there are a number of mechanisms and signaling pathways that intertwine. It is very important to develop specific and reliable predictive algorithms in order to enable early initiation of therapy due to facts that incidence of preeclampsia has upward trend and that cause adverse maternal and fetal outcome. Some of the most commonly used methods for prediction of preeclampsia include uterine artery Doppler velocimetry, determination of some microRNA, such as miR-210, and assessment of various pro-angiogenic and anti-angiogenic factors from blood. Angiogenic factors that possibly have most important role in pathogenesis of preeclampsia are vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), which promote angiogenesis, and soluble fms-like tyrosine kinase-1 (sFlt1) and soluble form of endoglin (s-Eng), which exhibit anti-angiogenic properties. Aggravating circumstance is that preeclampsia has heterogeneous origin, and due to this fact, the value of individual markers can vary significantly. There is a constant tendency for creating comprehensive algorithm for prediction of preeclampsia which would be sufficiently specific and sensitive, and in the same time cheap and available. In that sense, new clinical studies are needed to show the most effective combination of parameters in the predeclampsia prediction.

Keywords: *preeclampsia; prediction; Doppler velocimetry; microRNA; angiogenic factors.*

SAŽETAK

Preeklampsija je jedan od vodećih uzroka materinskog i perinatalnog morbiditeta i mortaliteta, koju najčešće karakterišu hipertenzija i proteinurija. Uprkos visokoj incidenciji patofiziološka osnova preeklampsije nije dovoljno jasna i postoji veliki broj mehanizama i signalnih puteva koji se međusobno prepliću. Vrlo je važno razviti specifične i pouzdane algoritme za predviđanje preeklampsije kako bi se omogućilo rano započinjanje terapije u skladu sa činjenicom da incidencija preeklampsije ima uzlazni trend i uzrokuje neželjeni maternalni i fetalni ishod porođaja. Neki od najčešćih metoda koje se koriste za predviđanje nastajanja preeklampsije podrazumevaju Dopler uterine arterije, određivanje pojedinih mikroRNK, poput miR-210, kao i određivanje različitih proangigenih i antiangiogenih faktora iz periferne krvi. Angiogeni faktori koji možda imaju najznačajniju ulogu u patogenezi preeklampsije su vaskularni endotelni factor rasta (VEGF) i placentalni factor rasta (PlGF), koji pospešuju angiogenezu, i solubilna fms-slična tirozin kinaza-1 (sFlt1) i solubilni oblik endoglina (s-Eng), koji ispoljavaju antiangiogena svojstva. Otežavajuća okolnost je činjenica da preeklampsija ima heterogeno poreklo, i u skladu sa tim vrednosti pojedinih markera mogu znatno da variraju. Postoji konstantna težnja za stvaranje sveobuhvatnog algoritma za predviđanje nastajanja preeklampsije koji bi bio dovoljno specifičan i senzitivan, a u isto vreme jeftin i dostupan. U tom smislu, potrebne su nove kliničke studije kako bi se obrazovala najefikasnija kombinacija parametara za predviđanje nastajanja preeklampsije.

Ključne reči: *preeklampsija; predikcija; Doppler; mikroRNK; angiogeni faktori.*

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ABBREVIATIONS

AP-1 - activated protein 1
BMP - bone morphogenetic protein
CRH - corticotrophin releasing hormone
CYR61- cysteine-rich protein 61
DNA - deoxyribonucleic acid
eNOS - endothelial nitric oxide synthase
HDP - hypertensive disorders of pregnancy
LPS - lipopolysaccharides
microRNA - microribonucleic acid
MPV - mean platelet volume
NF-κB - nuclear factor-κB
NO - nitric oxide
PAPP-A - pregnancy associated plasma protein A
PC - platelet count
PDW - platelet distribution width
PI - pulsatility index
Plcr - platelet large cell ratio
PlGF - placental growth factor
PP13 - placental protein 13
qRT-PCR - quantitative reverse transcription-polymerase chain reaction
RI - resistance index
RNA - ribonucleic acid
sEng - soluble endoglin
sFlt-1 - soluble fms-like tyrosine kinase-1
TGF-β - transforming growth factor β
VEGF - vascular endothelial growth factor

INTRODUCTION

Preeclampsia represents one of the hypertensive disorders of pregnancy (HDP) and one of the leading causes of maternal and perinatal morbidity and mortality worldwide complicating 2%-8% of pregnancies (1, 2). According to the American College of Obstetricians and Gynecologists there are four major hypertensive disorders encompassed within HDP: 1) chronic hypertension, 2) preeclampsia and eclampsia, 3) hronic hypertension with superimposed preeclampsia, and 4) gestational hypertension (3). The International Society for the Study of Hypertension in Pregnancy as an additional category also lists the "white coat hypertension" In addition to the fact that HDP make up a large share of maternal and perinatal mortality, another worry is related to the results of the studies that indicate that incidence of the HDP continues to increase (4). Clinical diagnosis of preeclampsia is based on several criteria: 1) blood pressure above 140/90 mmHg in two separate measurements separated by an interval longer than 4 hours, or blood pressure above 160/110 mmHg in two measurements separated shorter interval, after 20 week of gestation in previously normotensive woman; 2) proteinuria which exceeds 300 mg/24 h; 3) in the absence of proteinuria, additional criteria include: thrombocytopenia, renal failure, disturbance of liver function, pulmonary edema, as well as any symptom related to the disorder of the function of the nervous system (3, 5). If preeclampsia progresses and seizures occur, preeclampsia passes into eclampsia. In the fetus preeclampsia causes premature birth and growth restriction, while in women with preeclampsia increases risk for other diseases, such as renal failure, stroke and cardiovascular disease (6).

There are many conditions recognized as risk factors for development of preeclampsia (Table 1). Nulliparous women have a three times greater risk for development of preeclampsia in comparison to multiparous women. Also in women with pregnancies complicated with preeclampsia there is increased risk for recurrence in subsequent pregnancies. Age is also a risk factor, so that pregnant women over 40 years also have an increased risk of developing preeclampsia (7). Risk factors for preeclampsia also include family history for preeclampsia, twin pregnancy, antiphospholipids antibodies and thrombophilia, preexisting diabetes, preexisting chronic hypertension or increased blood pressure, preexisting renal and autoimmune disease, as well as increased body mass index (8, 9).

Despite high and increasing incidence of preeclampsia the pathophysiological basis of preeclampsia is still not clear and there are a number of mechanisms and signaling pathways that intertwine. Preeclampsia is usually defined as a syndrome of maternal systemic inflammatory response that affects various organ systems, and according to the current hypothesis preeclampsia is caused by placental dysfunction which takes place in two stages (10, 11). The first stage implies poor placentation which occurs during first half of pregnancy, between 8 and 18 week of gestation. During this stage clinical symptoms are absent, but Doppler's ultrasound analysis of blood flow velocity in uterine arteries indicate changes that could affect blood flow in spiral arteries (12, 13). Ischemic and reperfusion injury of placenta due to disturbed perfusion of placenta causes increased production of reactive species and oxidative stress,

Table 1. Risk factors for preeclampsia onset.

Nulliparity
Advanced maternal age
Previous pregnancies complicated with preeclampsia
Pregnant women of advanced age
Family history of preeclampsia
Twin pregnancy
Preexisting chronic hypertension
Preexisting renal and autoimmune disease
Increased body mass index and obesity

as well as synthesis and release of proinflammatory mediators from syncytiotrophoblast. These mediators induce clinical manifestations of preeclampsia in second half of pregnancy, which represents the second phase. Proinflammatory mediators involved in occurrence of preeclampsia include: soluble fms-like tyrosine kinase-1 (sFlt-1), soluble endoglin (sEng), leptin, activin-A, corticotrophin releasing hormone (CRH), serum placental protein 13 (PP13), and pregnancy associated plasma protein A (PAPP-A) (14-17). There were various attempts to provide the best prediction algorithm for preeclampsia, as well as to set aside parameters which would enable the most adequate monitoring of preeclampsia which has already been diagnosed.

UTERINE ARTERY DOPPLER IN PREECLAMPSIA PREDICTION

Attempts to use ultrasound and Doppler's effects in the prediction of preeclampsia have a relatively long history. Namely, one of the first tries to use Doppler velocimetry in analysis of blood flow and pressures in women with HDP was conducted by Fleischer and colleagues during the eighties of the last century (18). These authors compared ratio between systolic and diastolic pressure in normal pregnancies and HDP, and concluded that systolic/diastolic ration physiologically does not exceed 2.6. On the other hand when this ratio is higher pregnancy can be complicated by premature birth, intrauterine growth retardation and maternal preeclampsia. During early pregnancy, as well as in nonpregnant state, Doppler analysis show low end-diastolic velocity and early diastolic notch. Namely, normal shape of uterine waveform consist steep systolic slope, early diastolic notch and a small flow of blood during the diastole. As the pregnancy progresses the resistance to blood flow decreases, diastolic notch is gradually removed and diastolic blood flow increases (19). Increase of blood flow reaches peak between 20th and 24th week of pregnancy, and at the end of pregnancy uterine arteries blood flow amounts 970 ml/min (20). In HDP resistance to blood flow in uteroplacental circulation is high, which is transmitted upstream to the uterine arteries and can be detected through increased pulsatility index (PI) or resistance index (RI) (13).

In cohort study performed by Sharma and colleagues it was shown that single uterine artery Doppler scan in second trimester of pregnancy (between 20 and 23 week of gestation), combined with other risk factors such as maternal age over 34 years and chronic hypertension, would clarify what pregnancies need further supervision (21). Pedroso and coworkers analyzed 30 large cohort studies and randomized trials which dealt with prediction possibility of uterine artery Doppler in occurrence of preeclampsia and fetal growth retardation (22). They concluded that of analysis of uterine artery PI by Doppler has low sensitivity and prediction capability, but in combination with other biochemical markers or analysis of maternal risk factors its sensitivity increases, making it an acceptable and useful part of the preeclampsia prediction algorithms. Tan and coauthors analyzed results from three previously reported prospective non-intervention screening studies, in a combined total of 61,174 singleton pregnancies (23). From this total number of analyzed pregnancies, 2.9% developed preeclampsia. These authors confirmed conclusion of previously mentioned study, and showed that analysis of maternal factors, uterine artery PI by Doppler, mean arterial pressure and serum placental growth factor (PLGF) enabled prediction over 90% of preeclampsia. Abdel Razik and colleagues investigated the interconnection between Doppler ultrasound parameters: diastolic notch, PI and RI, and platelet indices: platelet count (PC), mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (Plcr) (24). They analyzed 270 normal pregnant women between 20th and 24th week of gestation and showed that patients with preeclampsia had higher frequency of diastolic notching, as well as higher PI and RI, combined with significant increase of MPV and PDW. Also, patients with disturbed Doppler parameters and platelet indices had higher risk to develop a severe form of preeclampsia. Another investigation dealt with possibility of prediction of early and late preeclampsia through analysis of placental volume and placental blood flow, and results showed that pregnant women with preeclampsia have had lower placental volume and higher uterine PI compared to healthy pregnancies (25). Results of longitudinal study conducted by Porto and coworkers indicated that all pregnancies complicated with early onset of preeclampsia have had significantly higher uterine artery PI between 16+0 and 19+6 weeks of gestation (26). Navaratnam and colleagues went a step further, and compared PlGF tests and the sFlt-1/PlGF ratio with abnormal uterine artery Doppler (27). Results of their investigation showed significant association between PlGF or sFlt-1/PlGF ratio with results of Doppler parameters.

Taken all together, uterine artery Doppler can be used for prediction and monitoring of preeclampsia, but in order to increase sensitivity and specificity uterine artery Doppler should be combined with estimation of other markers.

ROLE OF MICRO-RNA IN PREECLAMPSIA

Ribonucleic acid (RNA) is polymeric molecule that have crucial role in deciphering of information within the deoxyribonucleic acid (DNA). Three various forms of RNA molecules take part in this process: messenger RNA (mRNA), which arises from the transcription process from DNA and contains information regarding the structure composition of proteins, transfer RNA (tRNA), which enables transfer of appropriate amino acid to ribosome, and ribosomal RNA (rRNA), which participates in the protein synthesis itself as part of the ribosome. In 1993 another form of RNA was discovered, micro RNA (microRNA) for

which it turns out to have a number of key roles in the body (28). The fact that microRNAs are ubiquitously present in viruses, plants and animals depict their evolutionary necessity and importance. These small molecules, containing 20 to 24 nucleotide bases, have pivotal roles in processes such as regulation of cell cycle and regeneration, immunity, normal function of various tissues and organs, but on the other hand their role is recognized also in different pathological states, including carcinomas (29-33). Until now, there are more than 28,000 microRNAs which have been indentified in various species, and of that number 2654 are human microRNAs (34).

Pineles and colleagues firstly described the discrepancy in expression of microRNAs in placental tissue from patients with preeclampsia and control group of women with preterm labor and delivery (35). Namely, authors analyzed expression of 157 different microRNAs, using real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR), and found that two microRNAs, miR-182 and miR-210, had significantly higher expression in samples of placenta tissues of women with preeclampsia. Further, Zhang and colleagues firstly described negative correlation between expression of miR-155 and cysteine-rich protein 61 (CYR61) (36). CYR61 is important angiogenic regulator and has important role in placental angiogenesis (37). It was previously showed that expression of CYR61 in preeclamtic placental tissue has been reduced, thus reveling new mechanism in development of preeclampsia. It is also shown that stimulation of human-trophoblast-derived cells with lipopolysaccharides (LPS) leads to increment of miR-155, transcription factor activated protein 1 (AP-1) and nuclear factor (NF)-κB, suggesting the role of miR-155 in process of syncytialization, as well as in pathogenesis of preeclamsia (38). Enquobahrie and colleagues found differences in expression of eight microRNAs in placental tissues of women with preeclampsia and women with normal pregnancy and labor using qRT-PCR (39). Results of this investigation showed up regulation of miR-210, and down regulation of seven other microRNAs (miR-328, miR-584, miR-139-5p, miR-500, miR-1247, miR-34c-5p and miR-1). Overexpression of miR-210 under hypoxic conditions, which exist in preeclampsia, is related to NF-κB transcriptional factor p50, suggesting another mechanism involved in pathogenesis of preeclampsia. Furthermore, miR-210 modulates mitochondrial respiration and reduces oxygen consumption (40). Gunel and coworkers found increased plasma levels of miR-210 in pregnant women with preeclampsia, as well as decreased levels of miR-152 (41). Placenta-associated serum exosomal miR-155 derived from patients with preeclampsia caused decreased expression of endothelial nitric oxide synthase (eNOS) in primary human umbilical vein endothelial cells, and thus decreased production of nitric oxide (NO) (42).

In order to assess the possibility of prediction of preeclampsia by analyzing microRNAs from peripheral blood buffy coat samples of pregnant women, Winger and coauthors investigated 30 microRNAs (43). Authors performed qRT-PCR analysis on samples of 48 pregnant women collected during first trimester of pregnancy (11±13 weeks gestation), of whom 8 had preeclampsia. Of 30 investigated microRNAs, eight is included in panel (miR-1267, miR-148a, miR-196a, miR-33a, miR-575, miR-582, miR-210 and miR-16), and data showed that investigation of maternal immune cells may provide early prediction of preeclampsia and enable adequate response. On the other hand, Luque and coworkers based on the results of previously conducted investigation made quite opposite conclusion (44). Of 754 analyzed microRNAs from pregnant women with preeclampsia and healthy pregnancies, 63 of them were continuously registered in samples, but only 15 were differently represented. Further statistical analysis indicated that there were not significant differences in presence any of them in sera of pregnancies with preeclampsia and healthy pregnancies, so author concluded identification of microRNAs from maternal serum samples during first trimester is not a reliable predictor for development of preeclampsia.

Bearing in mind all above results it can be concluded that microRNAs have important role in pathogenesis of preeclampsia. On the other hand, predictive value of microRNAs determination depends on type of sample and time of sampling (week of gestation). In order to provide good predictive model which include analysis of microRNAs future investigation are necessary. These investigations will also provide new insights into roles of microRNAs in pathophysiology of preeclampsia and other HDP.

ANGIOGENIC FACTORS IN PREECLAMPSIA

Various circulating factors that affect angiogenesis take part in pathogenesis of preeclampsia. These factors can be classified as pro-angiogenic and anti-angiogenic and their imbalance actually is crucial in onset of preeclampsia. Decreased expression of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), which promote angiogenesis, combined with increased expression of soluble fms-like tyrosine kinase-1 (sFlt1) and soluble form of endoglin (s-Eng), which exhibit anti-angiogenic properties, are most often referred to as the focus of the preeclampsia pathogenesis.

The vascular endothelial growth factor (VEGF) is pro-angiogenic factor mostly synthesized by endothelial cells, macrophages, T-cells, tumor cells and cytothrophoblast in response to stimulation by hypoxia or various mediators (interleukins, transforming growth factor β - TGF-β, platelet derived growth factors) (45). There are five members of VEGF family in mammals, VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF). When only the VEGF is mentioned, it is thought of VEGF-A, because it is predominant form. All VEGFs are generated as alternative splice variants of mRNA from the same gene located on chromosome 6 which contains 8 exons (46, 47). VEGFs can achieve their effect by acting on two receptor fami-

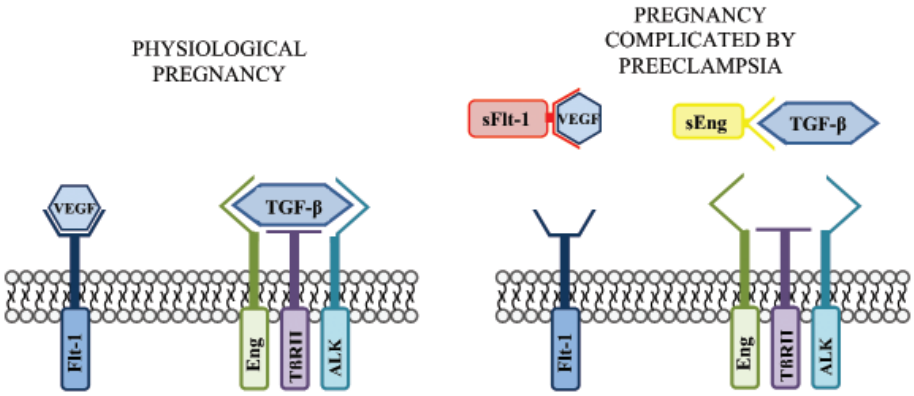

Figure 1. Role of angiogenic factors in pathogenesis of preeclampsia.
sEng - soluble endoglin; sFlt-1 - soluble fms-like tyrosine kinase-1; TGF-β - transforming growth factor β; VEGF - vascular endothelial growth factor.

lies, VEGF receptors (VEGFR) and neuropilin receptors. VEGFR belong to tyrosine kinase receptors, and there are three types of them: VEGFR-1 (or fms-like tyrosine kinase - Flt-1), VEGFR-2 (or murine Flk-1) and VEGFR-3 (or Flt-4), and there are two neutrophilin molecules: neutrophilin 1 and neutrophilin 2 (46). VEGF is very important in maintenance of fenestrated and sinusoidal epithelium, so the symptoms in preeclampsia are the most pronounced in organs in which these types of epithelia are present (kidney, liver). Angiogenesis is process of crucial importance in embryogenesis and growth, and due to that fact the physiological regulation of angiogenesis during pregnancy by above mentioned growth factors is also undoubtedly crucial. The main source of PlGF is placenta, and its level is low in the first trimester of normal pregnancy, with its concentration increasing from 11 week of gestation, reaching peak at 30 week of gestation, followed by decrease (48). PlGF enhances action of VEGF by competitive binding to VEGFR-1 and thus enabling the VEGF to bind the VEGFR-2 which has higher activity. PlGF also magnify the effects of VEGF binding to VEGFR-2, via mechanisms such as intermolecular transphosphorylation of VEGFR-2.

Soluble form of Flt-1 – sFlt-1, binds VEGF and PlGF, thereby reducing their availability in circulation (Figure 1). Due to the mentioned fact arise antagonistic properties of Flt-1 in relation to VEGF and PlGF. In preeclampsia sFlt-1 is mainly produced by syncytiotrophoblast, while its expression is regulated by hypoxia-inducible transcription factor (HIF) (49). Certain amount of sFlt-1 is present in circulation of healthy and non-pregnant women, indicating the physiological role of sFlt-1 in regulation of VEGF level (50). Hypothesis regarding action of sFlt-1 implies the existence of endothelial threshold for sFlt-1 concentration. When concentration of sFlt-1 exceeds that threshold, concentration of VEGF and PlGF decreases leading to endothelial dysfunction and preeclampsia (51). In women with previously disturbed endothelial function, regarding states such as hypertension, diabetes or obesity, mentioned threshold is lower, so they are consequently more vulnerable to preeclampsia. Endoglin is an integral transmembrane glycoprotein, which is also referred as endothelial marker CD105 (52). Endoglin is part of TGF-β complex, and act as co-receptor for TGF-β1 and TGF-β3 but not TGF-β2 (53). Membrane endoglin, through its short cytoplasmic domain, is connected with endothelial nitric oxide synthase (eNOS) and thus has important role in regulation of eNOS activity and NO bioavailability (54). The form of endoglin that could be found in circulation in some conditions including preeclampsia is referred to as soluble endoglin (sEng) (55). TGF-β has important role in regulation of endothelial and vascular homeostasis through migration and proliferation of endothelial cells (56). sEng act as anti-angiogenic factor through inhibition of TGF-β signaling and consequent lack of activation of eNOS (Figure 1). Furthermore, sEng binds to the bone morphogenetic protein (BMP)-9 and induces synthesis of endothelin-1 from endothelial cells, which has highly vasoconstrictor properties (57, 58). Production of sEng is enhanced in hypoxic conditions and has opposite action in comparison to membrane endoglin.

Tarasevičienė and colleagues investigated the possibility of prediction of preeclampsia by detection of sFlt-1, PlGF, sFlt-1/PlGF ratio and uterine artery Doppler parameters: PI and RI (59). These authors designed case-control study which involved 72 pregnant women with preeclampsia and 72 women with physiological pregnancy, and based on obtained results thay concluded that sFlt-1/PlGF ratio and PlGF are more powerful predictors of preeclampsia compared to sFlt-1, PI and RI, and moreover, that these two parameters are quite sufficient for diagnostic of early preeclampsia. Benovská and coworkers assessed possibility of early prediction of preeclampsia thorough analysis of sFlt-1, PlGF, sFlt-1/PlGF ratio in 120 pregnant women whose gestational age was between 16 and 20 weeks (60). They made conclusion that all mentioned parameters, but in particular sFlt-1/PlGF ratio, can predict onset of preeclampsia in early pregnancy, even 10 to 15 weeks before it occurs. Similar conclusion made Caillon and coauthors based on analysis of sFlt-1/PlGF ratio of 67 high-risk pregnant women (61). The investigation of AbdelHalim and colleagues included 107 pregnant women with preeclampsia and 93 healthy, normo-

tensive pregnant women (62). Authors, among others, measured sEng in serum and showed that higher values of sEng were present in blood of women with preterm labor and adverse fetal outcome compared to term labor and favorable fetal outcome. Meta-analysis conducted by Allen and coauthors included 30 studies and 65,538 women and dealt with questioning of abnormal values of circulating biomarkers and preeclampsia (63). Among other biomarkers, these authors concluded that sEng was significantly associated with early as well as late onset of preeclampsia. Moore Simas and coworkers recommended assessment of sFlt-1 and sEng for prediction of preeclampsia (64).

Based on results on above mentioned investigations it can be concluded that angiogenic biomarkers are relatively reliable tool in prediction of preeclampsia. However, in order to improve the accuracy of prediction it is always better to determine several biomarkers in the same time.

CONCLUSION

Bearing in mind that preeclampsia can be severe and result in adverse fetal and maternal outcome, as well as that incidence of preeclampsia has a continuous upward trend, it is very important to develop specific and reliable predictive algorithms in order to enable early initiation of therapy. Aggravating circumstance is that preeclampsia has heterogeneous origin, and due to this fact, the value of individual markers can vary significantly. There is a constant tendency for creating comprehensive algorithm for prediction of preeclampsia which would be sufficiently specific and sensitive, and in the same time cheap and available. In that sense, new clinical studies are needed to show the most effective combination of parameters in the predeclampsia prediction.

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ANTITUMOUR EFFECT OF A MIXTURE OF N-PROPYL POLYSULFIDES *IN VITRO*

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ANTITUMORSKI EFEKTI SMEŠE N-PROPIL POLISULFIDA *IN VITRO*

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ABSTRACT

Copper serves as a limiting factor for multiple steps of tumour progression, including angiogenesis, growth and metastasis. High levels of copper have been found in a wide spectrum of human cancers. Antitumour activities of copper-chelating drugs have been reported in animal models. Organosulfur compounds (diallyl sulfide, DAS; diallyl disulfide, DADS; S-ethylcysteine, SEC; N-acetylcysteine, NAC) derived from garlic exhibit marked copper-chelating activity. We analysed a mixture of fifteen n-propyl polysulfides (DPPS) for potential antitumour activity against several murine tumour cell lines, including colon carcinoma (CT26), mammary carcinoma (4T1) and melanoma cell lines (B16F10), and compared the effects with the antiproliferative effect in highly proliferative murine mesenchymal stem cells (mMSCs). The effects of the mixture of n-propyl polysulfides (100%) on cell viability were determined using MTT assays. Cell apoptosis was analysed using Annexin V-FITC/PI assays.

The results of the MTT assays indicate that this standardized mixture of n-propyl polysulfides has a strong, dose-dependent cytotoxic effect against all three of the tested tumour cell lines (CT26, 4T1, B16F10). The cytotoxic effect of the n-propyl polysulfide mixture against the CT26 and B16F10 cell lines was much stronger than that of cisplatin and was significantly weaker in mMSCs, which are non-cancerous and highly proliferative cells, than in cancer cells. Flow cytometric analysis of CT26 and 4T1 cells revealed that apoptosis was not the dominant mechanism of cell death induced by the n-propyl polysulfide mixture. The n-propyl polysulfide mixture exerted highly cytotoxic activity against murine colon carcinoma and melanoma cell lines, but its antiproliferative activity against mMSCs was significantly lower than that of cisplatin.

Keywords: *n-propyl polysulfides, tumour cell lines, cytotoxicity, in vitro*

SAŽETAK

Bakar učestvuje u različitim fazama progresije tumora, u angiogenezi, rastu i metastaziranju. Povećane vrednosti bakra u serumu i u tkivu tumora, karakteristika su različitih vrsta tumora kod ljudi. U animalnim eksperimentalnim modelima, supstance (lekovi) koje heliraju bakar ispoljavaju anti-tumorski efekat. Helatori bakra su i organosumporna jedinjenja, izolovana iz belog luka. U ovoj studiji analizirali smo potencijalnu anti-tumorsku aktivnost smeše petnaest različitih n-propil polisulfida na nekoliko mišjih ćelijskih linija tumora: karcinom kolona (CT26), karcinom dojke (4T1) i melanom (B16F10). Aktivnost ove smeše na tumorskim linijama, uporedili smo sa antiproliferativnim efektom na mezenhimalne matične ćelije miša (engl. murine mesenchymal stem cells, mMSC). Efekat smeše n-propil polisulfida (100%) na vijabilnost ćelija ispitali smo MTT testom. Apoptozu ćelija smo analizirali koristeći Annexin V-FITC/PI test.

Rezultati MTT testa ukazuju da standardizovana smeša n-propil polisulfida ima jak citotoksični, dozno-zavisni, efekat na sve tri testirane ćelijske linije tumora (CT26, 4T1, B16F10). Smeša n-propil polisulfida ispoljava izraženiji citotoksični efekat na CT26 i B16F10 linije u odnosu na cisplatinu. Citotoksični efekat ove smeše na mMSC je značajno slabiji poredeći sa efektom cisplatine, što ukazuje na selektivnije dejstvo. Analiza CT26 i 4T1 ćelija protočnom citometrijom pokazala je da apoptoza nije glavni oblik smrti ćelija, koju uzrokuje smeša n-propil polisulfida. Smeša n-propil polisulfida ispoljava jaču citotoksičnu aktivnost na ćelijskim linijama mišjeg karcinoma kolona i melanoma i slabiju aktivnost na mMSC u poređenju sa efektom cisplatine.

Ključne reči: *n-* propil polisulfidi, ćelijske linije tumora, citotoksičnost, *in vitro*

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INTRODUCTION

Copper is an essential trace element for most living organisms. It serves as a structural and catalytic cofactor for enzymes that play crucial roles in various biochemical processes (1). Copper is a cofactor in redox enzymatic reactions required for the normal growth and development of organisms. Since copper has roles in many enzymatic reactions (1, 2), its requirement in different aspects of cancer progression, such as immortalization, angiogenesis, and metastasis, is clear (3, 4). Copper can induce angiogenesis by directly binding to pro-angiogenic factors (such as VEGF) and by stimulating the migration of endothelial cells (5). It also triggers proliferative and metabolic enzymes that increase the ability of cancer cells to metastasize (3). Reduced or elevated levels of copper have been connected to various pathological conditions in humans. Many tumours tend to accumulate high concentrations of copper; the concentrations of copper in serum and tumour tissue are significantly higher in cancer patients than in healthy subjects, and the copper concentration correlates with cancer progression and therapeutic response (6, 7). High levels of copper have been found in a wide spectrum of human cancers, including breast, prostate, colon, lung, and brain cancer (6, 8-10). Furthermore, in comparison with normal tissues, tumours have a greater demand for copper and are more sensitive to reductions in systemic copper levels (11).

Antiangiogenic activities of copper-chelating drugs have been reported in animal models (11) (12). Another study reported impaired oxidative phosphorylation and tumour growth after pharmacological suppression of systemic copper, without concomitant effects on tumour angiogenesis (13). It has also been shown that copper chelation inhibits epithelial-to-mesenchymal transition and decreases the expression of vimentin and fibronectin, thus inhibiting the migratory and invasive properties of cells (14). Organosulfur compounds (diallyl sulfide, DAS; diallyl disulfide, DADS; S-ethylcysteine, SEC; N-acetylcysteine, NAC) derived from garlic exhibit marked copper-chelating activity (15).

Here, we analysed the potential antitumour activity of the mixture of fifteen n-propyl polysulfides against several murine tumour cell lines, including the colon carcinoma (CT26), mammary carcinoma (4T1) and melanoma cell lines (B16F10), and compared these effects with its antiproliferative activity against highly proliferative murine mesenchymal stem cells (mMSCs).

We show that the n-propyl polysulfide mixture exerts highly cytotoxic activity against murine colon carcinoma and melanoma cell lines, while its antiproliferative activity against mMSCs is significantly weaker than that of cisplatin.

MATERIALS AND METHODS

Preparation of drug solutions

The oil mixture of n-propyl polysulfides (100%) and 10 mM cisplatin water solution were diluted in cell culture medium immediately before use. MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, was dissolved (5 mg/mL) in phosphate-buffered saline with a pH of 7.2, and the solution was filtered through a 0.22 mm Millipore filter before use. All reagents were purchased from Sigma Chemicals.

Cell culture

CT26, 4T1, and B16F10 cells were purchased from American Type Culture Collection (ATCC, Manassas, USA). Mouse bone marrow-derived MSCs were purchased from Gibco. Cells were maintained in DMEM (Sigma Aldrich, Munich, Germany) supplemented with 10% foetal bovine serum (FBS, Sigma Aldrich, Munich, Germany), penicillin (100 IU/mL), and streptomycin (100 μg/mL) in a humidified atmosphere of 95% air and 5% CO2 at 37°C. Subconfluent monolayers in the log growth phase were harvested by a brief treatment with 0.25% trypsin and 0.02% EDTA in phosphate-buffered saline (PBS, Sigma Aldrich, Munich, Germany), and the cells were washed three times in serum-free PBS. The number of viable cells was determined by trypan blue exclusion.

Cytotoxicity assay

The effects of the tested compounds on cell viability were determined using the MTT colorimetric technique. All examined cells were diluted with growth medium to $5x10^4$ cells/ml, and aliquots ($5x10^3$ cells/100 ml) were placed in individual wells in 96-well plates. The next day, the medium was exchanged with 100 μL of test compound, which had been serially diluted 2-fold in growth medium to concentrations ranging from 1 mg/ml to 0.008 mg/ml. Both the n-propyl polysulfide mixture and cisplatin were tested in triplicate. Cells were incubated at 37°C and 5% CO_2 for 24 h. After incubation, the supernatant was removed, and 15% MTT solution (5 mg/mL in PBS, 10 μL) in DMEM without FBS was added to each well. After an additional 4 h of incubation at 37°C in 5% CO_2, the medium with MTT was removed, and DMSO (150 μL) with glycine buffer (20 μL) was added to dissolve the crystals. The optical density of each well was determined at 595 nm using a Zenyth 3100 Multimode microplate detector. The percentage cytotoxicity was calculated using the formula: % cytotoxicity = 100-((E-B)/(S-B)*100), where B is the background optical density of medium alone, S is the total viability/spontaneous death of untreated target cells, and E is the experimental well. Each of the tested complexes was evaluated for cytotoxicity in three separate experiments.

Apoptosis assay

For the detection of apoptosis, CT26 and 4T1 cells were plated in T25 culture flasks and allowed to grow overnight. After the cells reached subconfluency, the medium was replaced with the test substances (0.01 mg/ml). Treated cells were placed at 37°C in a 5% CO_2 incubator for

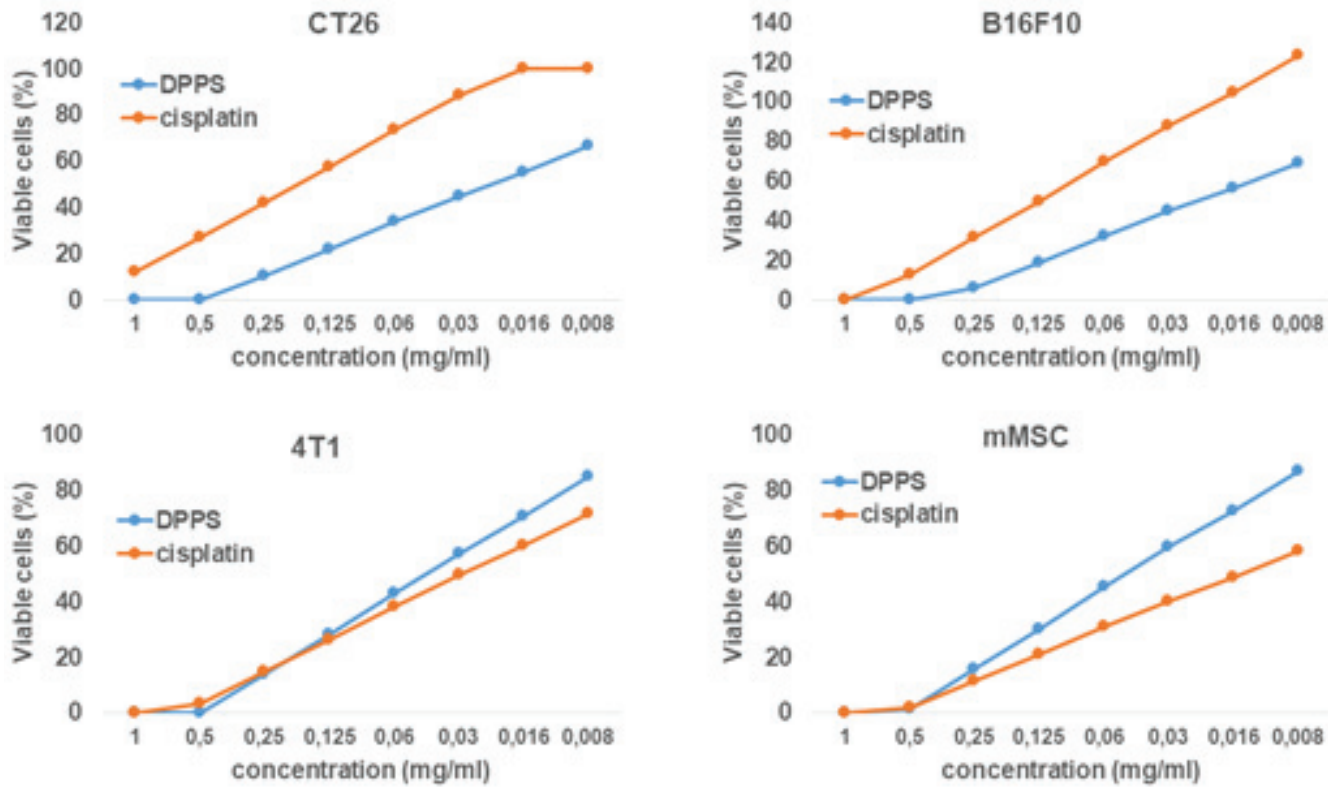

Figure 1. Graphs presenting the survival of CT26, B16F10, and 4T1 cells, as well as MSCs, after 24 h of growth in the presence of DPPS and cisplatin. Data are presented as the mean of three independent experiments.

24 h. The cultured cells were washed twice with PBS and resuspended in 1X binding buffer (10X binding buffer: 0.1 M Hepes/NaOH (pH 7.4), 1.4 M NaCl, 25 mM CaCl2) at a concentration $1x10^6$/mL. Annexin FITC and propidium iodide (PI) were added to 100 mL of cell suspension, which was then incubated for 15 min at room temperature (25°C) in the dark. After incubation, 400 mL of 1X binding buffer was added to each tube, and the stained cells were analysed within 1 h using a FACS Calibur (BD, San Jose, USA) and Flow Jo software (Tri Star). Since Annexin V FITC staining precedes the loss of membrane integrity that accompanies the later stage identified by PI, an Annexin FITC-positive, PI-negative staining pattern indicates early apoptosis, while viable cells are Annexin V FITC negative and PI negative. Cells that are in late apoptosis or are already dead are positive for both Annexin V FITC and PI.

RESULTS

Anticancer activity of copper complexes

The results of the MTT assays indicate that the standardized mixture of n-propyl polysulfides has a strong, dose-dependent cytotoxic effect on all three of the tested carcinoma cell lines (CT26, 4T1, B16F10) (Figure 1). The n-propyl polysulfide mixture had almost the same cytotoxic activity as cisplatin against the 4T1 murine mammary carcinoma cell line. Interestingly, the cytotoxic effects of the n-propyl polysulfide mixture against the murine colon cancer and melanoma cell lines (CT26 and B16F10, respectively) were much stronger than those of cisplatin (Figure 1). Significant cytotoxic effects of the n-propyl polysulfide mixture against CT26 and B16 F10 cells were detected at even the lowest tested concentration (0.008 mg/ml). Importantly, compared with cisplatin, the tested mixture had significantly lower cytotoxicity against mMSCs, which are non-cancerous and highly proliferative cells (Figure 1). The similar effects of the tested mixture and cisplatin on 4T1 cells and the significantly greater effects of the mixture against CT26 and B16F10 cells, as well as the lower cytotoxicity against mMSCs, were confirmed by analysis of the IC50 values (Table 1).

To determine the possible mode of death of the cells treated with the n-propyl polysulfide mixture, flow cytometry analysis of CT26 and 4T1 cells stained with Annexin V and PI after exposure to the test mixture (at a

Table 1. IC50 values (in mM) for 4T1, CT26, and B16F10 cells, as well as mMSCs, after exposure to DPPS and cisplatin for 24 h as determined by MTT assays. The data are presented as the mean ± SD (standard deviation) from three experiments.

	IC50 (mg/ml)			
Compound	4T1	CT26	B16F10	mMSC
DPPS	0.043±0.006	0.024±0.018	0.023±0.002	0.059±0.002
Cisplatin	0.029±0.004	0.160±0.092	0.123±0.082	0.028±0.006

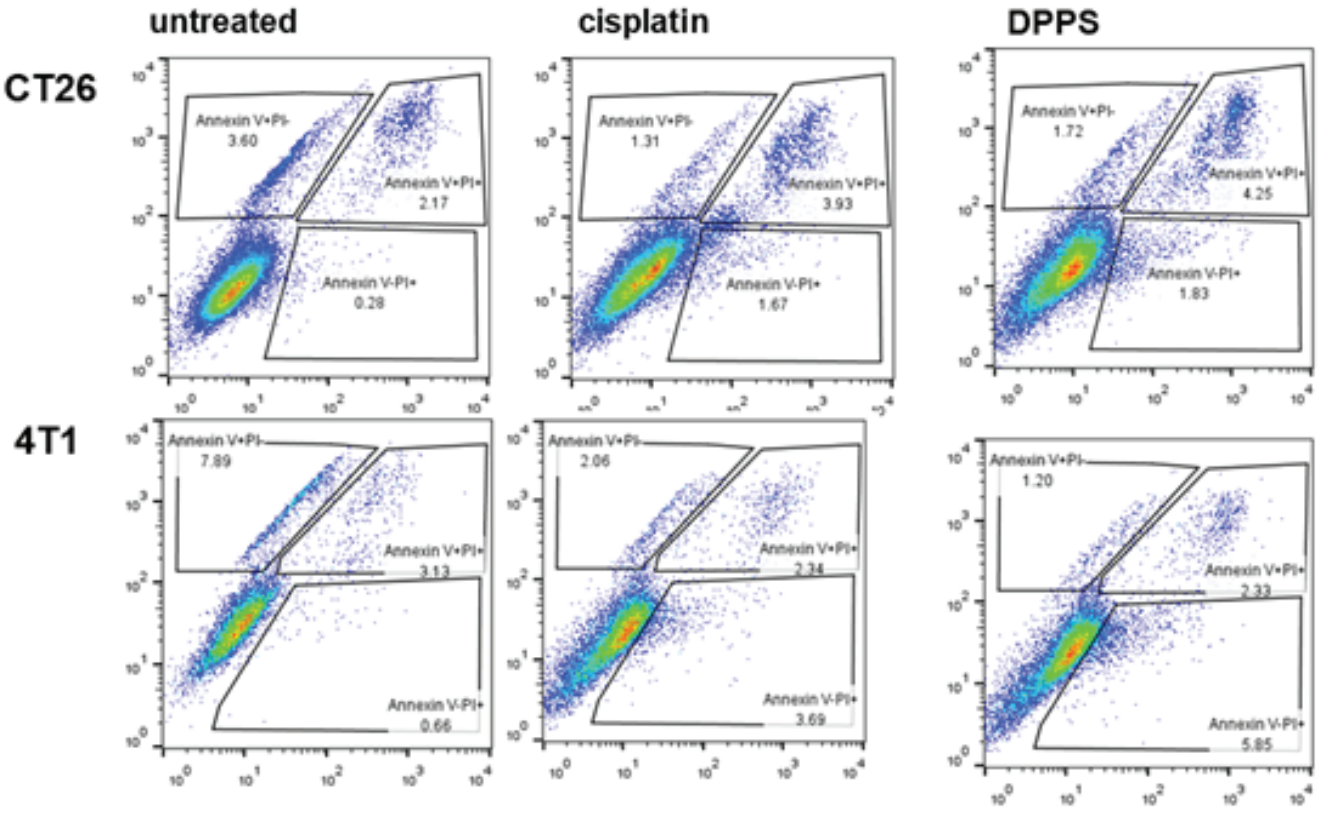

Figure 2. Representative flow plots showing the percentages of early- and late apoptotic and viable CT26 and 4T1 cells after 24 h of treatment with DPPS (0.01 mg/ml).

concentration of 0.01 mg/ml) for 24 h was performed. In agreement with the results of the MTT assays, a stronger cytotoxic effect of the n-propyl polysulfide mixture than of cisplatin, as determined by Annexin V PI staining, was observed in CT26 cells, while better cytotoxicity in 4T1 cells was shown for cisplatin (Figure 2). Furthermore, analysis of the percentages of stained CT26 and 4T1 cells after treatment with the test mixture and cisplatin indicated that apoptosis is not the dominant mechanism of cell death induced by the n-propyl polysulfide mixture. The percentage of PI+ cells (Annexin V+ PI+ and Annexin V- Pi+) was higher in the populations of both CT26 and 4T1 cells treated with the test mixture than in those treated with cisplatin (Figure 2).

DISCUSSION

This study shows for the first time a greater cytotoxic effect of an n-propyl polysulfide mixture compared with a standard chemotherapeutic agent (cisplatin) in murine colon carcinoma (CT26), melanoma (B16F10), and mammary carcinoma (4T1) cell lines.

The biological activities of various organosulfur compounds are partially the result of their chelating activity (16). Metal cations, including Cu^{+} and Cu^{2+}, may be involved in the formation of chelating complexes (17). Four organosulfur compounds (diallyl sulfide (DAS), diallyl disulfide (DADS), S-ethylcysteine, and N-acetylcysteine) show marked copper-chelating capability. The method used to determine the chelating effects of DAS and DADS on copper is based on restoring the activity of xanthine oxidase, which is inhibited in the presence of copper (15).

Our finding that a mixture of n-propyl polysulfides, which may have chelating activities, reduces the viability of tumour cells agrees with previous findings. The copper chelating agent trientine supresses tumour development (18, 19). Curcumin, a polyphenol that belongs to the ginger family, chelates copper with high affinity and inhibits cell proliferation, invasion, metastasis, and angiogenesis (20). Administration of curcumin in animal tumour models resulted in the suppression of tumour growth associated with reduced copper concentrations in the serum of the treated groups (20). Furthermore, organic copper-binding compounds, such as clioquinol and pyrrolidine dithiocarbamate, bind copper and form new complexes that function as cancer-specific proteasome inhibitors and apoptosis inducers in human breast cancer cells (21).

More than 95% of the total copper in human plasma is associated with ceruloplasmin, while the remaining plasma copper is associated with albumin and transcuprein (22). It has been shown that the concentration of ceruloplasmin and transcuprein in tumour tissue is increased. Furthermore, tumour tissue can take up copper from the ceruloplasmin fraction of the plasma (22). Copper enters the cell through various transporter molecules in the plasma membrane, known as copper

transporter protein 1 CTR1 (23), and binds to different factors, such as metallothionein, cytochrome oxidase, superoxide dismutase and the cytosolic copper chaperones Cox17 and Atox1 (24). Inhibitors of the copper trafficking proteins Atox1 and CCS significantly reduce the proliferation of cancer cells with no effects on normal cells. Blocking copper trafficking induces cellular oxidative stress and reduces the cellular levels of ATP (25).

Our findings indicate that the reduction in the viability of murine MSCs (noncancerous and highly proliferative cells) in response to the n-propyl polysulfide mixture was smaller than that in response to cisplatin (Figure 1). This finding agrees with previous reports that copper chelating agents selectively kill human colon cancer cells without affecting the viability of noncancerous colon or intestinal cells (26). The selective cytotoxic activity of the n-propyl polysulfide mixture towards tumour cells may be the consequence of more pronounced chelating activity in tumour cells, as they contain higher amounts of copper. The higher percentage of necrotic tumour cells exposed to the n-propyl polysulfide mixture is in line with a previous finding that the organosulfur compound diallyl disulfide induces mainly necrotic death in *Candida albicans* (27).

Based on our findings, further studies should be done to explore the mechanisms of the antitumour action of this n-propyl polysulfide mixture, to elucidate the basis of the selective activity towards tumour cells and to evaluate for *in vivo* effects in animal tumour models.

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THE EFFECTS OF DPP4 INHIBITORS ON LIPID STATUS AND BLOOD PRESSURE IN RATS WITH DIABETES MELLITUS TYPE 2

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EFEKTI INHIBITORA DPP4 NA LIPIDNI STATUS I KRVNI PRITISAK PACOVA OBOLELIH OD DIABETES MELLITUS-A TIP 2

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ABSTRACT

The aim of the present study was to examine, evaluate and compare the effects of administered dipeptidyl peptidase -4 (DPP4) inhibitors saxagliptin and sitagliptin on lipid status parameters and blood pressure in rats with streptozotocine induced diabetes mellitus type 2. Forty-eight Wistar albino rats were divided randomly into 4 groups: 1. group I: control healthy group; 2. group II: rats with diabetes mellitus type 2; 3. group III: rats with diabetes mellitus type 2+ treated with 0.6 mg/kg of sitagliptin; 4. group IV: rats with diabetes mellitus type 2 treated with 0.45 mg/kg of saxagliptin. The rats from experimental groups were fed with a high-fat diet for 4 weeks and after 6–8 h of starvation received one dose of streptozotocin (STZ) intraperitoneally (25 mg/kg body weight) to induce type 2 diabetes mellitus (T2DM). Animals with fasting glucose above 7 mmol/L and insulin over 6 mmol/L were included in the study as rats with T2DM. Upon completion of the experiments, the blood was collected from the anesthetized animals and serum triglyceride (TG), total cholesterol (TCH), high density lipoprotein (HDL), and low density lipoprotein (LDL) were measured using spectrophotometry and commercial kits. At the beginning of the study and the day before sacrificing animals, the blood pressure and heart rate were measured by a tail-cuff noninvasive method. DPP4 inhibitors, as glucagon-like peptide-1 (GLP-1) agonists, were associated with modest reductions in DBP, LDL-C, TCH, and TGL and significant improvement in HDL, SBP and HR.

Keywords: *DPP4 inhibitors, lipid status, blood pressure, diabetes mellitus type 2, rats.*

SAŽETAK

Cilj ove studije je bio da ispita, proceni i uporedi efekte administriranih dipeptidil peptidaza-4 (DPP4) inhibitora saksagliptina i sitagliptina na parametre lipidnog statusa i krvnog pritiska kod pacova sa dijabetes mellitusom tipa 2 izazvanim streptozotocinom. Četrdeset osam Wistar albino pacova je svrstano u 4 grupe: 1. grupa I: kontrolna grupa zdravih pacova; 2. grupa II: pacovi sa diabetes mellitus tipom 2; 3. grupa III: pacovi sa diabetes mellitus tipom 2 tretirani sa 0,6 mg/kg sitagliptina; 4. grupa IV: pacovi sa diabetes mellitus tipom 2 tretirani sa 0.45 mg/kg saksagliptina. Pacovi iz eksperimentalnih grupa su hranjeni hranom sa visokim sadržajem masti 4 nedelje i nakon 6-8 sati gladovanja primili su jednu dozu streptozotocina (STZ) intraperitonealno (25 mg/kg telesne težine) radi izazivanja dijabetes melitusa tipa 2 (T2DM). Životinje sa glukozom natašte iznad 7 mmol/L i insulinom preko 6 mmol/L uključene su u studiju kao pacovi sa T2DM. Po završetku eksperimentalnog perioda, krv je sakupljena od anesteziranih životinja i serumski trigliceridi (TG), ukupni holesterol (TCH), lipoproteini visoke gustine (HDL) i lipoproteini niske gustine (LDL) su određivani spektrofotometrijski i korišćenjem komercijalnih kitova. Na početku studije i dan pre žrtvovanja životinja, krvni pritisak i srčana frekvenca su mereni neinvazivnom metodom repne pletizmografije. Inhibitori DPP4, kao agonisti glucagonu-sličnog peptida-1 (GLP-1), bili su povezani sa blagim redukcijama DBP, LDL-C, TCH i TGL i pozitivno su uticali na HDL, SBP i HR.

Ključne reči: *DPP4 inhibitori, lipidni status, krvni pritisak, diabetes melitus tip 2, pacovi.*

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INTRODUCTION

Cardiovascular diseases, such as coronary heart disease, cerebrovascular disease, and peripheral artery disease have remained the leading cause of morbidity and mortality worldwide. Across Europe and the USA, myocardial infarction represents direct cause of death in 40–50% of patients with diabetes, and the number of patients with diabetes that die as a direct result of ischemic heart disease is reported to be increasing (1). It is well known and explained that existing atherosclerotic vascular lesions and diabetes mellitus are with no doubt the strongest risk factors for further life-threatening vascular diseases. Hyperlipidemia and diabetes mellitus increase cardiovascular events via increasing atherosclerosis. What is more, collateral artery growth (arteriogenesis), which function is to compensate the loss of an artery due to atherosclerosis, is compromised in diabetes mellitus, a disease which is almost always associated with dyslipidemia (2). According to novel investigation, there is a high need for effective drugs that show positive effects in patients with diabetes, even with insulin resistance, but also express cardioprotective effects and therefore postpone or decrease the risk of cardiovascular complications in diabetic patients. Particularly, these drugs should have the capacity to promote arteriogenesis, which is a tissue-, and even life-saving, process. Dipeptidyl-peptidase 4 (DPP4/CD26) inhibitors might present such drugs.

Dipeptidyl peptidase-4 (DPP4) is a widely expressed protease that cleaves the N-terminal of peptides containing a penultimate alanine or proline, such as the incretins, glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic polypeptide (or gastric inhibitory polypeptide GIP) (1). Drugs that inhibit DPP4 exhibit a decrease in degradation of these hormones, which improves glycemic control through increased glucose-mediated insulin secretion, decreased glucagon release and delayed gastric emptying. These hypoglycemic drugs of newer generation have shown to be effective, well tolerated and therefore, increasingly prescribed. In addition, recent research put the focus on potential cardioprotective and antihyperlipidemic effects of these drugs, beyond glycemic control, thus, making them an interesting and attractive therapeutic strategy, alone or in combination with other hypoglycemic drugs (2, 3). Not all representatives of this group of drugs express the same pharmacodynamics. For example, *in vitro* study has shown stronger inhibition of DPP4 and slower rate of dissociation from its active site of saxagliptine in comparison with sitagliptin and vildagliptin (4).

Besides outstanding glucose-lowering effects, DPP4 inhibitors have also shown other beneficial effects that are not of metabolic nature. This refers to anti-inflammatory effect and cardioprotective effect especially via blood pressure (BP) regulation. Recent findings suggest that sitagliptin could decrease systolic blood pressure (SBP), independently of glucose-lowering effect (5). Additionally, it was shown that sitagliptin exerts BP reduction effect both in diabetic and non-diabetic patients (6-9). Data provided from other studies have also confirmed hypotensive effects of DPP-4 inhibitors in animal models and supported this phenomenon (10-13). On the other hand, there is a growing number of evidence claiming the opposite - that no changes in BP were measured when compared to control group (14-17). Furthermore, some research groups have come to conclusion that DPP-4 inhibitors might even lead to the increase in BP when combined with ACE (angiotensin-converting enzyme) inhibitors (18).

Taken into consideration all mentioned above, the aim of the present study was to examine, evaluate and compare the effects of administered DPP4 inhibitors saxagliptin and sitagliptin on lipid status parameters and blood pressure in rats with streptozotocine induced diabetes mellitus type 2.

MATERIALS AND METHODS

Ethical approval

This research was carried out in the laboratory for cardiovascular physiology of the Faculty of Medical Sciences, University of Kragujevac, Serbia. The protocol of the current study was approved by the Ethical Committee for the experimental animals' well-being of the Faculty of Medical Sciences, University of Kragujevac, Serbia. All experiments were performed according to EU Directive for welfare of laboratory animals (86/609/EEC) and principles of Good Laboratory Practice (GLP).

Animals and design of the study

Forty-eight Wistar albino rats (males, six weeks old, body weight 200 ± 20 g, at the beginning of experiments) were included in the study. They were housed in a room with a 12/12-hour light/dark cycle, an ambient temperature of 22 ± 2°C. The rats had free access to food and water - *ad libitum*. Rats were divided randomly into 4 groups (12 animals per group):

1. Group I: Control healthy group (n=12);
2. Group II: Rats with diabetes mellitus type 2 (n=12);
3. Group III: Rats with diabetes mellitus type 2 treated with 0.6 mg/kg of sitagliptin (n=12);
4. Group IV: Rats with diabetes mellitus type 2 treated with 0.45 mg/kg of saxagliptin (n=12).

Except for the control healthy group, the rats were fed with a high-fat diet for 4 weeks and after 6–8 h of starvation received one dose of STZ intraperitoneally to induce T2DM. STZ was prepared *ex tempore* by dissolving in citrate buffer and, depending on the body weight, it was administered in a dose of 25 mg/kg (18). Three days after STZ injection and 12 h after starvation fasting glucose and insulin level as well as blood pressure were measured. Animals with fasting glucose level above 7 mmol/L and insulin level over 6 mmol/L were included in the study and were used in the study as rats with T2DM. The T2DM rats were then randomly divided into three groups: T2DM rats (n = 12), T2DM rats treated with 0.6 mg/kg body weight of sitagliptin (n=12) and T2DM treated with 0.45 mg/kg body weight of saxagliptin (n=12). Sitagliptin and saxagliptin were applied intraperitoneally once a day for three weeks.

Drugs

Streptozotocin (MW= 265,221), sitagliptin (MW= 523,32) and saxagliptin (MW= 315,41) were purchased from Sigma-Aldrich Chemie GmbH Eschen str. 5, 82024 Taufkirchen, Germany.

Evaluation of blood pressure and heart rate

At the beginning of the study and the day before sacrificing animals, the blood pressure and heart rate were measured by a tail-cuff noninvasive method BP system (Rat Tail Cuff Method Blood Pressure Systems (MRBP-R), IITC Life Science Inc., Los Angeles, CA, USA) (19). At least ten determinations were made in each session, with mean values taken.

Lipid profile

Upon completion of the experiments, the blood was collected from the anesthetized animals into blood collection tubes after an overnight fast (12 h). After standing for 30 min, the serum was prepared by centrifugation of blood at 1000 × g for 10 min at 4°C and stored at −80°C until analysis.

Serum triglyceride (TG), total cholesterol (TCH), high density lipoprotein (HDL), low density lipoprotein (LDL) were measured in the serum using spectrophotometry and commercial kits from Siemens Healthcare Diagnostics (Frimley, Camberley, Surrey, UK) and according to the manufacturer's instructions on the programmed analisator (Dimension Xpand, Siemens, IL, USA).

Statistical analysis

We used traditional parameters of descriptive statistics: average value±standard deviation (SD), and minimal and maximal values. Normality of the parameter distribution was evaluated with the Shapiro–Wilk and Kolmogorov-Smirnov tests. Additionally, data was analyzed using a one-way analysis of variance (ANOVA) and the post hoc Bonferroni test for multiple comparisons. The statistical significance was based on $p<0.05$. Complete statistical evaluation was performed with SPSS Statistics 22 (SPSS, Chicago, IL).

RESULTS

Lipid profile

Level of triglycerides (TGL), total cholesterol (TCH) and low-density lipoprotein (LDL) were significantly increased in experimental groups (rats with T2DM) compared to the control group, while the level of high-density lipoprotein (HDL) was decreased. Sitagliptin and saxagliptin have significantly decreased the level of TGL, TCH, and LDL compared to the T2DM group. There was no significant change in the level of HDL (Figure 1).

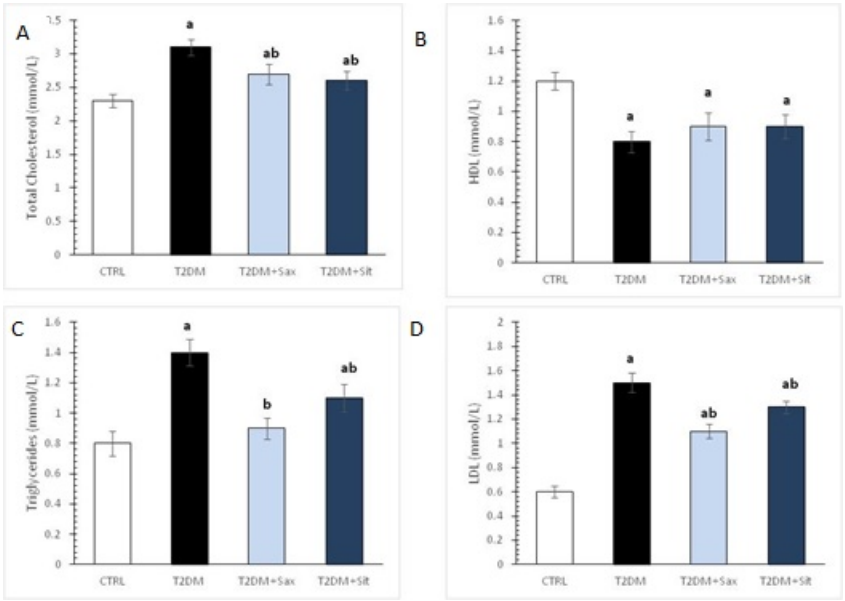

Figure 1: Changes in lipid profile in healthy and rats with T2DM:
(**A**) Total Cholesterol (TCL, mmol/l); (**B**) high-density lipoprotein (HDL, mmol/l);
(**C**) Triglycerides (TGL, mmol/l); (**D**) Low-density lipoprotein (LDL, mmol/l).

Values are expressed as mean ± standard deviation (SD) for 12 animals, for each group. Values p < 0.05 were considered statistically significant. a - statistical significance in relation to control (CTRL) group; b - statistical significance in relation to T2DM group.

Blood pressure and heart rate

Systolic and diastolic blood pressures were significantly increased in the T2DM group compared to the control group. Diastolic pressure was significantly decreased in groups with sitagliptin and saxagliptin compared to the T2DM group. There was no significant change in the heart rate (Figure 2).

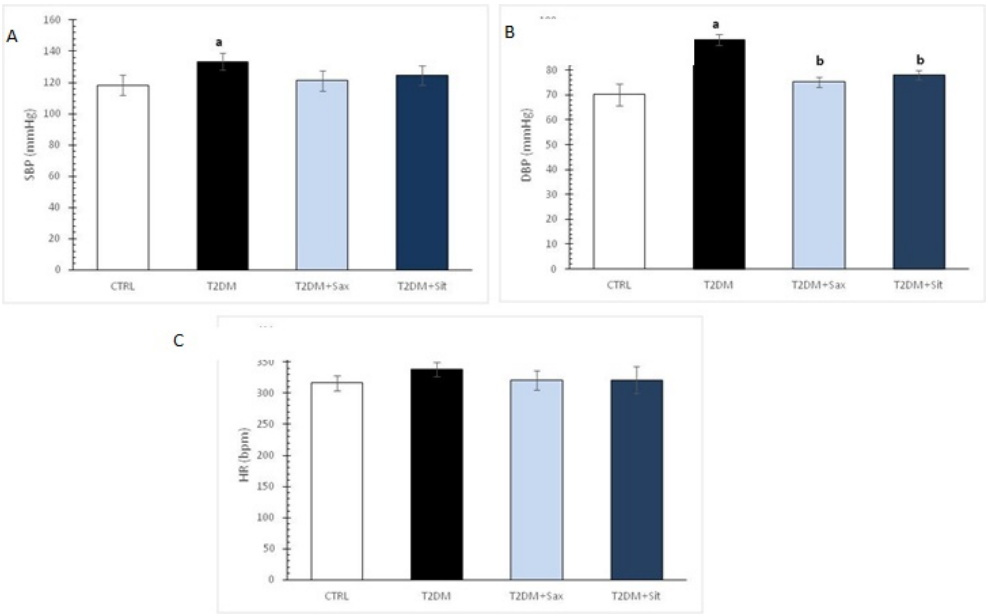

Figure 2: Changes in blood pressure and heart rate in healthy and rats with T2DM: (**A**) systolic blood pressure (SBP, mmHg); (**B**) diastolic blood pressure (DBP, mmHg); (**C**) heart rate (HR, bpm). Values are expressed as mean ± standard deviation for 12 animals, for each group.

Values $p < 0.05$ were considered statistically significant. a - statistical significance in relation to control (CTRL) group; b - statistical significance in relation to T2DM group.

DISCUSSION

The prevalence of hypertension in individuals with T2DM is estimated to be twice higher compared to non-diabetic individuals (20, 21). Possible reasons for this include diabetes related metabolic disorders, such as chronic hyperglycemia and hyperlipidemia along with low grade inflammation and oxidative stress. Blood pressure responses to DPP-4 inhibitor therapy are shown to be either neutral or modestly reduced (22-25). In addition, by measuring the blood pressure, we have confirmed the existence of a model diabetes mellitus type 2, which is quite obvious by noticing a statistically significant difference in the systolic and diastolic pressure in the T2DM group compared to the control. Using this parameter, we have managed to show that the drugs from the group of DPP-4 inhibitors lower diastolic blood pressure in comparison with T2DM group (Figure 2).

Recent studies highlighted that sitagliptin might induce changes in blood pressure in different ways, but this depends primarily on the presence of coronary disease, antihypertensive co-therapy (mostly ACE inhibitors and AT blockers), and thus, in the case of healthy animals (without coronary disease) this medicine decreased diastolic and systolic pressure, but not in the case of spontaneously hypertensive rats (17, 26). On the other hand, when dealing specifically with patients with T2DM, the study indicates a statistically significant decrease in systolic pressure after intravenous treatment with DPP-4 inhibitors (vildagliptin and sitagliptin). Pathophysiological mechanism responsible for BP reduction of DPP4 inhibitors are well explained by many authors. Firstly, DPP4 inhibition surely increases incretin level (GLP-1), which is associated with cardiac friendly lipid status in recent meta-analyses (27). There are DPP-4 non-incretin substrates that are involved in inflammation, immunity and cardiovascular system and its expression on endothelial surface suggests that its inhibition might reduce the vascular tone. Furthermore, animal studies have shown NO- dependent or independent arterial relaxation induced by GLP-1 (28). These vasodilator properties might also be mediated through GLP-1 metabolites and independently of the GLP-1 receptor, acting instead through an NO/cGMP-dependent mechanism (29). It is explained in the literature that

immune systems, both innate and adaptive might contribute to low-grade inflammation, which is connected with the development and progression of hypertension. DPP4 (CD26), besides being included in glucose and lipid metabolism, also participates in non-specific inflammation by regulating the activity and chemotaxis of macrophages, monocytes, NK and T cells. These cells secrete inflammatory mediators that can disturb the function of vascular endothelium (reactive oxygen species, cytokines, chemokines, adhesion molecules) in the form of increased proliferation of smooth muscle cells and vascular remodelling. Recent studies provided evidence about DPP4 inhibitors treatment inhibiting production of cytokines controlling the proliferation of T lymphocites and therefore express hypotensive effect via reduction of inflammation (28, 30, 31). Other research groups claimed the opposite since no effect on BP was noticed with DPP4 inhibitors treatment. There is also smaller amount of evidence regarding possible increase in BP by using ACE inhibitors and DPP4 inhibitors co-therapy (14, 15, 17). As for heart rate, values did not vary between groups and no statistically significant differences were noticed. DPP-4 inhibitors have not shown any effect on this parameter in our research (Figure 2). These results are consistent with various studies, where the DPP4 inhibitors therapy did not ameliorate cardiovascular outcomes in T2DM patients.

Lipid profile is a strong determinant of cardiovascular risk in T2DM. Current guidelines recommend an accurate control of hypercholesterolemia in order to reduce macrovascular complications. The fact that type 2 diabetic patients are more likely to be dyslipidemic than the general population is well known for decades. Lipid abnormalities associated with T2DM refer to high serum triglyceride levels, a high proportion of small dense low-density lipoprotein (LDL) particles, higher triglyceride-enriched, verylow-density lipoprotein (VLDL) particles, and lower protective high-density lipoprotein cholesterol (HDL) levels, together with glycation of apolipoproteins and increased LDL oxidation, all of which contribute to genesis of foam cell in atherosclerosis (32, 33). According to Derosa et al, the addition of sitagliptin to existing hypoglycemic therapy might lead to a better and durable (over 7 years of therapy) improvement of lipid profile. This beneficial effect is supposed to be due to delayed gastric emptying (34). Our study results elucidated that sitagliptin and saxagliptin improve lipid status in T2DM rats, via significant reduction of TCH, LDL and TGL (Figure 1). In line with these results are also the results of other research groups (35, 36). Possible explanation for beneficial lipid effects of DPP4 inhibitors may be connected to its stimulating effect on the activated proteine-kinase pathway, which leads to increase in glucose and lipid catabolism (37). On the other hand, no improvement in HDL parameters was achieved in our study, which is in correlation with the findings of Saad et al. (36). What is more, GIP can purify chylomicron in the circulation by stimulating the lipoproteine lipase from adipose tissue, while GLP-1 could decrease the postprandial secretion of triacylglycerol after meal (38-40). Since DPP inhibitors potentiate the GLP-1 and GIP function, it is clear why it improves lipid profile. Clinical trials have also confirmed beneficial effects of sitagliptin in diabetic patients, referring to amelioration of lipoprotein and lipid profile, which is explained by decrease in atherogenic remnant lipoproteins (RemL-C) (41).

CONCLUSION

DPP4 inhibitors, as GLP-1 agonists, were associated with modest reductions in DBP, LDL-C, TCH, and TGL and significant improvement in HDL, SBP and HR. These drugs might be helpful in achieving homeostasis in lipid status and blood pressure in T2DM patients. Hence, further evidence is needed to determine if improvements in lipid profile and BP might translate into reductions and amelioration of cardiovascular outcomes.

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EFFECT OF PATELLAR RESURFACING ON CLINICAL OUTCOMES, RANGE OF KNEE MOTION AND ANTERIOR KNEE PAIN IN PATIENTS WITH TOTAL KNEE ARTHROPLASTY

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EFEKTI PATERLARNE REPOZICIJE NA KLINIČKE ISHODE, OPSEG POKRETA KOLENA I BOLA U PREDNJEM DELU KOLENA KOD PACIJENATA SA TOTALNOM ARTOPLASTIKOM KOLENA

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ABSTRACT

Objectives: In this study, we retrospectively compare the clinical results, range of knee motion and anterior knee pain in patients on whom we performed knee arthroplasty with and without patellar resurfacing.

Thirty-eight patients were evaluated in the study. Knee Society scores, knee range of motion and anterior knee pain before and 12 months after surgery were detected. Patients were divided into two groups: resurfaced patellas and nonresurfaced patellas. There were 18 patients in the resurfaced group and 20 patients in the nonresurfaced group.

Mean Knee Society score was 40.72±13.09 in the resurfaced group and 38.55±5.88 in the nonresurfaced group before surgery. Mean Knee Society score was 80.38±7.78 in the resurfaced group and 80.10±3.22 in the nonresurfaced group in the last control. Mean knee range of motion was 92.83±12.12 degrees in the resurfaced group and 91.05±10.10 degrees in the nonresurfaced group before surgery. Mean range of motion was 106.22±9.13 degrees in the resurfaced group and 97.25±8.50 degrees in the nonresurfaced group after surgery. There were twelve patients with anterior pain before surgery in the resurfaced group and 13 patients with anterior knee pain before surgery in the nonresurfaced group. After surgery, there was one patient with anterior pain in the resurfaced group and 9 patients with anterior knee pain in the nonresurfaced group.

Anterior knee pain ratio was smaller in the resurfaced group than in the nonresurfaced group, and there was a significant difference in range of knee motion as a result of our study. We offered to resurface the patella.

Keywords: *patella, motion of knee, surgical intervention*

SAŽETAK

U ovoj retrospektivnoj studiji, uporedili smo kliničke karakteristike, stepen pokretljivosti zgloba kolena i prisustvo bola u prednjem delu kolena kod pacijenata kod kojih je uradjena artoplastika kolena sa ili bez repozicije patele.

Trideset osam pacijenata je uključeno u studiju. Knee Society skorovi, opseg pokreta u zglobu kolena i prisustvo bola su praćeni pre i 12 meseci posle hirurške intervencije. Pacijenti su bili podeljeni u dve grupe: grupa pacijenata sa repozicijom patele i bez nje. U grupi sa repozicijom bilo je 18, a u grupi bez repozicije 20 pacijenata.

Srednja vrednost Knee Society skora u grupi sa repozicijom patele bila je 40,72±13,09 dok je u drugoj grupi bio je 38,55±5,88 pre operacije. Posle operacije, u grupi ispitanika sa repozicijom patele isti skor iznosio je 80,38±7,78 dok je u drugoj grupi iznosio 80,10±3,22. Srednja vrednost obima pokreta u zglobu kolena bila je 92,83±12,12 stepena u grupi sa repozicijom kolena, dok u drugoj grupi je bila 91,05±10,10 pre operacije. Nakon hirurške intervencije, ovaj skor je u prvoj grupi iznosio 106,22±9,13 a u drugoj 97,25±8,50 stepena. Od ukupnog broja pacijenata, kod 12 pacijenata je bio prisutan bol prednjeg kolena u grupi sa repozicijom, dok u drugoj grupi je bilo 13 pacijenata pre operacije sa prisutnim bolom. Nakon operacije, 1 pacijent u prvog grupi i 9 pacijenata sa pristunim bolom je zabeleženo.

Odnos prisutnog prednjeg bola kolena je bio manji u grupi pacijenata sa repozicijom kolena u odnosu na drugu grupu, sa značajnom razlikom u obimu pokreta zgloba kolena. Preporučujemo repoziciju patele u zglobu kolena.

Ključne reči: *patela, pokretljivost zgloba kolena, operacija*

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INTRODUCTION

Knee arthroplasty is the last-stage treatment modality for gonarthrosis. New knee arthroplasty designs are obtaining better clinical results with a long survival rate period. In particular, patellar components had complications, which resulted in revisions in the early designs (1).

Anterior knee pain is an unwanted complaint that can be seen after knee arthroplasty. The rate of anterior knee pain is approximately 8% after primary knee arthroplasty (2). The exact aetiology of the anterior knee pain after primary knee arthroplasty is unknown. According to one opinion, high- compressive forces create pressure on the unresurfaced patellae, and this pressure may result in cartilage erosion on the patellar surface after knee joint replacement (3). Some study results offer patellar resurfacing to reduce anterior knee pain after knee arthroplasty (4, 5). Re-operation rates, because of anterior knee pain, are decreased by patellar resurfacing after total knee arthroplasty (6). However, patellar osteonecrosis, loosening of a patellar component, wear, extensor mechanism rupture and patellar maltracking are some of the complications that can be faced after patellar resurfacing (7).

Exact indications of patellar resurfacing include severe destruction of the patellofemoral joint, patellar maltracking, and inflammatory arthritis, such as rheumatoid arthritis and patellar and femoral component incongruency (8).

There are currently 3 approaches for patellar resurfacing. The first approach is always to resurface the patella; the second one is never to resurface the patella, and the third one is to resurface the patella, selectively, according to the quality of the articular cartilage and the congruence of the patellofemoral joint at the time of surgery (9).

We aimed to compare the clinical results, range of knee motions and anterior knee pain in patients on whom we performed knee arthroplasty with and without patellar resurfacing in this study.

MATERIALS AND METHODS

Approval for the study was granted by the University Ethics Committee with decision no 62 in 05.02.2018. Informed consent was obtained from all the patients. The inclusion criteria for this retrospective study were patients who had received posterior cruciate retaining knee arthroplasty and follow-up of more than 1 year. There were 52 patients, but 14 patients had a follow-up period of less than 1 year and were thus excluded from the study. A total of 38 patients in our clinic met these criteria. Demographic data were obtained from a total of 38 patients retrospectively. KSS scores, knee range of motion and anterior knee pain before and 12 months after surgery were detected from patient hospital files. Patients were divided into two groups: resurfaced patellas and nonresurfaced patellas. There were 18 patients in the resurfaced group and 20 patients in the nonresurfaced group.

SURGICAL TECHNIQUE AND POST-OPERATIVE PHYSICAL THERAPY

All knees were operated with midline incision and a medial parapatellar capsular incision to expose the knee joint. Dissection of the vastus medialis started from the quadriceps tendon with distal extension through the medial patellar retinaculum to the medial border of the patellar ligament. The synovium was incised in line with the capsular incision. The patella was everted or subluxated laterally. Cruciate retaining, fixed-bearing prostheses were implanted to all knees. Cement was used for the fixation of components. Patellar resurfacing was performed with the onlay technique; an equivalent thickness of bone was removed with the patellar component. In the nonresurfaced patellas, osteophytes were removed. An extramedullary alignment guide was used for the tibia, and an intramedullary alignment guide was used for the femur. The tibial cut was with an angle of 3°–5° for the tibial posterior slope. The distal femur was resected at 7° valgus. The patella was resurfaced using a standard cemented polyethylene patellar button.

The decisions on performing either patellar resurfacing or patellar retention were based on the surgeon's subjective judgement.

Postoperatively, mobilisation was performed on the first day after surgery. Static quadriceps exercises, straight leg raising exercise and range of motion exercise were started from day 1 after surgery. Below-knee thromboembolic disease stockings were used for both lower extremities, and chemical prophylaxis for deep vein thrombosis was in the form of tablet acetylsalicylic acid 100 mg once a day for 6 weeks. Perioperative intravenous cefazolin sodium was given to all patients for 24 hours.

The same physical therapy protocol applied to all patients after discharge from hospital.

Table 1: Demographic and clinical results of the groups.

	Number of patients	Follow up period (month)	Mean Pre operative knee sociaty score	Mean Post operative knee sociaty score	Mean pre operative knee range of motion (Degree)	Mean post operative knee range of motion (degree)	Number of patients with anterior knee pain pre operatively	Number of patients with anterior knee pain pre operatively
Total	38	20,42±4,94	39,57±9,89	80,23±5,76	91,89±10,99	101,50±9,8	25	10
Resurfaced group	18	18,27±2,98	40,72±13,09	80,38±7,78	92,83±12,12	106,22±9,13	12	1
Non resurfaced group	20	22,35±5,59	38,55±5,88	80,10±3,22	91,05±10,10	97,25±8,50	13	9

STATISTICAL ANALYSIS

Parametric tests were used because the data had normal distribution. Student's t test for continuous variables, chi-square test for categorical data and paired t test for comparison of pre- and post-operative values were used in the comparison of the two groups. At the 95% confidence interval, p <0.05 was considered significant.

RESULTS

Mean age of the patients was 70.55±6,03 years. Mean follow-up period was 20.42±4.94 months. Mean Knee Society score was 39.57±9.89, and mean knee range of motion was 91.89±10.99 before surgery. In the last control, mean Knee Society score was 80.23±5.76, and mean knee range of motion was 101.50±9.8 degrees. Mean follow-up period was 18.27±2.98 months in the patellar resurfaced group and 22.35±5.59 months in the nonresurfaced group. Mean Knee Society score was 40.72±13.09 in the resurfaced group and 38.55±5.88 in the nonresurfaced group before surgery. Mean Knee Society score was 80.38±7.78 in the resurfaced group and 80.10±3.22 in the nonresurfaced group in the last control. Mean knee range of motion was 92.83±12.12 degrees in the resurfaced group and 91.05±10.10 degrees in the nonresurfaced group before surgery. Mean range of motion was 106.22±9.13 degrees in the resurfaced group and 97.25±8.50 degrees in the nonresurfaced group after surgery. A total of 25 patients had anterior knee pain before the surgery. Twelve patients with anterior pain before surgery were in the resurfaced group, and 13 patients had anterior knee pain in the nonresurfaced group. After surgery, there was 1 (5,5%) patient with anterior knee pain in the resurfaced group, and there were 9 (45%) patients with anterior knee pain in the nonresurfaced group in the last control. There was no significant difference between Knee Society scores of both groups in the last control after surgery (p>0,05). A significant difference was detected in Knee Society scores before and after surgery in both groups (p<0,05). There was a significant difference between groups in range of knee motion in the last control after surgery (p<0,05). The resurfaced group had higher angular values than did the nonresurfaced group. We detected a significant difference in anterior knee pain after surgery in the last control between groups (p<0,05). The resurfaced group had less anterior knee pain than did the nonresurfaced group. No reoperation was performed and no reinfection was detected.

DISCUSSION

Anterior knee pain is one of the important conditions where the exact aetiology is not understood. Excessive patello-femoral loads and abnormal patellar tracking can be the reason for anterior knee pain (10). According to the results of the study that was done by Erduran et al., patellar tilt had an increase in gonarthrotic patients compared to the control group. The congruence angle was lower in knees with gonarthrosis at 0° and 10° of knee flexion but higher at 20°, 30°, 40° and 60° than in knees in the control group. They found an increase in congruence angle with the contraction of the quadriceps in the control group, but no significant changes were observed in patients. As a result of their study, the differences in dynamics of the patellofemoral joint in patients with gonarthrosis can be the reason for patellofemoral complaints after knee arthroplasty (11). Another study about the effect of lower extremity torsional deformities on anterior knee pain showed that lower extremity torsional deformities are not the primary aetiologic reason for patellofemoral instability and anterior knee pain (12). Heergaard et al. showed that after total knee arthroplast, patellar tracking changes, and this change increases patellofemoral contact pressures. These increased pressures can be the reason for anterior knee pain after knee arthroplasty (13). According to our results, there was 1 patient with anterior knee pain in the patellar resurfaced group, but there were 9 patients in the nonresurfaced group. Our clinical results are consistent with the results of these biomechanical studies.

In a study with 116 patients who had been performed cruciate-retaining knee arthroplasty, 68 knees had patellar resurfacing and 48 had patellar retention, and the mean follow-up period was 14,8 years. No significant difference was detected in either clinical or radiological results between the two groups (14). Another study with medium follow-up period found patellar resurfacing had no advantage on knee function and patient satisfaction (15). No significant difference in functional outcome was detected between patellar resurfacing and retention in a randomised controlled trial including 1715 patients (16). There was no significant difference in Knee Society scores between resurfaced and nonresurfaced groups, but there was a significant difference in knee range of motion. Higher values of range of motion angles were detected in the resurfaced group.

Results of the meta-analysis done by Tang et al., which compared patellar resurfacing and retention in total knee arthroplasty with a follow-up period of 1 to 2 years, showed patellar resurfacing increases the Knee Society Clinical Score and reduces the reoperation rates (17). There was no significant difference in Knee Society scores between groups, and there was no reoperation in either group in our study. Proponents of the patellar resurfacing emphasise the low reoperation rates and low incidence of anterior knee pain after knee arthroplasty with patellar resurfacing (18, 19). Theratio of anterior knee pain after patellar resurfacing was 1%-5%, but in the retention group, the anterior knee pain ratiowas 10%-14% (20, 21). Our results showed 45% anterior kneepain in the nonresurfaced group, and this result is higherthan in the literature. The anterior knee pain ratio in the re-surfaced group is compatible with the literature.

In the results of the study done by Pilling et al., there was no significant difference in patient satisfaction, infection rate, anterior knee pain and knee rating systems between patellar resurfacing and retention, although there

was a difference in Knee Society Score. However, the reoperation rate is significantly lower in the patellar resurfacing group (22). There was no reoperation in our series, but our follow-up period was short. The short follow-up period is one the most important restrictions of our study.

Barrack et al. showed as a result of their randomised controlled study (23) that, postoperatively, the ratio of anterior knee pain was 28% with resurfaced patellae, despite the fact that none of the patients had any anterior knee pain before the knee arthroplasty procedure. Our results are not consistent with the results of this study

CONCLUSIONS

Smaller anterior knee pain ratio in the resurfaced group than in the nonresurfaced group and a significant difference in range of knee motion are the results of our study. We offered to resurface the patella.

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EARLY AND LATE OUTCOME AFTER SUPRASONIC EXCISION OF INFECTED MESH IMPLANTS AFTER HERNIOPLASTY

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RANI I KASNI ISHODI SUPRAZVUČNE EKSCIZIJE INFICIRANIH MREŽNIH IMPANTATA NAKON NERNIOPLASTIKE

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ABSTRACT

The purpose of this research is to analyze early and postponed complications of the new method to eliminate mesh implants during full-grown infection process.

The Republican clinical hospital (Russia) was the location to carry out a post-hoc analysis of 149 cases on surgical removal of infected mesh implants for ventral hernias of different localization in the period 2000 to 2017. The control group were 78 patients who underwent meshes removal with traditional surgical instruments. The study group included 71 patients, who underwent our method of supra-sonic excision of the implants.

The duration of surgery in the group of patients subjected to supra-sonic excision of the implants was significantly lower (84.3 min vs. 141.5 min) than in the group of traditional surgical techniques. Complications of early postoperative period was most often registered in the control group: foreign bodies (92.8% vs. 7.2%), infection (81.8% vs. 18.2%) and bleeding (87.5% vs. 12.5%). In the long term the recurrence of hernias in the control group were detected 1.8 times more frequently for ventral hernias than in the group of supra-sonic excision of the implants.

Thus, supra-sonic excision of the implant prevents damage to viable tissues of the abdominal wall during the allocation of the implant and provides a good bactericidal effect, which promotes normal tissue regeneration and prevents possible recurrence of the herniation.

Keywords: *hernias, suprasonic excision of infected mesh, early and late outcome*

SAŽETAK

Cilj ovog istraživanja je analiza ranih i odloženih komplikacija novog metoda za eliminaciju mrežnih impantata tokom celokupnog procesa infekcije.

Republička klinička bolnica (Rusija) je predstavljala lokaciju sprovođenje post-hoc analize 149 slučajeva hirurškog uklanjanja inficiranih mrežnih impantata ventralnih kila različite lokalizacije u periodu od 2000. do 2017. Kontrolnu grupu je činilo 78 pacijenata kod kojih je mrežica uklonjena tradicionalnim hirurškim instrumenti. Eksperimentalna grupa se sastojala od 71 pacijenta koji su bili podvrgnuti našem metodu suprazvučne ekscizije impantata.

Trajanje hirurške intervencije u grupi pacijenata podvrgnutih suprazvučnoj eksciziji implantata značajno je bila niža (84,3 min naspram 141,5 min) nego u grupi u kojoj su korišćene tradicionalne hirurške tehnike. Komplikacije ranog postoperativnog perioda su najčešće registrovane u kontrolnoj grupi: strana tela (92,8% naspram 7,2%), infekcija (81,8% naspram 18,2%) i krvarenje (87,5% naspram 12,5%). U pogledu kasnih ishoda ponovna pojava kila u kontrolnoj grupi je zabeležena 1.8 puta češće nego u grupi sa suprazvučnom ekscizijom implantata.

Prema tome, suprazvučna ekscizija implantata prevenira oštećenja tkiva abdominalnog zida prilikom ugradnje implantata i pruža dobar baktericidni efekat, što stimuliše normalnu regeneraciju tkiva i sprečava mogućunost ponovne pojave kila.

Ključne reči: *kila, suprazvučna ekscizija inficirane mreže, rani i kasni ishodi*

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INTRODUCTION

Surgical infection that occurs after installing mesh implants in cases of ventral and inguinal hernia, is a serious problem that does not have a definite approach to its solution (1). The majority of authors believe that to eliminate this complication it is necessary to use all existing methods of conservative therapy (2). However, as practice shows, these methods are not always efficient, especially when performing radical hernioplasty method "on lay". Besides, when it becomes clear that conservative treatment of the wound infection is ineffective, the mesh implant gets firmly overgrown by the connective tissue and removing it is a major challenge (3). By using conventional surgical instruments, in separation of the foreign body (implant) from the soft tissue in the area of surgical intervention the surgeon always takes a risk to resect along with it an unreasonably large amount of tissue, which often results in the subsequent recurrence of the hernia. In addition, the operation to eliminate the implant comes with bleeding that requires hemostasis by suturing the blood vessels with introducing into the wound additional foreign bodies (suture), and most likely with leaving the undetected parts of the implant, "disguised" in the connective tissue and difficult to be discerned (4-6). This circumstance is unlikely to completely help get rid of wound infection, which later remind of itself by repeated inflammatory phenomena in the surgery location.

MATERIALS AND METHODS

The Republican clinical hospital (city of Ufa, Russia) was the location to carry out a post-hoc analysis of 149 cases on surgical removal of infected mesh implants for ventral hernias of different localization in the period 2000 to 2017. The study was approved by the permission of the Ethical Committee of the Bashkir State Medical University (city of Ufa, Russia). The necessary condition was to receive an informed voluntary consent of the patients to participate in the stated study.

To participate in the study one had to meet the following criteria: an infected mesh implant installed for ventral or inguinal hernia; no effect in relation to conservative therapy. The study excluded patients with complicated perioperational period, diagnosed intestinal fistulas and/or peritonitis, sepsis. The time, from placing the implant before admission of the patient to the surgical treatment to its removal, ranged from 3 weeks to 1.5 years, on average it was equal to 9 months.

Depending on the surgical technique the patients were divided into 2 groups. The control group (group I) were 78 patients who underwent meshes removal with traditional surgical instruments. The study group (group II) included 71 patients, which underwent our methodology of suprasonic excision of implants (table 1).

The developed method of surgical intervention with elimination of infected mesh implants is the following. After excision of the postoperative scar on the skin along with fatty tissue within healthy tissue a previously installed implant gets allocated. To do this, it is fixed on the edge with a mouse-tooth forcets and gets maximum pulled up, and then it gets separated from the aponeurosis and muscles of the abdominal wall by destroying the connective tissue adhesions using ultrasound, cavitated by "SONOCA-180" produced by the "Söring" company (Germany). The ultrasonic generator produces electrical oscillations at an ultrasonic frequency which is converted by a piezostrictive transducer located in the handpiece, into reciprocating motion of the titanium waveguide with the frequency of the system ultrasonic oscillations of 25 kHz. The energy of the ultrasonic vibrations is transferred to the liquid, supplied through the central channel into the wave-water, resulting in the abovementioned fluid cavitation processes (7).

The surgery technique provides for the devastating effect of ultrasonic waves on the connective tissue through a liquid antiseptic - 0.2% solution of aqueous chlorhexidine of bi-gluconate. For this purpose, 0.2% aqueous chlorhexidine of bigluconate solution is continuously fed between the installed implant and the abdominal wall during the entire ultrasonic treatment. The average speed of the ultrasonic processing is about 2 cm^2/min. The amount of the used antiseptic solution depends on the size of the wound surface. During the postoperative period the patients are provided with a rational antibacterial, analgesic therapy and prophylaxis of thromboembolic complications, individually to each patient (8, 9).

Table 1. The characteristics before surgery according to the procedure

Characteristic	Group I, n = 78	Group II, n = 71	p
Age, years	30.7 (8.8-84.1)	39.4 (6.1-87.7)	0.037*
Gender, M/F	23/55	20/51	0.653†
Time between operation, month	9.2 (6.5-11.3)	8.4 (7.1-10.2)	0.7*
WBC (10^3 /μL)	12.3 (4.3-26.5)	13.0 (4.4-36.4)	0.160*
Alb (g/dL)	4.4 (2.8-5.3)	4.4 (2.5-5.3)	0.154*
Neutro. (%)	80.9 (43.0-95.5)	83.6 (27.6-95.5)	0.674*
Lymph (%)	13.0 (3-46)	10.9 (3-57.8)	0.666*

Showing medians and interquartile ranges. *Tested by Mann-Whitney U-test. †Tested by Fisher's exact test. WBC: white cell count, Alb: Albumin, Neutro: neutrophil, Lymph: lymphocyte.

Table 2. The outcomes according to the procedure

Characteristic	Group I, n = 78	Group II, n = 71	p
Operation time, min	141.5 (101-153)	84.3 (54.2-93.7)	0.002*
Bleeding, n (%)	7 (8.9)	1 (1.4)	0.035†
Residual fragments of meshes, n (%)	13 (16.6)	1 (1.4)	0.003†
Eventration, n (%)	3 (3.8)	0 (0.0)	0.003†
LHS, days	14 (7-23)	7 (3-18)	0.001*
SOI, days	1 (0-6)	1 (5-14)	0.016*
SSI, n (%)	9 (11.5)	2 (2.8)	0.044†

Showing medians and interquartile ranges. *Tested by Mann-Whitney U-test. †Tested by Fisher's exact test. LHS: length of hospital stay, SOI: started an oral intake, SSI: surgical site infection, Clavien-Dindo classification IIIa.

Statistical analyses were performed with the SPSS statistical software package (version 13.0; SPSS Inc., Chicago, IL). Univariate and multivariate analyses were performed to clarify the laboratory parameter and clinical factors most significantly associated with supra-sonic excision and traditional surgical operation. Univariate analyses, Mann-Whitney U-test, and Fisher's exact test were utilized, and Odds ratios with 95% CI were calculated using logistic regression model analyses. P values of less than 0.05 were considered to be statistically significant.

RESULTS

The mediana operation time for both groups was 127 minutes. The duration of surgery in the group of patients subjected to supra-sonic excision of the implants was significantly lower (84.3 min vs 141.5 min) than in the group of traditional surgical techniques.

Among the complications (table 2) of the early postoperative period, the most frequently registered were foreign bodies (fragments of the mesh). The control group showed residual foreign bodies in 13 patients, which required a total of 17 repeated surgical operations. Herewith two patients had to undergo the mesh elimination in two stages. The study group registered only one patient with a residual mesh.

The second most common complication of early postoperative period was wound infection. In the group of traditional surgical technique the postoperative wound purulence was registered in 9 patients, in the study group - in 2 patients (81.8 % vs 18.2 %). It should be noted that at the stage of necrectomy remnants of the mesh were found in all 9 patients of group I. In group II, the wound infection was not associated with a foreign body.

Table 3. Recurrence of ventral hernias

Reherniation	Group 1 (n=78)	Group II (n=71)	p
Patients, n (%)	11 (14.1)	6 (8.5)	0.002

Showing medians and interquartile ranges. Tested by Fisher's exact test.

Bleeding and eventration characteristically exhibit less occurrence for both groups. However, in the group of traditional surgical technique the bleeding (87,5% for group I vs 12,5% for group II) happened more often which required a second surgery. Out of 7 patients of control group only two patients proved that conservative hemostatic therapy was effective. It should be noted that eventration was not registered in the group of patients subjected to supra-sonic excision of the implants.

In the late postoperative period (5 years), we were able to examine 137 out of 149 patients: Group I - 68 patients and group II - 69 patients. Dynamic observation primarily showed recurrent hernia formation, which was diagnosed in 46 patients under research (table 3).

Table 3 shows that the hernia recurrence was most frequent with conventional surgical techniques.

In multivariate analysis, bleeding, length of hospital stay and reherniation were significantly lower in Group II than in Group I (p=0.035, 0.001 and 0.02, Table 4).

DISCUSSION

According to the data (10) every year more than 20 million patients undergo hernia repair. Just like any invasive intervention hernia repair is associated with several complications, such as migration of the mesh into the ab-

Table 4. Multivariate analysis clinical and operative factors according to the procedure

Indicators	Group I (n=78)	Group II (n=71)	Odd ratio	95% CI	p
Bleeding, n (%)	7 (8.9)	1 (1.4)	2.29	1.04-4.97	0.035
LHS, days	14 (7-23)	7 (3-18)	2.03	1.12-3.71	0.001
Reherniation , n (%)	11 (14.1)	6 (8.5)	2.4	1.09-3.92	0.002

Showing medians and interquartile ranges. Tested by Fisher's exact test.

dominal cavity (11, 12), development of persistent seroma posterior (13-15), development of chronic pain (16, 17), and surgical infection. Although incisional hernia repair is classified as a clean surgery, it still has a high incidence of surgical site infection (SSI) (0.7%-26.6%). The presence of an SSI could increase early recurrence rates after hernia repair (18).

To reduce the frequency of complications different authors suggest applying different surgical techniques of hernia repair and using different materials (19-21).

The purpose of this research is to develop and assess advantages of the new method of mesh implants elimination in cases of full-grown infection process. Traditionally, the meshes installed for ventral hernias, are eliminated using the classic open method of elimination on the previous access. And our research used the open access to the mesh, when the initial absence of infection in the abdominal cavity allows avoiding the spread of this process. This technique was justified because, according to our data the group of the original method to eliminate the mesh registered the least number of early complications, including, in addition to wound infection, partial removal of the mesh and eventrations. Longer operative time and massive blood loss during operation are another issue in the comparison of operation techniques. Generally, those two factors are dependent on surgeon's experience. Though most surgical staffs in general has performed basic and advanced procedures, operating time is long when performed by inexperienced surgeons, and is shortened by accumulating experience. Also blood loss is dependent on surgeon's skill and on the situation of hernia. In our study, amount of blood loss and operation time were significantly lower in study group.

With regard to the postponed results and, namely, recurrence of the hernia after mesh is eliminated, Rehman *et al*. analyzed data from 40 patients, which showed that after the mesh is eliminated the recurrence rate of hernia amounted to an average of 5% (22). According to our data, the recurrence of hernias in the group, where the mesh is eliminated with ultrasound, was the smallest. Herewith the control group showed the recurrence rate which was consistent with Rehman's findings on the ventral hernias.

Thus, supra-sonic excision of the implant prevents damage to viable tissues of the abdominal wall during the allocation of the implant and provides a good bactericidal effect, which promotes normal tissue regeneration and prevents possible recurrence of the herniation.

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IS 3 WEEKS OF EXERCISE ENOUGH TO CHANGE BLOOD PRESSURE AND CARDIAC REDOX STATE IN HYPERTENSIVE RATS?

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DA LI JE 3 NEDELJE VEŽBANJA DOVOLJNO DA PROMENI KRVNI PRITISAK I SRČANI REDOKS STATUS KOD HIPERTENZIVNIH PACOVA?

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ABSTRACT

The investigation was aimed to evaluate the effects of 3-weeks swimming exercise on blood pressure and redox status in high-salt-induced hypertensive rats. Male Wistar albino rats (n=40, 6 weeks old) were divided into 4 groups: 1. hypertensive rats that swam for 3 weeks; 2. sedentary hypertensive control rats; 3. normotensive rats that swam for 3 weeks; 4. sedentary normotensive control rats. Hypertensive animals were on high concentrated sodium (8% NaCl) solution for 4 weeks (period of induction of hypertension). After sacrificing, hearts were isolated and perfused according to Langendorff technique at gradually increased coronary perfusion pressure from 40-120 cmH_2O. The oxidative stress markers were determined in coronary venous effluent: the index of lipid peroxidation (measured as TBARS), nitrites (NO_2^-), superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2). Swimming did not lead to significant changes in levels of TBARS, NO_2^-, O_2^- in any of compared groups while levels of H_2O_2 were significantly higher in swimming hypertensive group comparing to swimming normotensive group at coronary perfusion pressure of 80-120 cmH_2O. Our results indicate that the short-term swimming start to reduce blood pressure. In addition it seems that this type of swimming duration does not promote cardiac oxidative stress damages.

Keywords: *hypertension, oxidative stress, swimming, rat heart*

SAŽETAK

Studija je imala za cilj da ispita uticaj plivanja od tri nedelje na krvni pritisak i redoks status kod pacova sa hipertenzijom izazvanom visokim unosom soli. Muški Wistar albino pacovi (n=40, 6 nedelja starosti) su podeljeni u 4 grupe: 1. hipertenzivni pacovi koji su plivali 3 nedelje; 2. sedentarni hipertenzivni kontrolni pacovi; 3. normotenzivni pacovi koji su plivali 3 nedelje; 4. sedentarni normotenzivni kontrolni pacovi. Hipertenzivne životinje su 4 nedelje pile visoko koncentrovani rastvor natrijuma (8% NaCl). Nakon žrtvovanja, srca su izolovana i perfundovana prema Langendorff-ovoj tehnici pri rastućem koronarnom perfuzionom pritisku od 40-120 cmH_2O. Parametri oksidativnog stresa su određivani u koronarnom venskom efluentu indeks lipidne peroksidacije (meren kao TBARS), nitriti (NO_2^-), superoksid anjon radikal (O_2^-) i hidrogen peroksid (H_2O_2). Plivanje nije dovelo do značajnih promena u nivoima TBARS, NO_2^-, O_2^- ni u jednoj od poređenih grupa dok su nivoi H_2O_2 bili značajno veći u grupi hipertenzivnih pacova koja je plivala u poređenju sa grupom normotenzivnih pacova koja je plivala pri vrednostima koronarnog perfuzionog pritiska od 80-120 cmH_2O. Naši rezultati ukazuju da kratak period plivanja počinje da snižava krvni pritisak. Osim toga, čini se da ovaj tip plivanja ne podstiče srčana oštećenja oksidativnim stresom.

Ključne reči: *hipertenzija, oksidativni stres, plivanje, srce pacova*

ABBREVIATIONS

BP- blood pressure
NO_2^- - nitrites
H_2O_2 - hydrogen peroxide
O_2^- -superoxide anion radical
ROS - reactive oxygen species
TBARS - Thiobarbituric Acid Reactive Substances

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INTRODUCTION

Environmental factors, such as psychological stress, exercise, and dietary sodium intake, have long been considered relevant to both the etiology and control of human essential hypertension. Reduction in the stresses of everyday life, increased levels of exercise, and reduced dietary sodium intake have been recommend as having a beneficial effect in controlling arterial blood pressure. Similarly, high levels of life stress, sedentary lifestyle, and high dietary sodium intake have frequently been linked to cardiovascular diseases (1, 2). Hypertension is considered to be major risk factor for coronary heart diseases contributing to morbidity and mortality worldwide. Importantly, experimental animal studies clearly demonstrate a well-documented promotion of endothelial dysfunction in hypertension by production of oxidative stress markers (3, 4). This occurs due to imbalance between reactive oxygen species (ROS) and antioxidant capacity in favour of oxidants (5). Therefore, treatment of hypertension can be focused on oxidative stress as a possible target.

Regular physical exercise has been considered an effective method which can perform a multitude of beneficial effects for health, such as promotion of health and lifespan, betterment of quality of life and reduce the incidence of disease (6). In addition it has been published in some papers that physical exercise lowers blood pressure (BP) patients with essential hypertension (7) and in male spontaneously hypertensive rats (SHR) (8, 9). Interestingly, controversial effects were determined in female SHR (10). Although the antihypertensive mechanisms of exercise are not completely understood yet, numerous mechanisms are supposed to be involved in reduction of BP while exercising such as decrease in levels of angiotensin II and melioration of nitric oxide (NO) production (11), higher concentration of the plasmatic atrial natriuretic peptide (8), lower production of oxidative stress markers (12). Changes in redox status caused by swimming represent modification in an antioxidant enzyme, alters muscle gene expression thus contributing to exercise-induced adaptations to skeletal muscle. However, it should be taken into consideration that various factors influence the oxidative stress response to swimming training, such as type of exercise, intensity, duration, gender and age of athletes etc (13). Physical exercise may be of potential importance for prevention or treatment of hypertension or hypertension-associated pat.

In recent years there are increased interest on effects of short time physical load on cardiovascular system. Therefore, the objective of this investigation was to explore effects of short-term exercise training on BP and oxidative stress parameters in a rat model of high-salt-induced hypertension.

MATERIAL AND METHODS

Ethical approval

The study was performed in the laboratory for the cardiovascular physiology of the Faculty of Medical Sciences, University of Kragujevac, Serbia. It was approved by Ethical committee of the Faculty, and performed according to the Faculty's rules for the welfare of laboratory animals, which are in consent with Good laboratory practice and European Council Directive (86/609/EEC).

Animals and high-salt-induction of hypertension

Forty male Wistar albino rats, body weight between 180 and 200 g (at the beginning of the experiment), six weeks old (obtained from the Military Medical Academy, Belgrade, Serbia) were housed under controlled environmental conditions, with a temperature of 22±2 °C and a 12-h light/dark cycle. The rats had *ad libitum* access to food and tap water or NaCl soluted in water (8% solution).

At 6 weeks old rats were randomly divided into the following groups (10 animals per group):

1. hypertensive rats that swam for 3 weeks (S-HTA-3);
2. sedentery hypertensive control rats (HTA-3);
3. normotensive rats that swam for 3 weeks (S-NTA-3);
4. sedentery normotensive control rats (NTA-3).

Hypertensive animals were on high sodium (8% NaCl solution) diet for 4 weeks (period of induction hypertension), and this animals did not drink tap water during the experimental protocol.

BP was monitored before (initial values), after period of induction hypertension (confirmation of hypertension) and after the training period (assessment of the impact of swimming on hypertension).

Swimming training protocol

Rats swam in a specially constructed swimming pool made of glass (80x60x100 cm) in which water temperature (37 ± 1 °C) was maintained by an electric heater, and a pump continuously made waves in order to prevent rats from floating. The training protocol was conducted during the same period of the day (8:00–10:00 am) for all the training sessions. The first week consisted of an adaptation period, initiated with 10 min of continuous swimming training on the first day. Swimming time was increased daily until reaching 60 min at the end of the fifth day. From the second week, the exercise duration was kept constant (60 min/day, 5 days/week) with 2 days of rest. Rats from the control group were put in water for 1 min a day, 5 days a week, in order to achieve the water-induced stress effect. This was maintained until the end of the training period, which lasted 3 weeks. To avoid effects related to acute exercise, animals rested for 48 h before being sacrificed for all additional procedures. The swimming was continuously supervised. Body weight was monitored weekly.

Hemodynamic parameters determination

Systolic BP (SBP), diastolic BP (DBP), mean arterial pressure (MAP) and heart rate (HR) were evaluated in conscious rats before and after period of induction of hypertension and after the training or sedentary period and was determined by an indirect tail-cuff method (IITC Life

Science, Inc., USA). Animals were restrained for 5–10 min and conditioned to the procedure with cuff inflation-deflation cycles. The results of three stable measurements of BP were averaged.

Isolated rat heart preparation

The hearts of male *Wistar albino* rats (n=40, 10 in each experimental subgroup) were excised and retrogradely perfused according to Langendorff technique (Experimetria Ltd,1062 Budapest, Hungary). After a short-term narcosis induced by intraperitoneal application of ketamine (10 mg/kg) and xylazine (5 mg/kg), animals were sacrificed by cervical dislocation (Schedule 1 of the Animals/ Scientific Procedures, Act 1986, UK), and premedicated with heparin as an anticoagulant. After emergency thoracotomy and rapid cardiac arrest by superfusion with ice-cold isotonic saline, hearts were rapidly excised, the aortas were cannulated and retrogradely perfused at gradually increased coronary perfusion pressure (CPP) from 40 to 120 cmH_2O in order to establish coronary autoregulation.

The composition of the non-recirculating Krebs-Henseleit perfusate was as follows (mM): NaCl 118, KCl 4.7, $CaCl_2x2H_2O$ 2.5, $MgSO_4x7H_2O$ 1.7, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, pyruvate 2, equilibrated with 95 % O_2 plus 5% CO_2 and warmed to 37 °C (pH 7.4).

Biochemical analysis

Samples of coronary venous effluent were collected on each value of perfusion pressure (40-120 cmH_2O). The following parameters of oxidative stress were determined spectrophotometrically (UV-1800 Shimadzu UV spectrophotometer, Japan): the levels of index of lipid peroxidation, measured as thiobarbituric acid-reactive substances (TBARS), nitrites (NO_2^-), superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2).

Determination of index of lipid peroxidation (TBARS)

The degree of lipid peroxidation in the coronary venous effluent was estimated by measuring TBARS, using 1 % thiobarbituric acid in 0.05 NaOH, which was incubated with the coronary effluent at 100 °C for 15 min and measured at 530 nm. Krebs–Henseleit solution was used as a blank probe (14).

Determination of nitrites (NO_2^-)

Nitric oxide decomposes rapidly to form stable nitrite/ nitrate products. The nitrite level (NO_2^-) was measured and used as an index of nitric oxide (NO) production, using Griess's reagent. A total of 0.5 ml of perfusate was precipitated with 200 µl of 30 % sulpho-salicylic acid, vortexed for 30 min, and centrifuged at 3000 x g. Equal volumes of the supernatant and Griess's reagent, containing 1 % sulphanilamide in 5 % phosphoric acid/0.1 % naphthalene ethylenediaminedihydrochloride were added and incubated for 10 min in the dark and measured at 543 nm. The nitrite levels were calculated using sodium nitrite as the standard (15).

Determination of hydrogen peroxide (H_2O_2)

The measurement of the level of hydrogen peroxide (H_2O_2) was based on the oxidation of phenol red by hydrogen peroxide in a reaction catalyzed by horseradish peroxidase (HRPO). Two hundred microliters of perfusate was precipitated using 800 ml of freshly prepared phenol red solution; 10 µl of (1:20) HRPO (made ex tempore) was subsequently added. For the blank probe, an adequate volume of Krebs–Henseleit solution was used instead of coronary venous effluent. The level of H_2O_2 was measured at 610 nm (16).

Determination of superoxide anion radical (O_2^-)

The level of the superoxide anion radical (O_2^-) was measured via a nitro blue tetrazolium (NBT) reaction in TRIS buffer with coronary venous effluent, at 530 nm. Krebs–Henseleit solution was used as a blank probe (17).

Statistical analysis

Complete statistical evaluation was performed with SPSS Statistics 18. Normality of parameter distribution was checked with the Kolmogorov–Smirnov test. Mann–Whitney U test was used for comparison of groups. Statistic p values less than 0.05 were considered to be statistically significant.

RESULTS

Body weight weight

The mean values of body weight of S-HTA-3 and S-NTA-3 rats and their controls did not significantly differ throughout the first week of research, while the values of body weight of S-HTA-3 compared to S-NTA-3 rats were

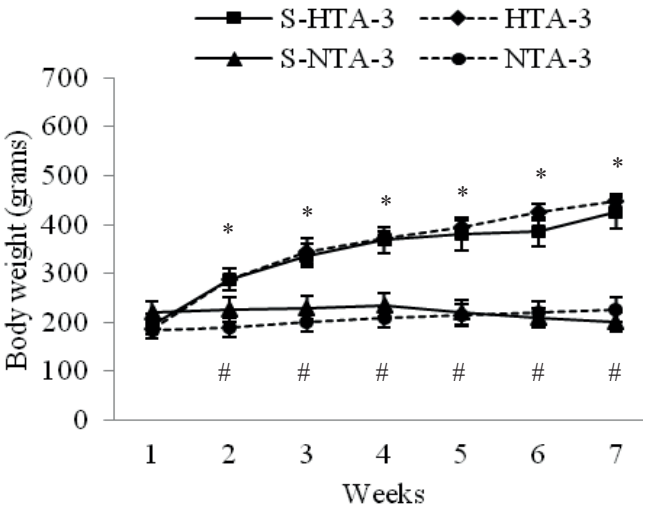

Fig. 1 - Mean body weight of hypertensive and normotensive rats. The values are represented as mean ± SD. S-HTA-3- hypertensive rats that swam for 3 weeks; HTA-3-sedentery hypertensive control rats; S-NTA-3-normotensive rats that swam for 3 weeks; NTA-3-sedentery normotensive control rats. * statistical significance between S-HTA-3 vs S-NTA-3; # statistical significance between HTA-3 vs NTA-3.

Table 1. The average values of hemodynamic parameters of hypertensive and normotensive rats that swam for three weeks and their controls in last week

Groups	Hemodynamic parameters			
	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	HR (bpm)
S-HTA-3	196,37 ± 6,87 [a,b]	104,31 ± 7,12 [a,b]	137,68 ± 6,64 [a,b]	330,19 ± 19,27
HTA-3	205,69 ± 7,19	115,81 ± 8,73	142,23 ± 7,12	325,17 ± 14,37
S-NTA-3	120,01 ± 4,27	80,18 ± 6,45	92,03 ± 6,75	339,28 ± 17,21
NTA-3	123,98 ± 6,15	83,23 ± 7,65	96,13 ± 4,27	327,48 ± 16,57

Data are means ± SD. SBP, systolic pressure; DBP, diastolic pressure; MAP, mean arterial pressure; HR, heart rate. Statistical significance was considered for a p value less than 0.05 (*p<0.05); [a]p < 0.05- S-HTA-3 vs. HTA-3; [b]p < 0.05- S-HTA-3 vs. S-NTA-3; S-HTA-3- hypertensive rats that swam for 3 weeks; HTA-3-sedentery hypertensive control rats; S-NTA-3- normotensive rats that swam for 3 weeks; NTA-3-sedentery normotensive control rats.

significantly higher from the second week to the end of the study (II week - p = 0.043; III week - p = 0.033; IV week - p = 0.028; week - p = 0.028; VI week - p = 0.028; VII week - p = 0.011). The mean values of body weight of hypertensive controls compared to normotensive controls were significantly higher from the second week to the end of the study (II week - p = 0.033; III week - p = 0.023; IV week - p = 0.018; V week - p = 0.018; VI week - p = 0.008; VII week - p = 0.008) (**Figure 1**).

Hemodynamic parameters

Initial levels of BP (period of induction of hypertension) were not different between the groups (average value 115.58/82.340 ± 8.14/5.18). After 4 weeks of treatment with high salt water, hypertension was confirmed in hypertensive groups (198.85/114 ± 9.31/6.23). There was significant differences between HTA-3 and S-HTA-3 group in values of SBP, DBP, MAP. The value of pressures (SBP, DBP, MAP) in S-HTA-3 rats were significantly higher compared with S-NTA rats, while there were no difference in values of HR between groups (**Table 1**).

Oxidative stress parameters

Parameters of oxidative stress in coronary effluent at different coronary perfusion pressures in swimming and sedentary hypertensive and normotensive rats are shown in **Figures 2-5**, statistical significance is presented in **Table 2**.

There were no significant differences in values of TBARS, NO_2^- and O_2^- between the groups (swimming vs sedentary and normotensive vs hypertensive) (**Figures 2-4**).

Comparing S-HTA-3 and S-NTA-3 with sedentary groups significant changes was not observed in values of H_2O_2 **(Figure 5)**. Significantly higher levels of H_2O_2 was noticed in S-HTA-3 group comparing to S-NTA-3 group at CPPs (80 cmH_2O - p=0.049, 100 cmH_2O - p=0.037, 120 cmH_2O - p=0.029).

DISCUSSION

First research that was conducted in 1978, gave information about the association between exercise and oxidative stress (18). After many investigations in this field, it was

Table 2. Significance in level of oxidative stress parameters between hypertensive and normotensive rats who swam for three weeks at different values of coronary perfusion pressures

CPP (cmH_2O)	**O_2^- (nmol/min/g wt)**				**NO (nmol/min/g wt)**			
	S-HTA-3 vs. HTA-3	S-NTA-3 vs. NTA-3	S-HTA-3 vs. S-NTA-3	HTA-3 vs. NTA-3	S-HTA-3 vs. HTA-3	S-NTA-3 vs. NTA-3	S-HTA-3 vs. S-NTA-3	HTA-3 vs. NTA-3
40	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050
60	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050
80	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050
100	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050
120	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050
	H_2O_2 (nmol/min/g wt)				**TBARS (µmol/min/g wt)**			
	S-HTA-3 vs. HTA-3	S-NTA-3 vs. NTA-3	S-HTA-3 vs. S-NTA-3	HTA-3 vs. NTA-3	S-HTA-3 vs. HTA-3	S-NTA-3 vs. NTA-3	S-HTA-3 vs. S-NTA-3	HTA-3 vs. NTA-3
40	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050
60	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050
80	p>0,050	p>0,050	**p=0,049**	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050
100	p>0,050	p>0,050	**p=0,037**	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050
120	p>0,050	p>0,050	**p=0,029**	p>0,050	p>0,050	p>0,050	p>0,050	p>0,050

S-HTA-3- hypertensive rats that swam for 3 weeks; HTA-3-sedentery hypertensive control rats; S-NTA-3- normotensive rats that swam for 3 weeks; NTA-3-sedentery normotensive control rats.

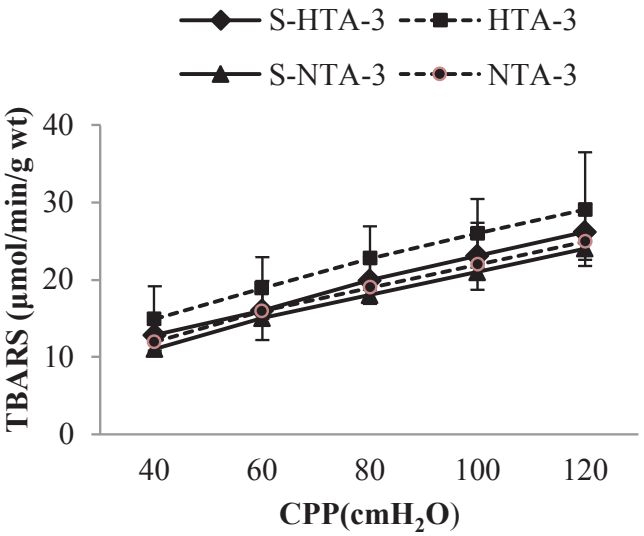

Fig. 2- Effects of 3 weeks of swimming on TBARS levels of hypertensive and normotensive rats. The values are represented as mean ± SD. S-HTA-3- hypertensive rats that swam for 3 weeks; HTA-3-sedentery hypertensive control rats; S-NTA-3- normotensive rats that swam for 3 weeks; NTA-3-sedentery normotensive control rats. *statistical significance at the level of $p < 0.05$ is shown in **Table 2**.

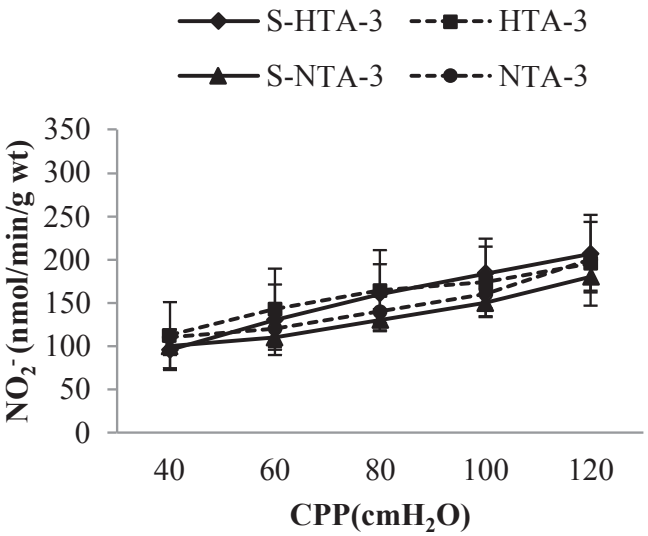

Fig. 3- Effects of 3 weeks of swimming on NO_2^- levels of hypertensive and normotensive rats. The values are represented as mean ± SD. S-HTA-3- hypertensive rats that swam for 3 weeks; HTA-3-sedentery hypertensive control rats; S-NTA-3- normotensive rats that swam for 3 weeks; NTA-3-sedentery normotensive control rats. *statistical significance at the level of $p < 0.05$ is shown in **Table 2**.

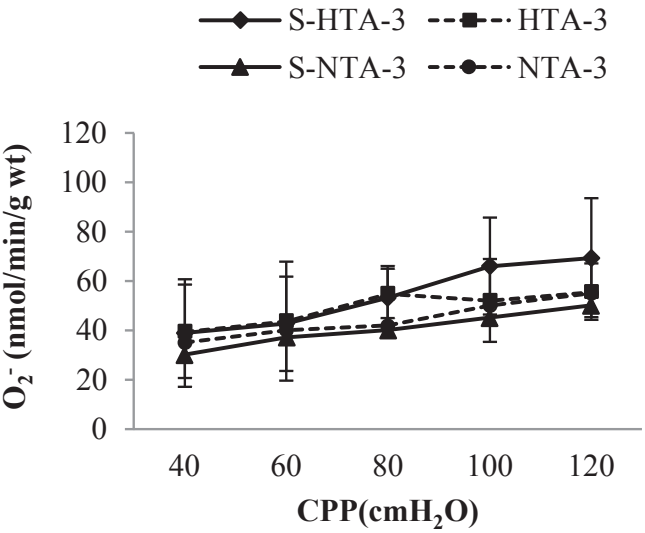

Fig. 4- Effects of 3 weeks of swimming on O_2^- levels of hypertensive and normotensive rats. The values are represented as mean ± SD. S-HTA-3- hypertensive rats that swam for 3 weeks; HTA-3-sedentery hypertensive control rats; S-NTA-3- normotensive rats that swam for 3 weeks; NTA-3-sedentery normotensive control rats. *statistical significance at the level of $p < 0.05$ is shown in **Table 2**.

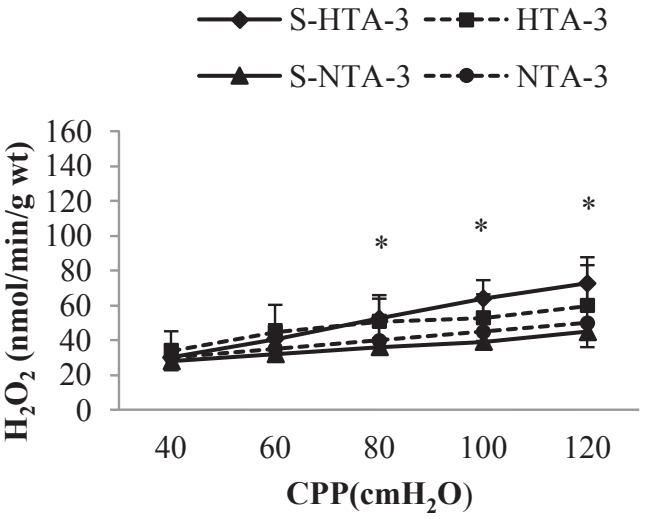

Fig. 5- Effects of 3 week of swimming on H_2O_2 levels of hypertensive and normotensive rats. The values are represented as mean ± SD. S-HTA-3- hypertensive rats that swam for 3 weeks; HTA-3-sedentery hypertensive control rats; S-NTA-3- normotensive rats that swam for 3 weeks; NTA-3-sedentery normotensive control rats. *statistical significance at the level of $p < 0.05$ is shown in **Table 2**.

described that increased aerobic meabolism is prospective sourse of oxidative stress during anaerobic exercise (19). Futrhermore, aerobic exercise has been shown to be effective in a significant reduction of ROS and in decrease of the occurrence of ROS associated diseases, including hypertension (20). Although many studies aimed to determine the influence of tredmill or cycle ergometer exercise on oxidative stress markers (21), our aim was to examine the effects of short-term swimming exercise considering that swimming has been proposed as a convenient model for identifying the physiological, biochemical and molecular responses to acute exercise training and the adaptations to chronic exercise training (22, 23).

Due to the fact that numerous patients choose a non-weight-bearing physical activity such as swimming, it is important to determine if this kind of practice has potential antihypertensive effects (24). Regular physical activity contributed in reduction of elevated BP in hypertensive patients and hypertensive animals in which hypertension was induced by N(ω)-nitro-L-arginine methyl ester (L-NAME)

(25, 26), deoxycorticosterone acetate (DOCA) (27) as well as in spontaneously hypertensive (28), Dahl saltsensitive and salt-resistant rats (29).

Taken together, these studies suggest the beneficial effects of aerobic training upon arterial blood pressure which seems to be intensity-dependent (28, 30). Our findings are in accordance with previous work that showed decrease in arterial pressure and MAP after four and eight weeks of a swimming program in spontaneously hypertensive rats (31-33). Nevertheless, it is important to recognize that even a 5–6 mmHg decrease in arterial blood pressure which is similar to that observed in our study can be associated with a approximately 42% reduction in stroke incidence and a approximately 14% reduction in coronary heart disease as noted in several epidemiologic studies (34). Significant reduction of SBP, DBP and MAP in our study proved that swimming training can be prescribed to patients with hypertension as a non-pharmacological treatment. Reason for reduction of BP may be due to lower sympathetic activity induced by physical activity which can be a consequence of progression of arterial baroreflex and chemosensitive cardiopulmonary baroreflex sensitivity in SHR. In present study swimming also led to maintenance of body weight in hypertensive rats which certainly contributed to reduction of BP (35).

A link between hypertension and oxidative stress is well established. Redox imbalance, increased bioavailability of ROS or/and decreased antioxidant capacity has been demonstrated both in humans and animals (36). However the presence of oxidative stress within the myocardium have been very poorly investigated. Therefore we sought to assess the changes in cardiac oxidative stress parameters of hypertensive rats after short-term swimming or sedentary period.

Analysis of parameters that we determined in the coronary venous effluent during coronary autoregulation refers to the oxidative stress in the endocardium of the left ventricle. Our data have shown that values of pro-oxidant markers such as TBARS, NO_2^-, O_2^- were not affected by 3 weeks swimming protocol. According to Claudio et al excercise training prevented the increase of ROS production in ovariectomized hypertensive rats demonstrating increased expresion of antioxidative enzymes. Potential explanation for non excessive production of ROS besides above mentioned mechanism, may also be lower electron leakage from mitochondria or chronic exposure of tissue to ROS, induced by training, which makes the organ more resistant to the effects that derive from the mechanisms of oxidative stress (37).

On the other hand, investigation conducted on hypertensive rats trained 6 weeks showed that exercise lead to significant reduction of TBARS values in serum when compared to sedentary group. Also significant decrease of NO was noticed in sedentary hypertensive group but level of NO increased in trained hypertensive group (38). Furthermore, Bertagnolli and co-workers noticed similar changes in favor of lipid peroxidation in 10 weeks trained SHR group. These significant changes in ROS release may be due to longer duration of physical load (39).

Surprisingly, none of the researchers has not dealed with determination of H_2O_2 levels in hypertensive physicaly active subjects. We noticed that levels of H_2O_2 were significantly higher in S-HTA-3 group comparing to S-NTA at CPP of 80-120 cmH_2O. Other researchers measured tissue total oxidant status (TOS) in heart tissue and expressed their results in terms of micromolar hydrogen peroxide equivalent per liter (lmol H_2O_2 equiv/L/mg protein). Their observation was that hypertensive animals had higher tissue oxidative stress levels than normotensives (40). Assumption for high concentration of hydrogen peroxide in hypertensive swimming rats may be activation of angiotensin II probably through angiotensin AT1 receptor-dependent stimulation of NADPH oxidase (Nox) enzymes (41).

On the basis of all mentioned above it can be assumed that the most important determinating factors of exrcise induced modification of cardiac oxidative stress can be duration and/or intensity.

CONCLUSION

Our results clearly indicate that reduction of BP may start after 3rd week of training. In addition it seems that duration of three weeks of swimming does not promote cardiac oxidative stress damages. These findings could be one step closer for better understanding of short-time exercise on blood preassure and oxidative stress of the heart.

ACKNOWLEDGMENTS

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SOCIO-MEDICAL ASPECTS OF DEPRESSION AMONG ELDERLY ADULTS IN SERBIA

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SOCIJALNO-MEDICINSKI ASPEKTI DEPRESIJE KOD STARIH OSOBA U SRBIJI

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ABSTRACT

Depression is the most frequent mental health problem in older age with serious consequences on personal, interpersonal and social level. The aim of this study was to determine the association of demographic factors, socio-economic factors and health status characteristics, with the presence of depressive symptoms in the elderly persons. The survey was conducted as a part of the national study "Health Survey of the Serbian population" in 2013. Data on the population aged 65 years and over were used for the purposes of this study (3540 respondents). PHQ-8 questionnaire was used to assess the presence of symptoms of depression. The relations between the presence of depressive symptoms, as a dependent variable, and a set of independent variables was examined by univariate and multivariate logistic regressions. Depression (PHQ-8 score≥10) was registered in 10% of the population aged 65 and above, wherein it was statistically significantly higher in women (12.7%) than in men (6.5%). Limitations in performing of daily activities showed to be the strongest predictor of depression in the elderly, while respondents who have had serious limitations had even six times more chanse to develop depression (OR=6.84). Respondents who rated their health as "bad or very bad" for 49.5% more frequently manifested depressive symptoms compared to those who evaluated their health as "very good or good" (OR=3.49). Respondents who have had two or more chronic diseases were three times more likely to have depression (OR=3.1) compared to people without chronic disease.

Keywords: *depression, elderly adults, risk factors, national health survey*

SAŽETAK

Depresija je jedan od najčešćih problema mentalnog zdravlja u starijoj životnoj dobi sa ozbiljnim posledicama na ličnom, međuljudskom i društvenom nivou. Cilj ovog istraživanja je bio da se utvrdi povezanost demografskih, socio-ekonomskih faktora i karakteristik zdravstvenog stanja sa prisustvom depresivnih simptoma kod starih osoba. Istraživanje je sprovedeno kao deo nacionalne studije "Istraživanje zdravlja stanovništva Srbije" 2013. godine. Za potrebe ove studije korišćeni su podaci o populaciji starijih od 65 godina. Za procenu prisustva simptoma depresije je korišćen PHQ-8 upitnik. Povezanost prisustva depresivnih simptoma, kao zavisne varijable i skupa nezavisnih varijabli ispitivana je univarijantnom i multivarijantnom logističkom regresijom. Depresija (PHQ-8 skor≥10) je registrovana kod 10% stanovništva starosti 65 i više godina pri čemu je statistički značajno bila veća kod žena (12.7%) u odnosu na muškarce (6.5%). Kao najjaci prediktor pojave depresije kod starijih osoba, kada je u pitanju zdravstveno stanje izdvaja se ograničenost u obavljanju svakodnevnih aktivnosti, pa su ispitanici koji su imali ozbiljna ograničenja imali čak šest puta veću šansu za pojavu depresije (OR=6.84). Ispiatnici koji su svoje zdravlje ocenili kao "loše ili veoma loše" su za 49.5% češće ispoljavali depresivne simptome u poređenju sa onima koji svoje zdravlje ocenjuju kao "veoma dobro ili dobro" (OR=3.49). Ispitanici koji su imali dve ili vise hroničnih bolesti su imali tri puta veću šansu da imaju depresiju (OR=3.1) u odnosu na osobe bez hroničnih bolesti.

Ključne reči: *depresija, stare osobe, faktori rizika, nacionalno istraživannje zdravlja*

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INTRODUCTION

Depression is the most frequent mental health problem in older age (1) with serious consequences on personal, interpersonal and social level (2). At least one in ten people aged 65 or more have significant symptoms of depression (3). Depressive disorders significantly impair quality of life and social functioning in diseased person, increasing overall morbidity and disability (4), causing significant social and economic consequences (5), threatening primarily women (6) and persons of lower socioeconomic status (7). It is also an independent predictor of mortality and is the leading cause of suicide in elderly adults, which is present in approximately 85% of elderly adults who have died by suicide (2).

Depression results from a complex interaction of genetic, biological, physiological and social factors (8). Changes characteristic for an old age and meny stressful events that occur more often in older age, such as chronical medical conditions, physical or cognitive functional decline, limited mobility, loss of independence, retirement, financial difficulties, isolation, death of a spouse or friend, loss of social support, contribute to the worsening of life perspective and depression (9,10). Most authors consider that risk factors simultaneously affect a person's vulnerability for the development of depression, and that this vulnerability varies depending on the interaction between some factors, as well as on the life circumstances of each individual (11). Depression often occurs in conjunction with other mental disorders and physical illnesses and is often undiagnosed and untreated, because it is considered that the depression is common and natural reaction to the changes that older age brings (9,12).

The global burden of depression is on the rise, which is mainly attributed to the aging of the population and to the growing number of people with physical and mental diseases which are often accompanied by depressive disorders. If current trend continues, according to the WHO estimations, by the year of 2030 depressive disorders are going to become the leading diagnostic category among the causes of disease burden worldwide, primarily in middle and higher income countries (13).

The aim of this study was to determine the association of demographic factors, socio-economic factors and health status characteristics, with the presence of depressive symptoms in the elderly persons.

METHODS

Study population and sample

The survey was conducted as a part of the national study "Health Survey of the Serbian population" in 2013, by mass interviewing a random, representative sample of the population of Serbia. The survey was conducted according to the model of cross-sectional study in the Republic of Serbia and it did not include the population living on the territory of Kosovo and Metohija. The target population did not include persons living in collective households and institutions. The survey was conducted in accordance with the methodology and instruments of the European Health Survey - Second Wave (European Health Interview Survey - EHIS wave 2) (14). It was implemented by the Ministry of Health of the Republic of Serbia.

The sample consisted of all households listed in all enumeration areas in census conducted in 2011. The mechanism used to obtain the random sampling of the respondents and households was a combination of two sampling techniques: stratification and a multi-stage sampling. A stratified two-stage sample of the population of the Republic of Serbia has been chosen in such a way to provide a statistically reliable assessment of the indicators that indicate the health status of the population at the national level, as well as at the level of 4 geographic areas (Vojvodina, Belgrade, Shumadija and Western Serbia, Southern and Eastern Serbia) identified as the main strata in the sample. Their further division into urban and rural areas resulted in a total of 8 strata. In the first stage, a total of 670 enumeration areas was selected. The households were the units of the second stage. Within each enumeration area, 10 addresses were selected (+3 spare addresses) inhabited by households to be interviewed. Out of total 10,089 households contacted, 6500 of them agreed to participate in the survey, so that the response rate of households was 64.4%. Of the total of 16,474 registered household members aged 15 years and over, 14,623 of them agreed to be interviewed, giving a response rate of 88.9%. Out of this number of people who agreed to be interviewed, 13,756 of them accepted to fill in the questionnaire (response rate 94.1%). Data on the population aged 65 years and over were used for the purposes of this study (3540 respondents).

Instruments

The questionnaires constructed in accordance with the questionnaire created by the European health research- the second wave (wave EHIS 2) (14), defined by internationally accepted indicators and adapted to the particularities of the area, were used as the instruments in this research (15).

To assess the presence of symptoms of depression, a Patient Health Questionnaire-8 (PHQ-8 questionnaire) containing eight questions which relate to the following physical problems was used: a reduction of interest or of feeling of pleasure in performing the activities; discouragement, melancholy, hopelessness; sleep problems (trouble falling asleep, sleeping in a continuity or too much sleep); a feeling of fatigue or lack of energy; reduced or increased appetite; feeling bad about themselves, feeling of failure, disappointment of themselves or of their families (feeling of worthlessness or of excessive or inappropriate guilt); difficulties with concentration during the activities such as reading a newspaper or television viewing (decreased ability to think or to concentrate, or ambiguities - estimated

subjectively or by others); slowed movement or speech, or otherwise, psychomotor restlessness that caused that they were moving more than usual (observed by others, and not just a subjective feeling).

Based on questions from the PHQ-8 questionnaire, respondents answered how often they were bothered by any of these mental health problems during the previous two weeks. Possible answers were "not at all", "a few days", "more than 7 days" and "almost every day". Responses to each question were evaluated using the score of 0 ("not at all"), 1 ("a few days"), 2 ("more than 7 days") and 3 ("almost every day"), and after gathering points for each answer, the total score obtained the values that ranged from 0 to 24. The score values 0 to 4 indicate the absence of symptoms of depression, a score of 5 to 9 denotes mild depressive symptoms (subsyndromal depression), and values close to 10 and more clearly indicate a high probability of the existence of a depressive episode (depression), which is further qualified as moderate (score 10 to 14), moderately severe (score 15 to 19) and severe depressive episodes (score of 20 and above). Based on the value of the 8-PHQ score, the patients were divided into one of the 3 categories: no symptoms of depression, depressive symptoms are mild (subsyndromal depression) and a depressive episode (depression) (16).

Following variables were used in the study: demographics (gender, age, marital status and type of settlement), socio-economic (education level, employment status and financial status), diverse dimensions of health (self-assessment of health, limitations in activities of daily living (ADL), chronic diseases and presence of pain) and social support.

Social support score (Oslo-3 Social Support Scale) was formed on the basis of three questions from the questionnaire and assigning a certain number of points for each answer: "How many people are so close to you that you can count on them when you have serious personal problems?" (Points ranging from 1 ("None") to 4 ("6 or more")), "How many people are really interested in you, in what you are doing, or in what is going on in your life?" (scores ranging from 1 ("not interested at all") to 5 ("very interested")), "How easy is for you to get practical help from neighbors if you have a need for it?" (The number of points ranges from 1 ("very difficult") to 5 ("very easy")). After collecting points, the scores of social support were formed: a strong social support (12-14 points), moderate (9-11 points) and bad (3-8 points) (17).

Statistical analysis

All the data of interest were presented and analyzed by adequate mathematical-statistical methods appropriate for the data type. The Chi-square test was used to compare proportions between groups. The t-test was used to compare continuous variables between groups. All the results where the probability is less than 5% are considered to be statistically significant. The relations between the presence of depressive symptoms, as a dependent variable, and a set of independent variables was examined by univariate and multivariate logistic regressions. Univariate logistic regression models were used to examine the associations between potential factors and the depressive symptoms. Variables that were statistically significant ($p< 0.05$) were further examined in multivariable logistic regressions. The unadjusted odds ratios (ORs) with their corresponding 95% confidence intervals (CIs) were also obtained. All statistical calculations were performed using commercial, standard software package SPSS, version 18.0. (The Statistical Package for Social Sciences software (SPSS Inc, version 18.0, Chicago, IL)).

RESULTS

Depression (PHQ-8 score ≥ 10) was registered in 10% of the population aged 65 and above, wherein it was statistically significantly higher in women (12.7%) than in men (6.5%) (χ^2=95.294, p<0,001). In relation to the subcategories of depression, largest percentage of respondents had moderate depressive episode (5.8%), and severe depression was detected in 1.6% of the respondents. Mild depressive symptoms (subsyndromal depression) were present in every fifth women (21.2%) and every eighth man (12.7%). The average value of the 8-PHQ score was 3.5 and it was statistically significantly higher among women (4.1) than in men (2.6) (t=-10.493, p<0,001) (Table 1).

Among the subjects who have had major depressive episode, every second had moderate depressive episode

Table 1. Prevalence of depression in the population aged 65 and over by gender

PHQ-8 scor	Females		Males		Total		p
	n	%	n	%	n	%	
0-4 (none depressive)	1330	66.1	1235	80.8	2656	72.5	
5-9 (mild depresive)	427	21.2	194	12.7	621	17.5	<0.001
10-24 (depressive episode)	255	12.7	99	6.5	354	10.0	
10-14 (moderate)	149	7.4	56	3.7	205	5.8	
15-19 (moderately severe)	65	3.2	28	1.8	93	2.6	
20-24 (severe)	41	2.1	15	1.0	56	1.6	
Average value of score	2.6		4.1		3.5		<0.001

Table 2. Prevalence of depression and demographic and socio-economic characteristics of the respondents

PHQ-8 scor	No symptoms of depression		Moderate symptoms of depression		Depressive episode		p*
	n	%	n	%	n	%	
Age (years)							
65-74	1501	76.8	315	16.1	139	7.1	<0.001
75-84	945	68.2	271	19.6	169	12.2	
85+	119	59.5	35	17.5	46	23.0	
Marital status							
Single	26	60.5	9	20.9	8	18.6	<0.001
Married	1521	72.4	309	15.7	135	6.9	
Widowed	943	66.2	284	19.9	198	13.9	
Separated/Divorced	75	70.1	19	17.8	13	12.1	
Education							
Elementary school or lower	1269	64.9	425	21.7	262	13.4	<0.001
Middle school	881	79.8	154	13.9	69	6.3	
High school/College or higher	415	86.5	42	8.8	23	4.8	
Employment status							
employed	229	59.8	105	27.4	49	12.8	<0.001
unemployed	68	67.3	16	15.8	17	16.8	
inactive	2268	74.2	500	16.4	288	9.4	
Well-being index							
Poor class	1321	66.9	409	20.7	245	12.4	<0.001
Middle class	469	74.3	104	16.5	58	9.2	
Rich class	757	83	108	11.6	51	5.5	
Place of residence							
Urban	1471	77.3	283	14.9	149	7.8	<0.001
Rural	1094	66.8	338	20.6	205	12.5	
Social support							
strong	25	80.6	3	9.7	3	9.7	<0.001
moderate	291	61.8	96	20.4	84	17.8	
poor	1849	60.8	522	18.2	667	21.9	

* Chi-square test

(57.9%), 26.3% had a moderately severe, and 15.8% had severe depressive episode, with no statistically significant differences between genders. In patients with depressive episode, the most common symptoms that lasted more than seven days, or were present almost every day, were a feeling of fatigue or lack of energy (90.7%), sleep problems (75.7%), feelings of discouragement and hopelessness (66.5%).

The prevalence of depression continuously increases with age and it was highest among respondents aged 85 and over (23%), which is three times higher prevalence in relation to the age group of 65-74 years (7.1%) (χ^2=75.44, p<0.001). In terms of marital status, the results showed that the lowest percentage of depression was among respondents who were married or cohabiting (6.9%). The highest number of depressed persons was among those who have never been married or cohabiting (18.6%) (χ^2=68.49, p<0.001). In the relation to the education degree, the prevalence of depressive episodes was the highest in the category of the least educated persons (13.4%), and every forth respondent with primary or lower level of education (21.7%) had mild depressive symptoms (χ^2=136,61, p<0.001). Between the well-being index and the prevalence of depression, there was an inverse association, so the percentage of depressed patients was the highest among the poor (12.4%) (χ^2=84.84, p<0.001). When it comes to a place of residence, a significantly higher percentage of subjects with depression is present in the rural areas (12.5%), compared to the urban areas (7.8%) (χ^2=49.43, p<0.001). Among respondents who have had a strong social support, more than 90% of them didn't have symptoms of depression, while among those with a low score of social support every fifth respondent was depressed (21.9%) (χ^2=50.05, p<0.001) (Table 2).

There was a statistically significant correlation between the self-assessment of health status and the presence of the depressive symptoms (χ^2=713.08, p<0.001). Among respondents who rated their health as "bad or very bad", there was significantly higher percentage of those who had depressive episode (21.8%), compared to those who evaluated their health as "very good or good" (0.4%).

The presence of depression symptoms is significantly different relative to the degree of limitation in the performance of daily activities (χ^2=621.54, p<0.001). More than one third of patients with severe constraints on performing of daily activities (36.8%) had a major depressive episode, while only 1.7% of the subjects without limitations, manifested depressive episode. More than a quarter of subjects with severe or very severe pain in the previous four weeks had depressive symptoms (27.5%), while

Table 3. Prevalence of depression and health status of the respondents

PHQ-8 scor	No symptoms of depression		Moderate symptoms of depression		Depressive episode		p*
	n	%	n	%	n	%	
Self-assessment of health							
good	747	94.6	40	5.1	3	0.4	
moderate	1123	84.2	168	12.6	43	3.2	<0.001
poor	695	49.1	413	29.2	308	21.8	
Limitations in everyday activities							
no limitations	1843	88.9	196	9.5	35	1.7	
limited, but not serious	603	55.9	335	31.1	140	13.0	<0.001
seriously limited	42	36.8	30	26.3	42	36.8	
The presence of bodily pain							
didn't have a pain	1079	90.7	84	7.1	26	2.2	
weak pain	546	79.5	105	15.3	36	5.2	<0.001
moderate pain	578	22.5	216	34.8	73	20.6	
strong and very strong pain	362	45.4	216	27.1	219	27.5	
The impact of pain on daily activities							
not at all	214	87	25	10.2	7	2.8	
lightly and moderate	980	71.2	306	22.2	90	6.5	<0.001
high and very high	292	40.1	206	28.3	231	31.7	
Chronic diseases / conditions							
no chronic diseases	390	93.3	20	4.8	8	1.9	
have one chronic disease	601	89.7	51	7.6	18	2.7	<0.001
have two or more chronic diseases	1574	64.2	550	22.4	328	13.4	

* Chi-square test

Table 4. Odds ratios (OR) and 95% confidence intervals (CI) for the depression depending on demographics and socioeconomic characteristics

	Univariate model		Multivariate model	
	OR (95%CI)	p	OR(95%CI)	p
Gender				
male	1		1	
female	2.161 (1.847-2.530)	< 0,001	1.678 (1.395-2.018)	< 0,001
Age groups				
65-74	1		1	
75-84	1.539 (1.319-1.796)	< 0,001	1.373 (1.164-1.621)	< 0,001
85+	2.250 (1.665-3.041)	< 0,001	1.728 (1.250-2.389)	< 0,001
Marital status				
married / common-law marriage	1		1	
never married / common-law marriage	2.24 (1.204-4.165)	< 0,001	2.023 (1.056-3.879)	0.034
widower / widow	1.751(1.503-2.039)	< 0,001	1.287 (1.077-1.538)	0.006
divorced	1.462 (0.954-2.240)	< 0,001	1.433 (0.916-2.239)	0.115
Settlement type				
city	1		1	
other	1.690 (1.457-1.961)	< 0,001	1.255 (1.045-1.506)	0.015
Education				
high and senior high school	1		1	
high school	1.616 (1.197-2.181)	< 0,001	1.366 (1.002-1.862)	0.048
primary school and lower	3.456 (2.619-4.562)	< 0,001	1.886 (1.379-2.580)	< 0,001
Wellbeing Index				
rich class	1			
middle class	1.684 (1.315-2.155)	< 0,0005	1.433 (1.105-1.858)	0.07
poor class	2.413 (1.986-2.932)	< 0,0005	1.607 (1.272-2.033)	< 0,001
Social support				
strong	1		1	
moderate	1.725 (1.215-2.541)		1.523(1.051-2.264)	< 0,001
poor	3.749 (2.428-5.845)		3.045 (1.984-4.829)	< 0,001

Table 5. Odds ratios (OR) and 95% confidence intervals (CI) for the depression depending on health caracteristics

	Univariate model		Multivariate model	
	OR (95%CI)	p	OR(95%CI)	p
Self-assessment of health				
good	1		1	
average	3.264 (2.322-4.589)	< 0,001	1.561 (1.010-2.411)	0.045
poor	18.022 (13.027-24.932)	< 0,001	3.495 (2.276-5.365)	< 0,001
Limitations in everyday activities				
no limitations	1		1	
limited, but not serious	6.285 (5.238-7.540)	< 0,001	2.995 (2.389-3.756)	< 0,001
seriously limited	13.867 (9.268-20.760)	< 0,001	6.846 (4.047-11.582)	< 0,001
Chronic diseases / conditions				
no chronic diseases	1			
have one chronic disease	1.599 (1.012-2.526)	0.449	1.499 (0.743-3.026)	0.258
have two or more chronic diseases	7.770 (5.249-11.501)	< 0,001	3.109 (1.640-5.891)	0.001
The presence of bodily pain				
didn't have a pain	1			
weak / moderate pain	3.756 (2.995-4.702)	< 0,001	1.276 (0.957-1.700)	0.046
strong and very strong pain	11.787 (9.266-14.995)	< 0,001	3.710(1.619-5.826)	0.001
The impact of pain on daily activities				
not at all	1		1	
lightly and moderate	2.702 (1.831-3.989)	< 0,001	1.614 (0.978-2.663)	0.061
high and very high	10.008 (6.709-14.930)	< 0,001	1.319 (0.863-2.015)	0.201

among respondents with no presence of pain, depression was present in only 2.2% (χ^2=622.55, p<0.001). Research also confirmed an association between the presence of chronic diseases and depression (χ^2=276.64, p<0.001), subjects who had two or more of chronic diseases (multimorbidity) were seven times more likely to have a depression (13.4%), as compared to those without chronic diseases (1.9%) (Table 3).

All demographic and socio-economic variables were analyzed using the multivariate model. Results of the study showed that women (OR=1.68) and persons aged 85 years or more (OR=1.72) are 1.7 times more likely to be depressed in relation to the men and respondents aged 65-74 years. Also, a life without a partner (OR=2.02), the lowest level of education (OR=1.89) and poverty (OR=1.61), proved to be significant predictors of depression. A chance for the presence of depression was three times higher in people with poor social support (OR=3.04) (Table 4).

Limitations in performing of daily activities, when it comes to health status of respondents, showed to be the strongest predictor of depression in the elderly, while respondents who have had serious limitations had even six times more chanse to develop depression (OR=6.85). Respondents who rated their health as "bad or very bad" for 49.5% more frequently manifested depressive symptoms compared to those who evaluated their health as "very good or good" (OR=3.49). Respondents who have had two or more chronic diseases were three times more likely to have depression (OR=3.11) compared to people without chronic disease, while those with chronic pain were for 71% more likely to express depressive symptoms (OR=3.71) (Table 5).

DISCUSSION

Among citizens of Serbia aged 65 years and over, 17.5% of respondents had mild depressive symptoms, and a depressive episode was registered in 10% of the population. The average prevalence of depression in elderly adults varies between 10.0% and 15.0% (18), while some countries report significantly higher rates of prevalence. In Brazil, the prevalence of depression was 30.6% (1), while Cahoon reported that depression affects an estimated 15% to 19% of Americans age 65 and older (19). Large variations between countries concerning the prevalence of depression can be explained by socio-demographic and cultural differences, as well as by differences in the methodological approach to data collecting (1).

The prevalence of depression in our sample continuously increases with age and it has the highest values in the category of 85 years of age and over (23%). Studies suggest that people age 65 and older have twice the chance to develop a depression in comparison to younger adult population, as well as that an older age is the predictor of depression (20), and that the prevalence of depression is the highest in the population of the oldest persons (21).

The number of women reporting the depression symptoms is twice as high as the number of men (12.7% : 6.5%), which is in accordance with the results from the available literature that are supporting the fact that women are at greater risk to develop a depression (6,22,23). Gender difference in the prevalence of depression is explained by a number of factors (genetic, biological, psychological), as well as by a different social roles and status of women in society (24). Studies showed that the increased risk of depression in women, at least partly, is attributable to negative attitude towards them, to the lack of appreciation for their work, to fewer op-

portunities for education and employment and to a greater risk of domestic violence (25). The fact that men often do not recognize their symptoms of depression, as depression is generally considered as a "women's disease", should be taken into account in interpreting the differences in the prevalence of depression between women and men (26). Women are more open and willing to express the symptoms they feel, verbally as well as behaviorally, they more often complain of psychosomatic ailments and emotional instability, which is why they more frequently use the health care services (1). Power and health are traditionall masculine values and that is why men often avoid to express their emotions that are typical for females, and this may contribute to masking of depression (27).

The results of our study showed an association between depression and marital status. The smallest number of depressive was detected among respondents who were married or cohabiting, while multivariate regression model identified the best chance to develop a depression among people who have never been married/cohabiting (OR=2.02). Depressive simptoms are more common among persons who were either never married or who were previously married and currently have no partner, compared with married people (22). Yan et al. conducted a quantitative meta-analysis of the association between depression and marital status in people over the age of 55, based on 24 cross-sectional studies and eight longitudinal studies. Compared to married elderly people, unmarried elderly people (OR=1.55), widowed (OR=1.49) and never-married people (OR=1.32) had a higher risk for depression (28).

Empirical studies conducted in the past few decades showed that people who are married have a series of physical and mental benefits, achieve greater functionality, have better subjective assessmment of their health condition, have lower rates of morbidity and live longer than individuals who are not married (29). Married people have the advantage in terms of financial resources and stability, social and psychological support, as well as a support in choosing a healthy lifestyle (30).

The association between depression and socio-economic status was confirmed by the results of our study, where it was found that individuals with the lowest level of education (OR=1.88) and those belonging to the class of poor (OR=1.61) are significantly more likely to develop the symptoms of depression. Several studies confirm a strong inverse relationship between SES and depression (31,32). In a prospective cohort study, Koster and associates analyzed the correlation between depression and socio-economic status. The level of education and financial income at 2593 respondents aged 55-85 years were observed as indicators of socio-economic status. The obtained results showed that the persons of lower socioeconomic status have had a 50% greater chance of being depressed in comparison with those belonging to the highest SES group (33). Studies show that the lack of income and inability to satisfy the daily needs, along with the emotional stress due to an uncertain future, are significant predictors of depression (34). On the other hand, a higher level of education provides a better job and higher social status, better developed social networks and adopting healthy lifestyle habits (better access to informations). Better educated people have higher levels of health literacy, healthier lifestyle, better use of available informations in dealing with everyday problems that could negatively affect their health (35).

When it comes to a social support, among respondents who have a strong social support over 90% of them do not have symptoms of depression, while among those with poor social support every fourth respondent has a mild depressive symptoms or depressive episode, which is in accordance with results from the literature that indicate a strong relationship of depression and social support (36, 37). Good social support network increases the self-esteem of the individual and the resistance to the occurrence of negative emotions and depressive symptoms (38).

Poor physical health, functional disability, and chronic pain are proven to be a significant predictor of depression (18), but on the other hand, depression worsens outcomes of physical illness (39). As expected, our results showed that people who have two or more chronic diseases have three times higher risk of being depressed (OR=3.109), while people who have serious limitations in performing daily activities have almost seven times greater risk of depression (OR=6.846). Many epidemiological studies indicate a high comorbidity of depression and other psychiatric disorders, somatic symptoms and physical ailments, especially in population of the oldest persons (40, 41). Large 14-center, cross-national study conducted in nine western European countries, whose aim was to test an association between physical health and depressive symptoms of patients older than 65 years, showed that the association of depressive symptoms with functional disability was stronger than the one with chronic physical conditions (42).

CONCLUSION

Identification of risk factors associated with the development of depression in elderly, early diagnosis and timely, adequate and effective treatment and management of depression, are crucial factors to reduce the serious consequences to which these disorders can lead the individual, their families and the community as a whole. Mental health of older people can be improved through the promotion of active and healthy aging, which means creating the conditions and environment that support the well-being and enable people to live a healthy and integrated lifestyle.

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TREATMENT OF COMPLEX FEMORAL FRACTURES WITH THE LONG INTRAMEDULLARY GAMMA NAIL

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TRETMAN KOMPLEKSNIH PRELOMA BUTNE KOSTI DUGIM INTRAMEDULARNIM GAMA KLINOM

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ABSTRACT

The purpose of the current study was to present the authors' experiences with the long intramedullary Gamma nail in the treatment of patients with complex femoral fractures. This retrospective study included 48 patients with complex femoral fractures. All patients had received fracture fixation treatments with long intramedullary Gamma nails from January 2007 to December 2015. The complex fractures of all patients were classified into 3 types, according to the anatomical locations of the fractures. Type I included combined fractures of the shaft and the proximal femur. Type II included segmental fractures. Type III included combined fractures of the shaft and distal femur. According to the Harris Hip Score, 85.4% of our patients had excellent and very good functional outcomes of the operative procedure. Complications occurred in 7 (14.58%) patients. The most common complications occurred in patients with combined fractures of the shaft and distal femur (50%). Based on the findings of this study, we conclude that the clinical and radiological results after the treatment of complex femoral fractures with the long intramedullary Gamma nail show good outcomes, with a high rate of bone union and minimal soft tissue damage. Experience with this procedure is important to prevent and minimise technical complications.

Keywords: *long intramedullary Gamma nail; complex femoral fractures; Harris Hip Score*

SAŽETAK

Cilj ove studije bio je da predstavi iskustvo autora u lečenju kompleksnih preloma butne kosti dugim intramedularnim gama klinom. Ovo je retrospektivna studija koja obuhvata 48 pacijenata sa kompleksni prelomom butne kosti. Svi pacijenti su u periodu od januara 2007. do decembra 2015. godine lečeni metodom intramedularne fiksacije dugim Gama klinom. Kompleksni prelomi su prema anatomskoj lokalizaciji klasifikovani u tri tipa. Tip I obuhvata kombinovane prelome dijafize i proksimalnog dela femura. Tip II obuhvata segmentalne prelome. Tip III obuhvata kombinovane prelome dijafize i distalnog dela femura. 85,4% naših pacijenata imalo je odličan i vrlo dobar funkcionalni rezultat nakon operativne procedure, procenjen na osnovu Harris Hip Scor-a. Komplikacije su nastale kod 7 (14,58%) pacijenata. Najčešće komplikacije su primećene kod pacijenata sa kombinovanim prelomom dijafize i distalnog dela femura (50%). Na osnovu ove studije možemo zaključiti da nakon intramedularne fiksacije dugim Gama klinom klinički i radiološki rezultati imaju dobar ishod uz visok stepen sraščivanja kostiju i minimalno oštećenje mekog tkiva. Iskustvo je važno za sprečavanje i smanjenje tehničkih komplikacija.

Ključne reči: *dugi intramedularni Gama klin; kompleksni prelomi butne kosti; Harris Hip Scor*

ABBREVIATIONS

DHS-dynamic hip screw
DCP-dynamic compression plate
HHS-Harris Hip Score
LCP-locking compression plate

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INTRODUCTION

With aging populations and a prolonged average life expectancy, the incidence of femoral fractures has sharply increased and is expected to double in the next 25 years in industrialised countries (1). It is estimated that half of these fractures will be intertrochanteric, which have a high morbidity and mortality and seriously affect a patient's quality of life (1, 2).

Complex femoral fractures have been defined as unstable, combined fractures of the shaft and proximal or distal femur, as well as segmental fractures of the femur. These fractures are often difficult to treat and represent an important challenge in the field of traumatology (3). Complex femoral fractures occur more often in a younger population and are most commonly caused by high-energy trauma, either in traffic accidents or falls from heights (4). On the other hand, the most common cause of femoral fractures in older populations is a mild trauma (5). An early stabilisation of the fractured bone contributes to the preservation of blood supply and is essential for the healing process (6, 7). Surgical treatments are gradually becoming the first-choice treatments of complex femoral fractures. The primary goal of operative treatments for complex femoral fractures is to restore the anatomical alignment of the bony fragments, which allows early mobilisation of the patient and the limb (8). Although technologies and instruments have been continuously improved, fixation failure still poses a problem in the treatment of unstable femoral fractures. The operative treatment of complex fractures is very complicated because complex fractures are often associated with other serious injuries, and it is necessary to treat two or more levels of fractures with dislocation at the same time (9). The combination of two different fixation methods (such as dynamic hip screw (DHS) and dynamic compression plate (DCP), or condylar plate and DCP, or locking compression plate (LCP) and DHS) on the same femur is associated with numerous complications and poor functional results. Therefore, the use of a plate to achieve osteosynthesis of complex fractures necessitates a wide operative exposure and extensive stripping of soft tissue, which results in increased blood loss and a longer operating time. All of these techniques require a very long period of rest and an avoidance of support in the injured leg, which also leads to more frequent postoperative complications (10).

Considering previously mentioned facts, as well as long-term clinical experiences and follow-up observations, the use of an osteofixation system is necessary. The simultaneous repair of all fractures with an osteofixation system will improve the results of treatment of these complex injuries. Accordingly, the Gamma nail was developed in an attempt to overcome some of these problems. The Gamma nail, introduced in the late 1980s, combines intramedullary fixation in the shaft with a screw in the proximal fragment (8-12).In the 1990s, some authors were describing treatment with the Gamma nail as being equivalent to, or better than, treatment with the dynamic hip screw (13, 14). In contrast, other authors have observed that the Gamma nail has a higher rate of serious complications, such as perioperative fractures of the femoral shaft (15, 16). However, since 1988, the Gamma nail has been modified several times. Modifications in the nail design and attention given to specific details during the nail insertion have significantly decreased the rate of perioperative complications. These decreases in complications have been observed in recent papers, which have described the use of a newer generation of the Gamma nail (8, 17). The Gamma nail comes in two types; the standard nail (20 cm) is mainly used for trochanteric fractures, while complex fractures are treated with the longer (32-42 cm) version (12). Theoretically, the re-designed Gamma nail should reduce the occurrence of complications, such as the removal and extension of the nail, over-compression of the fracture, and collapse (18).

The use of the long Gamma nail during treatment has provided good results, in terms of less invasiveness, better fixation and faster rehabilitation of patients with complex femoral fractures. However, despite the good and reliable results, some typical failures and complications may occur (19). In addition to the quality of the implant, the success of femoral fracture treatment depends on the quality of the bones, the age of the patient, the general health status of the patient, the time interval between the fracture and treatment, the treatment adequacy, the patient's comorbidity, and the stability of fixation. (20, 21).

The purpose of the current study was to present the authors' experience with the long intramedullary Gamma nail in the treatment of patients with complex femoral fractures, in order to assess the success and safety of the technique.

PATIENTS AND METHODS

This retrospective study included patients with complex femoral fractures who had received fracture fixation treatments with the long intramedullary Gamma nail (produced by Synthes Switzerland, Stryker SAD, LIMA Italy) from January 2007 to December 2015. Forty-eight patients were included, and all patients were recruited from the Orthopedics and Traumatology Clinics of the Clinical Center Montenegro in Podgorica. A retrospective review of clinical charts and preoperative, perioperative, postoperative, and final radiographs were performed. The resulting outcomes and complications were recorded, while rehabilitation was supervised by physiotherapists.

Inclusion criteria were patients with complex femoral fractures who were treated with the long intramedullary Gamma nail and who were older than 16 years of age. Exclusion criteria were as follows: pathologic fractures, previous chemotherapy and/or radiotherapy, rheumatic diseases, a previous operation in the same hip/femur, and a traumatic amputation through or above the knee.

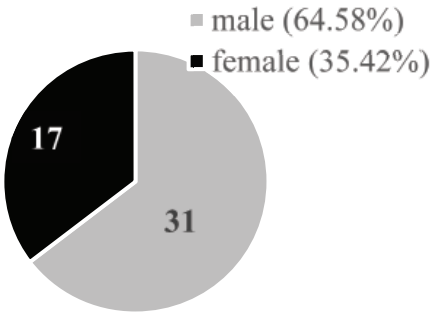

Figure 1. Gender distribution of the study population

The complex fractures of all patients were classified into 3 types, according to the anatomical locations of the fracture. Type I included combined fractures of the shaft and proximal femur. Type II included segmental fractures. Type III included combined fractures of the shaft and distal femur.To estimate the functional outcome of the operative procedure, we used the Harris Hip Score (HHS). This is a time-tested scoring system used for the evaluation of hip function, and is based on the best responses from the patient regarding different aspects of life. All patients were divided into 3 categories, based on the HHS: (1) excellent and very good HHS, (2) good HHS, and (3) poor HHS (22). Patients who were unable to come regularly were contacted via telephone, and the Harris Hip Score was evaluated and documented for analysis.

Bone healing was assessed both radiologically and clinically by using conventional X-ray studies, as well as by evaluating clinical symptoms, including pain associated with full weight-bearing. Healing was concluded with the formation of a bridging callus and the crossing of bone trabeculae on the fracture line in at least three out of four cortices, as well as the absence of pain with full weight-bearing (23).

The median duration of follow-up of all patients was 4.7 years (with a range of 30 months to 8.3 years).

RESULTS

Altogether, 48 patients, including 31 males and 17 females, were included in this retrospective study. The gender distribution of the study population is presented in **Figure 1**. The mean age was 42 years. The youngest patient was 17, while the oldest patient was 74 years old.As we mentioned before, the complex fractures of all patients were classified into 3 types (**Table 1**).

According to the Harris Hip Score, 41 patients were rated as excellent and very good, 4 patients were rated as good, and 3 patients were rated as poor (**Table 2**).Complications occurred in 7 (14.58%) patients with complex femoral fractures that were treated with the intramedullary long Gamma nail. In addition, one patient presented with both complications (infection and malunion). The overall complications encountered in the perioperative and postoperative periods are listed in **Table 2**.

Two patients with type I fractures, two patients with type II fractures, and three patients with type III fractures had some of the previously mentioned complications. The connections between the type of fracture and the frequency of perioperative and/or postoperative complications are presented in **Table 3**.

Table 1. Clinical characteristics of the study population

Classification of fracture	Number (percentage)
Type I	30 (62.5%)
Type II	12 (25%)
Type III	6 (12.5%)

Table 2. Functional outcome of the operative procedure

Harris Hip Score (HHS)	Excellent and very good	41 (85.4%)
	Good	4 (8.3%)
	Poor	3 (6.3%)
The presence of complications	Without complications	41 (85.42%)
	Infection	1 (2.08%)
	Non-union	2 (4.17%)
	Mal-union	2 (4.17%)
	Lag screw cut-out	2 (4.17%)
	Broken nail	1 (2.08%)

Table 3. Connection between the type of fracture and frequency of complications

Classification of fracture	Number of patients
Type I	2 (6.67%)
Type II	2 (16.67%)
Type III	3 (50%)

DISCUSSION

The treatment and surgical stabilisation of complex femoral fractures is a challenge for orthopaedics. Both design and technical problems have been found in various osteofixation systems. Consequently, the ideal implant for complex femoral fractures remains a matter of discussion (24, 25). Older implants (Jewett nails, McLaughlin nails, Ender intramedullary nails) have been reported to have unacceptably high complication rates (26, 27). Alternatively, the use of modern implants, such as dynamic hip screws, Gamma nails, and Medoff sliding plates, can reduce the incidence of complications and the patient's recovery time (10, 17).Consequently, the aim of the current study was to present the authors' experiences with the long intramedullary Gamma nail in the treatment of patients with complex femoral fractures.

In this retrospective study, 85.4% of our patients had excellent and very good functional outcomes of the operative procedure, according to the HHS. On the other hand, only 6.3% of patients were rated as poor by the HHS (Table 2). Considering the severity of the fractures, these findings suggests that treatment with the long Gamma nail produces more than satisfactory functional results in our hospital.

In the 48 reported cases, complications occurred in 14.58% of patients treated with the long intramedullary Gamma nail. Data from the literature data suggest that infections are the most common complications of fractures and predominantly occur in open fractures (28). Only one (2.08%) of the 48 patients had a mild infection. This rate is lower than that usually reported for Gamma nailing procedures (28-31). Other frequent complications, such as nonunions (where the fractured bone fails to heal) or malunions (where the fractured bone heals in a deformed manner) were noted in our patients. A union was defined as a callus formation at the fracture site, with the fracture line visible for less than a quarter of the circumference. Nonunions were reported in two patients (4.17%), and malunions were also reported in two patients (4.17%). Nellaiyappan and coworkers reported 2 (10.52%) cases of nonunions in 19 patients with complex femoral fractures (9). The most common nail-related complication (cut-out of the lag screw, mainly because of poor positioning in an osteoporotic bone) was observed in two patients (4.17%). The literature showed cut-out frequencies up to 10% (29, 30). In addition, a broken nail occurred in only one patient. The broken nail was easily removed using the technique involving an olive-tripped guide wire (32). The frequency of complications in our study (Table 2) is in accordance with previous literature data (14, 28-31). Most importantly, almost all of the complications occurring after Gamma nail fixation can be prevented by following strict observance of the recommended surgical technique, careful preoperative planning, and rigid postoperative protocols. The choice of the appropriate length for the lag screw, its best position in the femoral neck, and its dynamic proximal locking will considerably reduce the incidence of screw cut-out (32, 9).

Alternative devices described for the treatment of these fractures showed higher complication rates than the long Gamma nail. A study conducted by Aktselis and coworkers suggested that the intramedullary Gamma nail is superior to a sliding hip screw in the treatment of multi-fragmentary intertrochanteric fractures (33).

Many studies have examined long Gamma nail efficacy in the treatment of different femoral fractures. In a paper by Sehat and coworkers, it was shown that the long Gamma nail is effective in the treatment of proximal femoral fractures (31). Zhang and coworkers concluded that the locking intramedullary Gamma nail is a simple and safe treatment for unstable intertrochanteric femoral fractures, with a satisfactory clinical efficacy (34, 35). However, in a comparative study of the Gamma nail versus the proximal femoral nail, Woo-Kie and coauthors demonstrated no differences in clinical outcomes for the treatment of reverse obliquity intertrochanteric fractures (36).

Finally, we examined the connection between the type of fracture and the frequency of complications, in order to complete the picture of the efficacy of the long intramedullary Gamma nail. The most common complications occur in patients with combined fractures of the shaft and distal femur (50%), while patients with segmental and with combined fractures of the shaft and proximal femur had a lower rate of complications (Table 3). In combined shaft and distal femur fractures, the indications for treating fractures with the long intramedullary Gamma nail should be carefully considered.

The present study had certain limitations. First, this study was retrospective, which is not the best method when compared to a prospective study. Second, this study included a small number of patients, due to the uncommon nature of this injury.

CONCLUSION

Complex femoral fractures are generally difficult to treat and provoke high complication rates. From the findings of this study, we can summarise that the clinical and radiological results after the treatment of complex femoral fractures with the long intramedullary Gamma nail show good outcomes, high rates of bone union, and minimal soft tissue damage. Experience with this procedure is important to prevent and minimise technical complications.

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THE EFFECTS OF N-METHYL-D-ASPARTATE RECEPTOR BLOCKADE ON OXIDATIVE STATUS IN HEART DURING CONDITIONING MANEUVERS

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EFEKTI BLOKADE N-METIL-D-ASPARTATNOG RECEPTORA NA OKSIDACIONI STATUS SRCA TOKOM KONDICIONIRANJA

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ABSTRACT

N-methyl-D-aspartate receptor (NMDAR) belongs to ionotropic glutamate receptor family. The most prominent roles of the NMDAR are related to the physiological and pathophysiological processes of the central nervous system (CNS). The link between NMDAR and cardiovascular pathology came into focus due to detrimental effects of homocysteine on the cardiovascular system. Regarding the fact that NMDAR affects Ca^{2+} homeostasis in cells, one of the main mechanisms which mediate adverse effects of glutamate dyshomeostasis and abnormal NMDAR activity is oxidative stress. Both in ischemia and during reperfusion, there are imbalance in Ca^{2+} and production of reactive species, which remains one of the basic mechanisms underlining the overall cardiomyocyte death due to myocardial infarction. The aim of this study was to assess the effects of blockade of NMDAR in heart using MK-801, in preconditioning and postconditioning fashion and to compare the values of oxidative stress biomarkers. We used Langendorff technique of isolated heart. In the control group, all isolated rat hearts were subjected to global ischemia after stabilization period (perfusion of the whole heart with Krebs-Henseleit solution was stopped) for 20 minutes, followed by 30 minutes of reperfusion. In the preconditioning group, after stabilization period, hearts were perfused with MK-801 for 5 minutes, before global ischemia of 20 minutes which was followed by 30 minutes reperfusion. In the postconditioning group, hearts were perfused with MK-801 during the first 3 minutes of reperfusion. Results of this study showed antioxidative effects of NMDAR inhibition in pre- and postconditioning of the isolated rat heart.

***Keywords**: N-methyl-D-aspartate receptor, MK-801, preconditioning, postconditioning, isolated rat heart*

SAŽETAK

N-metil-D-aspartatni receptor (NMDAR) pripada grupi jonotropnih glutamatnih receptora. Najznačajnije funkcije NMDAR se odnose na fiziološke i patofiziološke procese u centralnom nervnom sistemu (CNS). Veza između NMDAR i patoloških procesa kardiovaskularnog sistema je došla u žižu naučnog interesovanja tokom ispitivanja štetnih efekata homocisteina na kardiovaskularni sistem. Imajući u vidu činjenicu da aktivacija NMDAR remeti homeostazu Ca^{2+} u ćeliji, jedan od osnovnih mehanizama uključen u ispoljavanje poremećaja glutamatne ravnoteže i patološke aktivnosti NMDAR je oksidacioni stres. Kako tokom ishemije, tako i u reperfuziji, postoji poremećaj ravnoteže Ca^{2+} i povećana produkcija reaktivnih vrsta, što predstavlja jedan od osnovnih mehanizama koji posreduju u izumiranju kardiomiocita tokom infarkta. Cilj ovog istraživanja je ispitivanje efekata blokade NMDAR upotrebom MK-801 tokom prekondicioniranja i postkondicioniranja i poređenje vrednosti biomarkera oksidacionog stresa. Korišćena je Langendorfova tehnika izolovanog srca. U kontrolnoj grupi sva srca su povrgnuta globalnoj ishemiji odmah nakon perioda stabilizacije (prekinuta je perfuzija čitavog srca Kreps-Henselejtovim rastvorom) u trajanju od 20 minuta, nakon čega je usledila reperfuzija u trajanju od 30 minuta. U grupi sa prekondicioniranjem, nakon stabilizacije aplikovan je MK-801 tokom 5 minuta u koncentraciji od 30 µmol/L, nakon čega je sledila globalna ishemija tokom 20 minuta, pa reperfuzija 30 minuta. U grupi sa postkondicioniranjem MK-801 je primenjen tokom prva tri minuta reperfuzije. Rezultati istraživanja ukazuju na antioskidacioni efekat inhibicije NMDAR tokom prekondicioniranja i postkondicioniranja izolovanog srca pacova.

***Ključne reči:** N-metil-D-aspartatni receptori, MK-801, prekondicioniranje, postkondicioniranje, izolovano srce pacova*

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INTRODUCTION

NMDAR belongs to ionotropic glutamate receptor family, together with AMPA, kainite and delta receptors (1). Structurally, NMDAR is a protein complex composed of four subunits (tetramer) which assembles forming a cation channel. There are three types of NMDAR subunits: GluN1 (binds glycine), GluN2 (binds glutamate) and GluN3 (binds glycine) (2, 3). Functional NMDARs are most often composed of two GluN1 and two GluN2 subunits, and based on this fact the necessity arises for the action of both glycine and glutamate, as co-agonists, for the activation of NMDAR, unlike other members of this receptor family which require only glutamate. Furthermore, in contrast to other ionotropic glutamate receptors, activation of NMDAR enables influx of a considerable amount of Ca2+ which triggers subsequent Ca2+-mediated intracellular cascades (4, 5). Another particularity of NMDAR is voltage-dependent block by Mg2+, which makes these receptors both ligand-dependent and voltage-dependent (3).

The most prominent roles of the NMDAR are related to the physiological and pathophysiological processes of the CNS, including synaptic plasticity during development, learning and memory, while impairment of their function are most often related to Huntington's, Alzheimer's or Parkinson's disease (6-8). Over the past decade or so, scientific interest in the roles of NMDAR outside the CNS has grown, and it has been shown that NMDAR takes part in a large number of processes in various non-neuronal tissues (9). Given the fact that NMDAR is Ca2+ channel, it was of great interest to estimate its presence and function in the heart, an organ whose function is strictly dependent on equilibrium in intracellular Ca2+ homeostasis (10-12). The link between NMDAR and cardiovascular pathology came into focus due to detrimental effects of homocysteine (Hcy) on the cardiovascular system and its underlying mechanisms (13). Actually, it is proposed that Hcy induces overactivation, as well as overexpression, of NMDARs in cardiovascular tissues during hyperhomocysteinemia (HHcy).

Regarding the fact that NMDAR affect Ca2+ homeostasis in cells, one of the main mechanisms which mediate adverse effects of glutamate dyshomeostasis and abnormal NMDAR activity is oxidative stress (14, 15). Namely, overactivation of NMDAR may induce the increase of intracellular Ca2+ level leading to increased production of prooxidants and shifting redox state of the cell toward oxidative stress and damage. Most of the effects regarding the role of NMDAR in affection of oxidation-reduction processes are described due to nerve tissue and related disorders (16). Furthermore, it was also shown that NMDAR activation has an impact on redox homeostasis in cardiomyocytes and cardiac tissue (16, 17).

Despite all the efforts of modern medicine, myocardial infarction remains one of the leading causes of death today. More than 30 years ago, the phenomenon of ischemic preconditioning (IPC) was first described, and since then tremendous scientific efforts have been made to examine new possibilities for myocardial preservation (18). Namely, both in ischemia and during reperfusion there are imbalance in Ca2+ and production of reactive species, which remains one of the basic mechanisms underlining the overall cardiomyocyte death (19). Besides ischemic preconditioning, many other conditioning maneuvers have been developed over the years that aim to prepare the myocardium in the best possible manner for the onset of ischemia or to reduce damage after ischemia and during reperfusion, including pharmacological possibilities of pre- and postconditioning (20). One of the potential advantages of pharmacological conditioning maneuvers is the ability to act on a large number of molecular targets.

THE AIM OF THE PAPER

Bearing in mind all the facts, the aim of this study was to assess the effects of blockade of NMDAR in the heart using MK-801, in preconditioning and postconditioning fashion and to compare the values of oxidative stress biomarkers using Langendorff technique of isolated rat heart.

MATERIAL AND METHODS

Isolated heart preparation according to Langendorff

Hearts of male Wistar Albino rats were used in this study (10 in each experimental group). All animals were 8 week old, 200±30g body weight, obtained from Military Medical Academy, Belgrade, Serbia. After intraperitoneally applied combination of ketamine (10 mg/kg) and xylazine (5 mg/kg), rats were sacrificed by cervical dislocation (Schedule 1 of the Animals/Scientific Procedures, Act 1986, UK). Sacrifice was followed by urgent thoracotomy and harvesting of the heart. After a quick removal of the excess of tissue, the hearts were attached to Langendorff apparatus by aorta cannulation. Immediately after aorta cannulation, the hearts were retrogradely perfused under a constant perfusion pressure (CPP) of 70 cmH_2O with complex Krebs-Henseleit solution (NaCl 118, KCl 4.7, $CaCl_2 \cdot 2H_2O$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.7, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, pyruvate 2, equilibrated with 95 % O_2 plus 5 % CO_2 and warmed to 37 ºC (pH 7.4)).

Experimental protocol

The hearts from all experimental groups underwent 20-minute stabilization period. During this period, each of the hearts was subjected to a short-term occlusion (20 s) followed by simultaneous bolus injections of 5 mmol/l adenosine (60 µl at a flow of 10 ml/min to elicit maximal coronary flow) to test coronary vascular reactivity. If coronary flow (CF) did not increase by 100% compared to control values, the hearts were discarded. Coronary flow was determined flowmetrically. When the CF was stabilized (three measurements of the same value repeated), the samples of coronary effluent were collected (control value) and the experimental protocol was initiated. In the control group (CG), all isolated rat hearts were subjected to global ischemia after stabilization period (perfusion of the whole heart with Krebs-Henseleit solution was stopped) for 20 minutes, followed by 30 minutes

of reperfusion. In the preconditioning group (PreC), after stabilization period, the hearts were perfused with MK-801 (30 µmol/L) for 5 minutes, before global ischemia of 20 minutes which was followed by 30 minutes reperfusion. In the postconditioning group (PostC), the hearts were perfused with MK-801 (30 µmol/L) during the first 5 minutes of reperfusion.

The samples of coronary venous effluent were collected during experiments in the same points of interest: after stabilization period (control), after application of MK-801 for PreC group, in the first, third, fifth minute of reperfusion, and further in intervals of 5 minute until the end of the experiment.

All research procedures were carried out in accordance with European Directive for welfare of laboratory animals No 86/609/EEC and principles of Good Laboratory Practice (GLP), approved by Ethical committee of the Faculty of Medical Sciences, University of Kragujevac, Serbia.

Determination of oxidative stress biomarkers

The following oxidative stress parameters were determined spectrophotometrically (Specord S-600 Analytik Jena) in collected samples of the coronary venous effluent:

1. Index of lipid peroxidation, measured as thiobarbituric acid reactive substances (TBARS)
2. Superoxide anion radical (O_2^-)
3. Hydrogen peroxide (H_2O_2) and
4. Nitrite (NO_2^-).

TBARS determination (index of lipid peroxidation)

The degree of lipid peroxidation in the coronary venous effluent was estimated by measuring TBARS using 1% thiobarbituric acid in 0.05 NaOH incubated with the coronary effluent at 100 ºC for 15 min and measured at 530 nm. Krebs–Henseleit solution was used as a blank probe (21).

Nitrite determination

Nitric oxide decomposes rapidly to form stable metabolite nitrite/nitrate products. Nitrite level (NO_2^-) was measured and used as an index of nitric oxide (NO) production using Griess's reagent. A total of 0.5 ml of perfusate was precipitated with 200 µl of 30 % sulfosalicylic acid, vortexed for 30 min, and centrifuged at 3000 x g. Equal volumes of the supernatant and Griess's reagent, containing 1% sulfanilamide in 5% phosphoric acid/0.1% naphthalene ethylenediamine-di hydrochloride was added and incubated for 10 min in the dark and measured at 543 nm. The nitrite levels were calculated using sodium nitrite as the standard (22).

Determination of superoxide anion radical

The level of superoxide anion radical (O_2^-) was measured by nitro blue tetrazolium reaction in TRIS buffer with coronary venous effluent, at 530 nm. Krebs–Henseleit solution was used as a blank probe (23).

Determination of hydrogen peroxide

A measurement of hydrogen peroxide (H_2O_2) was based on oxidation of phenol red by hydrogen peroxide, in a reaction catalyzed by horseradish peroxidase (HRPO) (24). 200 µl of perfusate was precipitated with 800 ml of freshly prepared phenol red solution and then 10 µl of (1:20) HRPO (made ex tempore) was added. For blank probe, an adequate volume of Krebs–Henseleit solution was used instead of coronary venous effluent. The level of H_2O_2 was measured at 610 nm.

Drugs

All drugs used in this experimental protocol were provided by Sigma-Aldrich.

Statistical analysis

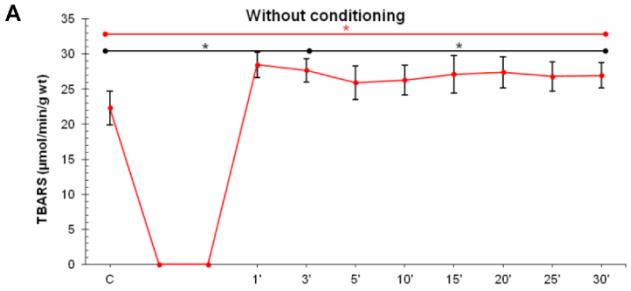
In CG values of measured biomarkers were compared in three points of interest: the last minute of stabilization (C), the third and the last minute of reperfusion period. In PreC group, we compared the last minute of stabilization (C), the last minute of application of MK-801, and the last minute of reperfusion period. In PostC group, three points of interest were same like in the CG: the last minute of stabilization (C), the third and the last minute of reperfusion. Values are expressed as mean ± SE. Statistical analysis was performed by ANOVA test. P values lower than 0.05 were considered to be significant.

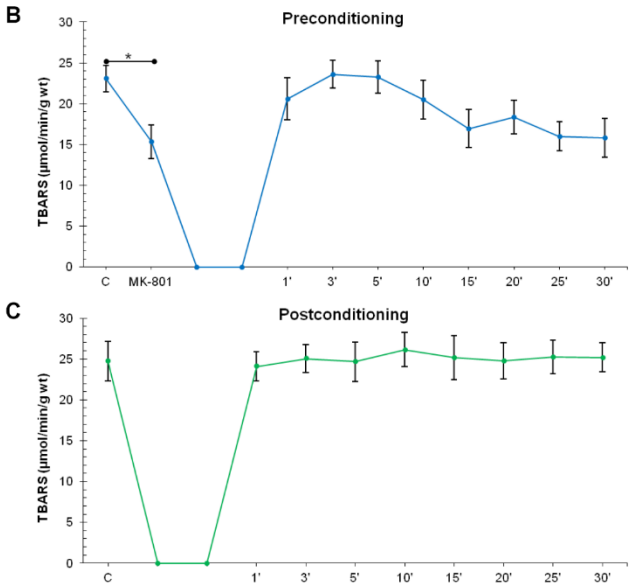
RESULTS

The effects of NMDAR conditioning on the values of the Index of lipid peroxidation measured as TBARS

In the CG, values of TBARS were significantly higher in the third and last minute of reperfusion in comparison to the control values. In the PreC group, the application of MK-801 induced significant decrease of TBARS and that decreasing trend continued to the end of the experiment. On the other hand, in the PostC group there were no statistically significant changes in TBARS values (Figure 1A, 1B, 1C).

Figure 1. Values of Index of lipid peroxidation measured as TBARS in group without conditioning (A), preconditioned with MK-801 (B), and postconditioned with MK-801 (C).

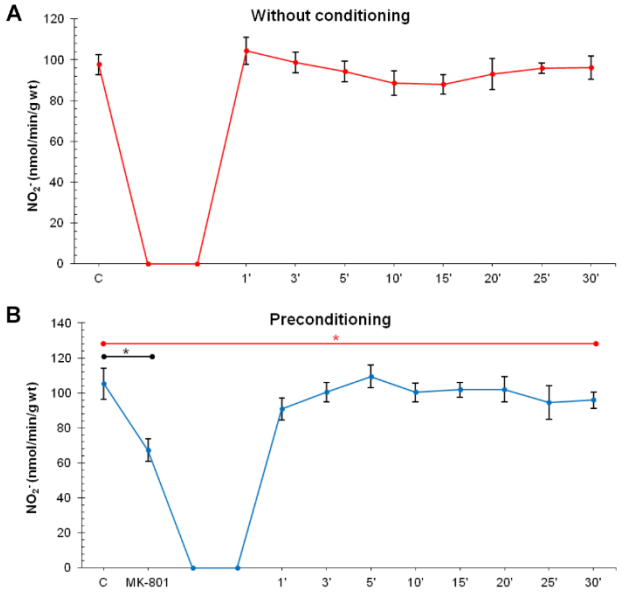


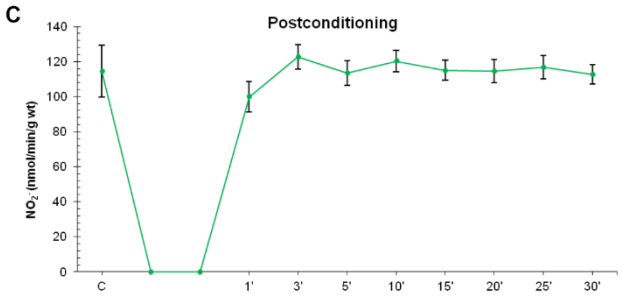


The effects of NMDAR conditioning on the values of the Nitrites

Nitrite (NO_2^-) levels did not change significantly in all points of interest in the CG and in the PostC group, while in the PreC group NO_2^- values were significantly lower after the administration of MK-801 in comparison to the control values, as well as in the last minute of stabilization (Figure 2A, 2B, 2C).

Figure 2. Values of nitrites in group without conditioning (A), preconditioned with MK-801 (B), and postconditioned with MK-801 (C).

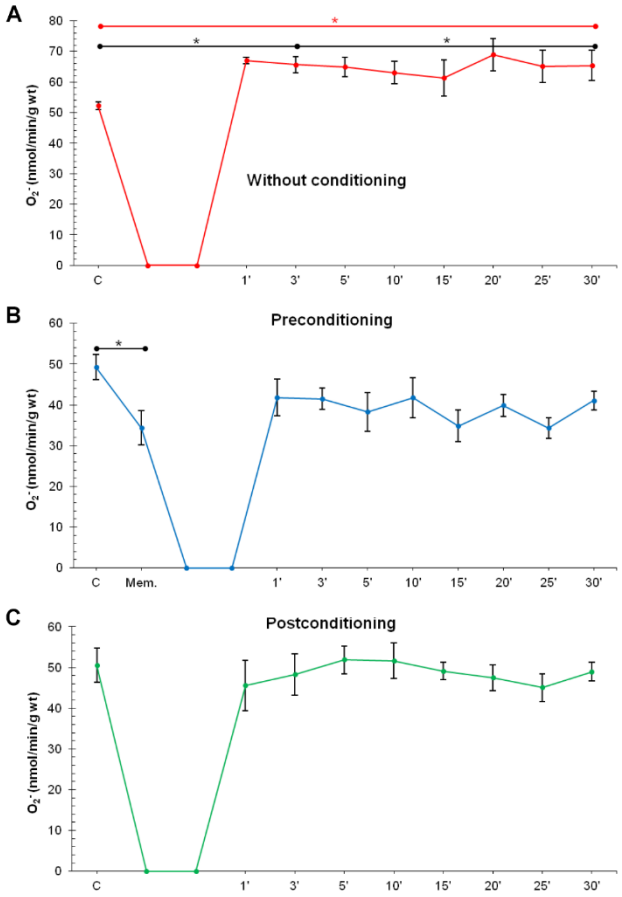




The effects of NMDAR conditioning on the values of the Superoxide anion radical

The values of superoxide anion radical (O_2^-) in the CG were significantly increased in the third and last minute of reperfusion compared to the control values, while in the PreC group MK-801 induced significant decrease of O_2^- levels, but in the postconditioning values were similar to the control values. In the PostC group, there were no statistically significant changes in O_2^- values (Figure 3A, 3B, 3C).

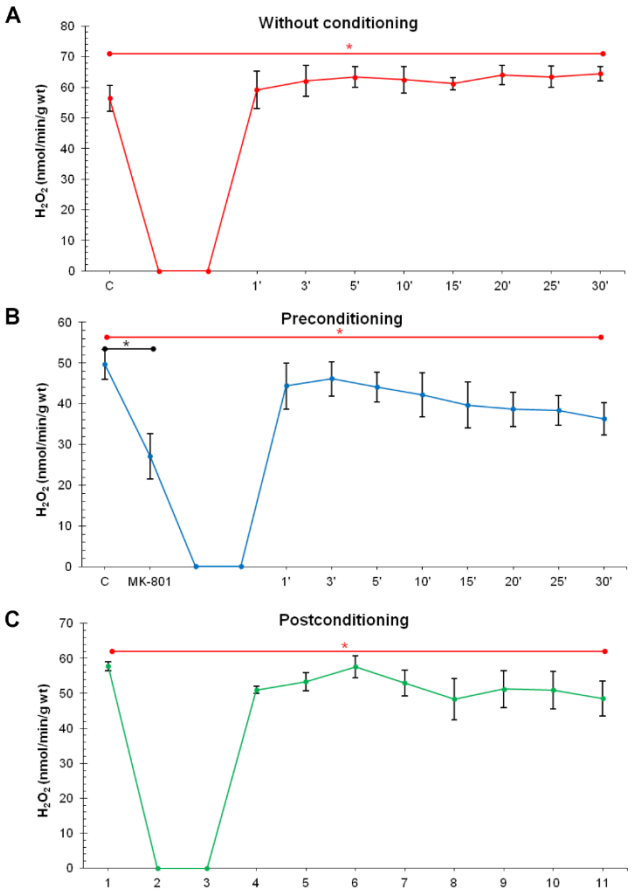
Figure 3. Values of superoxide anion radical in group without conditioning (A), preconditioned with MK-801 (B), and postconditioned with MK-801 (C).



The effects of NMDAR conditioning on the values of the Hydrogen peroxide

Hydrogen peroxide (H_2O_2) levels in the CG were significantly higher in the last minute of reperfusion compared to the control values. In the PreC group, H_2O_2 values were significantly lower after MK-801 application, and also significantly decreased in the last minute of reperfusion in comparison to the control values. Similarly, in the PostC group, H_2O_2 levels were significantly lower in the last minute of reperfusion compared to the control values (Figure 4A, 4B, 4C).

Figure 4. Values of hydrogen peroxide in group without conditioning (A), preconditioned with MK-801 (B), and postconditioned with MK-801 (C).



DISCUSSION

Most researchers dealing with the role of NMDAR in conditioning are related to the nervous system, and there is a lack of data regarding the possible role of these receptors in conditioning models outside the nervous system. Bearing in mind this fact, the aim of this investigation was to assess possible effects of NMDAR blocker, MK-801, applied in pre- and postconditioning fashion on oxidative stress biomarkers in isolated rat heart model.

It was shown that overactivation of extrasynaptic NMDAR actually has detrimental effect related to excitotoxicity, while activity of synaptic NMDAR, on the contrary, has neuroprotective effect (25). Dickie et al. indicated that the exposure of rat ventral mesencephalic slice cultures to high concentration of NMDA induced pronounced toxicity, while MK-801 abolished these toxic effects of NMDA (26). On the other hand, the use of small doses of NMDA had trophic effects, which also were annulled by MK-801. From these results it can be concluded that activation of NMDAR in nervous tissue may result in protection or damage depending on the localization of NMDAR, as well as on the degree of activation. Several authors indicated the beneficial effects of NMDAR antagonists, primarily MK-801 and memantine, in various models of neuron damage including hypoxia-ischemia and harmful effects of oxygen and glucose deprivation (27, 28). Doeppner et al. showed that acamprosate, as indirect NMDAR antagonist, exhibited neuroprotective effects if administered 12 hours before stroke, through inhibition of calpain-mediated pro-injurious signaling cascades (29).

Due to the role of NMDAR in postconditioning Li et al. pointed out that the beneficial effects of brief ischemic postconditioning against amyloid-β peptide induced damage in rat hippocampus (30). A brief ischemic postconditioning reduced neuronal loss through upregulation of NMDAR activity and consequent inhibition of MLK3-MKK3/6-P38MAPK signaling pathway, while amantadine as NMDAR antagonist induced canceling out the positive effects of ischemic postconditioning by restoration of MLK3-MKK3/6-P38MAPK.

There is a lack of research dealing with the role of cardiovascular NMDAR in heart conditioning techniques. Gao et al. assessed the activation of the NMDAR in cultures of neonatal rat cardiomyocytes (16). Stimulation with NMDA induced sustained irreversible increase of intracellular content of Ca^{2+} in cultured cardiomyocytes of neonatal rats, while pretreatment with 30 μmol of MK-801 induced partial inhibition of Ca^{2+} overload. Due to the increased Ca^{2+} levels, the increase of ROS production regarding NMDA application was also shown, and MK-801 abolished oxidative stress induced by NMDA suggesting the Ca^{2+} dependent increase of ROS in cardiomyocytes via NMDAR. Findings of McGee and Abdel-Rahman implicate that the increased activity of peripheral NMDAR and consequent increase of ROS production and Ca^{2+} entrance mediate pressor response (31). Namely, inhibition of phosphoinositide 3-kinase (PI3K)/Akt, protein kinase C, Ca^{2+} influx or NADPH oxidase attenuated pressor response induced by activation of peripheral NMDAR, suggesting the role of PI3K/Akt-protein kinase C signaling pathway in changes induced by NMDAR.

In the previous study from our laboratory, MK-801 also induced significant decrease of all oxidative stress biomarkers (32). Experimental protocol of this study implied subsequent administration of DL-Homocysteine and MK-801, as well as their combination, in duration of 5 minutes at constant perfusion pressure. All measured oxidative stress biomarkers, TBARS, NO_2^-, O_2^- and H_2O_2, significantly decreased during MK-801 application. On the other hand,

results of several studies from our research group, regarding the application of NMDAR agonists, showed the increase of oxidative stress parameters (11, 12).

Furthermore, there is the other side of the relation between oxidative stress and NMDAR. Betzen et al indicated the fact that oxidative stress increases expression of NMDAR subunits in endothelial cells (33). Using murine cerebrovascular endothelial cells which were exposed to superoxide, peroxynitrite or hydrogen peroxide it was shown that prooxidant environment increased the expression of GluN1 subunit. Increased expression of NMDAR subunits in turn resulted in increased functionality of NMDAR, and further to the increased susceptibility to disruption of blood-brain barrier induced by glutamate.

A considerable number of studies dealing with the role of NMDAR in the cardiovascular system refer to mechanisms mediating the adverse effects of homocysteine. Results of several studies performed by Tyagi et al. pointed out the role of NMDAR in a variety of changes induced by hyperhomocysteinemia (HHcy). Cardiac-specific deletion of NMDAR mitigated decrease in myocyte contraction induced by HHcy (34, 35). Actually, HHcy induced increased production of NO in mitochondria, leading to mitochondrial permeability and further to decline in myocyte mechanical function.

CONCLUSION

Results of this study undoubtedly showed antioxidative effects of NMDAR inhibition in pre- and postconditioning of the isolated rat heart. Oxidative stress remains one of the key mechanisms mediating cellular and tissue changes due to increased activity of NMDAR. Despite the fact that the results of this study are encouraging, in order to depict a possible role of NMDAR in cardiac protection more precisely and its underlining mechanisms, additional experiments are needed to involve multiple NMDAR agonists and antagonists, which have different effects and act in different ways.

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CONFLICT OF INTERESTS

None of the authors of the present study has any actual or potential conflicts of interest to disclose, including financial, personal, or other relationships with specific persons or organizations.

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THE COLLIS PROCEDURE AND THE ACQUIRED SHORT ESOPHAGUS

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COLLIS PROCEDURA I STEČENI KRATAK JEDNJAK

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ABSTRACT

One of the most intriguing problems in modern esophageal surgery is the acquired short esophagus. While some authors recognize this entity, others deny its existence. There is a consensus about types of the short esophagus, its etiology and pathophysiology. Definitive diagnosis can be established only intraoperatively. There are a few surgical procedures for this problem, and most frequently is used Collis gastroplasty with fundoplication. In this review we emphasize recent literature data and further perspectives of the Collis procedure.

Keywords: *Collis gastroplasty, acquired short esophagus, fundoplication*

SAŽETAK

Jedan od najinteresantnijih problema u modernoj hirurgiji jednjaka je stečeni kratak jednjak. Dok neki autori prepoznaju ovaj entitet, drugi negiraju njeno postojanje. Postoji konsenzus o vrstama kratkog jednjaka, njegovoj etiologiji i patofiziologiji. Definitivna dijagnoza se može postaviti samo intraoperativno. Postoji nekoliko hirurških procedura za rešavanje ovog problema, a najčešće se koristi Collis gastroplastika sa fundoplikacijom. U ovom radu dajeom pregled novijih podataka iz literature i dalje perspektive Collis procedure.

Ključne reči: *Collis gastroplastika, stečeni kratak jednjak, fundoplikacija*

INTRODUCTION

Modern esophageal surgery involves more controversial questions which do not include the census of leading world authors. One of them is related to the problem of the acquired short esophagus. Nowadays according to many surgeons the acquired short esophagus is present, on the other hand the others deny it and say that they have never seen it even nor in large series of patients (1, 2). The radiologists first provided the definition of this medical entity to describe the intrathoracic gastroesophageal juction. However, surgical definition is based on impossibility of reduction GE juction in the abdomen intraoperatively. In later literature, the definition of the short esophagus accepted by most groups of the appointed in this area includes: 1) the short esophagus is diagnosed intraoperatively; 2) only after extensive mediastinal mobilization of the esophagus; 3) when the intraabdominal part of the esophagus is shorter 2-3 cm (measured down tension-free) (3). Horvath and his coworkers divided the short esophagus into three categories: 1) a true, nonreducible short esophagus; 2) a true, but reducible short esophagus; 3) an apparent short esophagus (4). Early reports in the literature show the different frequency in patients with esophageal disease, from 0% (by Hill and his coworkers) to 60% (by Pearson and his coworkers) (the data collected for the time period 1980-1991) (2,3,5,6). Most data are based on the methods of precise gastroesophageal junction determination as well as the subjective gradation tension needed for the reposition of the suitable esophagus segment placed under the diaphragm. Later analysis shows that the actual incidence of the short esophagus is estimated to be 3-10% (4, 6). The reasons for the increased percentage values (compared to values in surgical practice) are tertial specialized objects as well as the data collected from the retrospective studies. Nowadays the usage of proton pump inhibitor and performing minimally invasive procedure decrease serious esophageal disease leading to the its shortening. The aim of this paper is to emphasize recent literature data and further perspectives of the Collis procedure.

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ETIOLOGY AND PATHOPHYSIOLOGY

Intrinsic shortening of the esophagus results most commonly from the chronic inflammation that accompanies gastroesophageal reflux disease. The dysfunction of the lower esophageal sphincter allows either acid or alkaline (in case of duodenogastric reflux) contents to regurgitate into the esophagus. The squamous epithelium of the esophageal mucosa is not an effective barrier to refluxed juices, a 'burn' to the esophagus results, similar to that occurring in the case of ingested corrosive chemicals. It leads to a cascade of events: an inflammatory response, the inevitable stages of edema, infiltration with inflammatory cells, subsequent healing, and eventual fibrosis (7). The process eventually may involve the deeper muscular layers of the esophageal wall and may even extend transmurally into the periesophageal tissues of the mediastinum. Thus, the connection between esophagitis, stricture formation and the shortening of esophagus was established (8). The components of reflux content are acid, biliary and pancreatic juice, which allow the H+ ion penetration into deeper wall layers of the esophagus, that consequently results in fibrosis. With repeated cycles of injury and repair overtime, functional and irreversible damage occurs to the involved esophagus. Contraction of the collagen in the trasmural fibrous scar can occur circumferentially, producing a peptic stricture or longitudinally, resulting in a short esophagus. Beside gastroesophageal reflux disease, there are other entities associated with the significant inflammatory reaction and the esophageal shortening: hiatus hernia type I and II, paraesophageal hernia hiatus type III, sarcoidosis, Barett's metaplasia, caustic ingestion, scleroderma and Crohn's disease.

PREOPERATIVE AND INTRAOPERATIVE DIAGNOSIS

As it is mentioned, the actual diagnosis of a short esophagus can be confirmed just in the operating room. However, very precise preoperative findings are necessary for each patient being prepared for an operation. In that way, preoperative findings can confirm surgeon's suspicion for a short esophagus. These indicators are: anamnestic data related to a long year gastroesophageal reflux disease (40% patients have complications - macroscopic esophagitis, Barett's esophagus, peptic stricture of the esophagus or an acquired short esophagus) and early unsuccessful antireflux procedure (6,9–11). Preoperative findings show the frequency of the acquired short esophagus at the following entities: manometric – absence of distal high pressure zone, hypo or aperistalsis of distal esophagus; esophagogastroduodenoscopy – identification of the GE junction (5 cm or more) above diaphragm hiatus; moderate or severe esophagitis, the presence of early peptic stricture, and Barett's esophagus; barium esophagram – a large esophageal hiatus hernia type I which can not reduce spontaneously in vertically position; a large hiatus hernia type III, and stricture (5,8,11,12). Intraoperative findings may cause a problem for a surgeon because of abnormal anatomic relations, complicated identification of gastroesophageal junction, presence of pneumoperitoneum raising the diaphragm and give a false diagnosis (1,5,10,12,13).

THE COLLIS PROCEDURE

If intraoperatively 2.5 to 3 cm length of the esophagus is confirmed, several surgical options for treatment are reported. One of well known is the lengthening procedure. Collis first described the operation that bears his name in 1957 because he had been unsatisfied with problems being caused with the short esophagus. This lengthening procedure was performed through a thoracoabdominal incision. Collis emphasized the fact that mediastinal mobilization of the esophagus to the level of the aortic arch was sufficient to perform the suitable antireflux procedure. When additional length was needed, he created a gastric tube (the Collis gastroplasty) by dividing the stomach between two clamps placed opposite of the small curvature with a bougie. Unfortunately, the Collis procedure alone as well as the lengthening procedure was not sufficient to control reflux for a long time. So, Pearson and his coworkers first completed transthoracic Collis gastroplasty with Belsey fundoplication (6). They pointed out an additional advantage of this combined procedure: the fundoplication sutures are placed into the healthy issue of the esophagus. Orringer, Henderson and Sloan advocated the Collis – Belsey procedure and reported the excellent results – as high as 85% symptomatic reflux control at 10 years of follow-up (14). Both ways considered a transthoracic approach because it was difficult to perform an adequate esophageal mediastinal mobilization. In 1986. the usage of newly developed gastrointestinal stapling devices helped Steichen and his coworkers to describe an effective open transabdominal procedure for performing Collis gastroplasty using EEA and GIA stapling devices (14–16). The first one was used to create a buttonhole in the gastric fundus after previous mobilization. The second one used to create neoesophagus was placed through the gastric buttonhole (17). Langer, Demos, Bingham first used 'uncut' Collis. Later, Evangelist, Paris, Van Kemmel, Piechler and Payne used it as well (18,19). This method considers the gastroplasty tube created by mucosal apposition of the anterior and posterior stomach wall from the proximal smaller curvature by the use of a 3 cm linear stapler, applied along an inlaying no. 48 or 50 French bougie held against the small curvature. The acquired tube like this is not separated from the gastric fundus by transection. Then, previous mobilized fundus is used for total fundoplication and the fundic wrap is sutured immediately anterior to the staple line. If the fundus is not appropriate because of earlier surgeries, the modified Collis-Nissen procedure by Jeyasingham is used. After formation of the gastroplasty tube, the fundus is opened

longitudinally and then closed transversely , as for a pyloroplasty. This closure provides a widened fundus which create the fundoplication wall. In that way, more fundi used as plication wall are given. The approach to these methods is transtoracal. Introducing laparoscopic surgery as well as maintaining the benefits of a less invasive approach reduces the usage of open approaches for creating neoesophagus and antireflux procedures. In the middle of the 1990s Swanstrom placed endoscopic linear stapler across the right mediastinal pleura and created neoesophagus in order to perform the fundoplication using laparoscopic techniques. In 1998. Johnson , Oddsdottir and Hunter performed laparoscopic procedure which initially had been described by Steichen (20). Awad placed the articular stapler through the left hemithorax and created the gastroplasty. One of the last modified Collis procedures started to be used in surgery in 2004. by Terry ,Vernon and Hunter, Lin and coworkers and Hoang (21). The authors performing laparoscopic gastroplasty and fundoplication noticed a significant number of apex fundus ischemia. It made them to modify the procedure and describe so-called stapled-wedge gastroplasty. The potential ischemic topic of fundus is removed with creating first transversal and then vertical stapled line. The transection of fundus is done transversely with the stapler and it creates another stapler which is used for performing the gastroplasty in a regular way (22).

DISCUSSION

The Collis procedure has been used clinically for 60 years as a standard lengthening technique in the treatment for the short esophagus. Initially, the procedure was performed with open approach through thorax or abdomen. Then its performing with development of gastrointestinal EEA, GIA, endoscopic and articular staplers became standard, safe and routine. Beside constant progress and modification, some complications are noticed by the long term following the patients. These ones may be early and late. All patients being prepared for the Collis gastroplasty followed the fundoplication experience complications relating to all antiflux procedure. Early complications include: dehiscence (leaks) of the gastroplasty tube, necrosis, postoperative dysphagia and early reflux. All these complications occur refer to technical omission during the performing operation. Postoperative dehiscence occurs in about 1% of all case, on the tubus alone, new created fundus or at the angle between them. Nowadays, for this reason, most authors oversew the staple line with a continuous one layer absorbable suture. Necrosis of gastroplasty is a very rare event and theoretically may occur when the gastroplasty is utilized following subtotal gastrectomy with division of the main left gastric artery. The incidence of postoperative leaks following gastroplasty is about 1%. Postoperative dysphagia may occur from the complications related to the antireflux procedure but may also result from a poorly created neoesophagus. Most authors preventively advocate utilizing a 50-60 French bougie in creating the gastroplasty, however Collis utilized as narrow a tube as a no. 32 French bougie. Technical failure of the antireflux procedure is the usual cause of early reflux. Late complications following the gastroplasty include: recurrent reflux, diverticulum of the gastroplasty tube and the carcinoma development. Henderson and Orringer described significant reflux (15-20% cases) occurring after partial fundoplication following each Collis procedure, so they proposed total fundoplication after this esophagus elongation procedure (16). Too long fundoplication results in prolonged dysphagia and now most authors recommend a total fudoplication of no more than 1.5 cm with the proximal suture placed in healthy part of esophagus. The incidence of persisting mild or moderate dysphagia occurs in 10-15% of gastroplasty operations. Diverticulum formation occurs in the proximal portion of the gastroplasty, if the fundoplication is too tight in the distal part which results from a technically bad performed antireflux procedure (23,24). Carcinoma in neoesophagus may occur unless early carcinoma of esophagus is diagnosed initially in the distal part or in the area of dysplasion of esophageal mucosa after gastroplasty formation (6,10,25). A special problem is existence of squamous mucosal islands producing hydrochloric acid. Mor and his coworkers said that reduction the amount of acid from new created tubus in the patients with Nissen-Collis gastroplasty took more time compared to the controllable group (patients without reflux) (24,26). For these reasons, the patients with reflux or abnormal production of acid in tubus need the usage of proton pump inhibitor for a long time (23,27,28). Generally speaking, this method solves the problem of short esophagus. It provides amotility segment producing acid in some cases, so it is called nonphysiologic treatment (10,12,14,16). The Collis procedure is used successfully in the cases of reoperation after failed antiflux procedure (Deschamps in 62.7% cases and Luketich in 52.5%) (18,25,29,30). Pearson and Orringer first described that mortality ranged from 0.5-1,1%. Long term results show a different percentage of successful neoesophagus formation, from 59-80 % (5,6,10,31,32).

CONCLUSION

In spite of possible complications occurring during the formation of neoesophagus, Collis designed a gastroplastic tubus in order to lengthen esophagus in the cases of the acquired short esophagus. The gastroplastic tubus combined with antireflux procedure was an excellent approach to solving the problem in distal esophagus with the decreased rate of mortality. It is utilized in the patients having a lot of risk of reccurence after standard antireflux procedures. It includes: the acquired short esophagus and peptic stricture, obesity, recurrent reflux after various antireflux procedure and combination of hiatus hernia type I and II and paraesophageal hiatal hernia type III. This procedure is contraindicated after myotomy of lower esopha-

geal sphincter and not utilized in the patients with serious motility disorder. The Collis-Nissen procedure has an established excellent long-term success rate for this complex problem. Methods to treat the short esophagus, aside from the Collis gastroplasty, have poorer long –term results, with increased rates of complications. With the development of endoscopic Collis techniques, coversion to an open laparotomy/thoracotomy when a short esophagus is encountered is no longer necessary. The choice of the laparoscopic /thoracoscopic single-stapler technique or the laparoscopic double – stapler technique should be left to the surgeon. Both procedures can be performed safely and offer all the benefits of laparoscopic surgery. Even though a lengthening procedure is the best choice, the Collis gastroplasty is a nonphysiologic treatment for a complex problem and requires long-term follow-up.

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LYMPHANGIOMA OF THE SMALL INTESTINE CASE REPORT AND REVIEW OF THE LITERATURE

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LIMFANGIOM TANKOG CREVA - PRIKAZ SLUČAJA I PREGLED LITERATURE

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ABSTRACT

Lymphangioma usually occurs in children and usually involves the skin. Mesenteric lymphangiomas are extremely rare in adults (1,2,3). Lymphangioma of the small-bowel mesentery is rare, representing less than 1% of all lymphangiomas (4).

We report a case of a 62-year-old female who presented with abdominal pain, discomfort, nausea and vomiting. Preoperative tests including abdominal ultrasonography and magnetic resonance imaging were performed, but they could not accurately determine the nature of the tumour. Laparotomy was performed; the tumour was excised completely, and a large cystic tumour of the small bowel mesentery was found. Histopathological examination diagnosed the tumour as a cystic lymphangioma. Lymphangiomas are extremely rare, especially in the abdomen of adults, and are asymptomatic for the most part; they often present as acute abdominal conditions, causing life-threatening complications such as secondary infection, rupture with haemorrhage, and volvulus or intestinal obstruction when the tumour increases in size, requiring emergent surgery. Lymphangioma is often difficult to diagnose, and surgical resection is selected in many cases for both diagnosis and treatment.

Keywords: *lymphangioma, small intestine, cystic, surgical excision*

SAŽETAK

Limfangiom se obično javlja kod dece, najčešće na koži. Mezenterični limfangiomi su izuzetno retki kod odraslih. Mezenterični limfangiom tankog creva je retka pojava i obuhvata manje od 1% svih limfangioma.

Predstavljamo slučaj žene stare 62 godine koja je imala tegobe u vidu bola u trbuhu, nelagodnosti, mučnine i povraćanja. Preoperativna ispitivanja, koja su rađena uključujući ultrasonografski pregled abdomena i magnetnu rezonancu, nisu mogla tačno odrediti prirodu tumora. Učinjena je laparotomija i tumor je izvađen u celosti. Otkriven je veliki cistični tumor mezenterijuma tankog creva. Histopatološkim pregledom dijagnostikovan je tumor kao cistični limfangiom. Iako su limfangiomi izuzetno retki, posebno u abdomenu odraslih osoba i asimptomatski u većini slučajeva, oni često prezentuju kao akutni abdomen, uzrokujući komplikacije opasne po život, kao što su sekundarna infekcija, ruptura sa hemoragijom, volvulus ili opstrukcija creva kada se tumor uveća i koje zahtevaju hitno operativno lečenje. Limfangiom je često teško dijagnostikovati, u mnogim slučajevima je izabrana hirurška resekcija u clju dijagnostike i lečenja.

Ključne reči: *limfangiom, tanko crevo, cistični, hirurška ekscizija*

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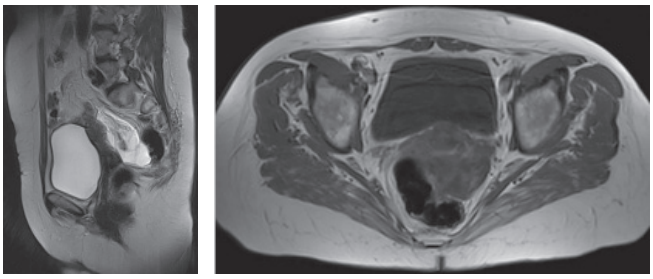
INTRODUCTION

Lymphangiomas most often occur in the head, neck, axilla, or groin of young children. Occasionally, the tumour is also discovered in adults in various other anatomical sites (3). Approximately 95% of lymphangiomas are found in the neck and axilla and the other 5% occur in the mediastinum and abdominal cavity, including the mesentery, retroperitoneum and bones (5). Intra-abdominal lymphangiomas (fewer than 5%) have been reported in the mesentery, gastrointestinal tract, spleen, liver and pancreas (6). Lymphangiomas are traditionally classified into three histologic types: capillary (simple), cavernous, and cystic (7). The capillary type usually originates in the skin and consists of uniform, small, thin-walled lymphatic spaces. The cavernous type is composed of various sizes of dilated lymphatic spaces associated with the lymphoid stroma and shows a connection with the adjacent normal lymphatic spaces. The cystic type consists of dilated lymphatic spaces of various sizes associated with collagen and smooth-muscle bundles in the stroma but lacks connection to the adjacent normal lymphatic spaces. Cystic lymphangioma findings are similar to cavernous lymphangioma findings in that dilated lymphatic spaces of variable size are seen for both (8). The aetiology of lymphangiomas is still unclear. They are considered to be a congenital dysplasia of lymphatic tissue and abnormal development of the lymphatic vessels during foetal life (9, 10). The histological features of lymphangioma were first described by Gaudier and Gorse in 1913 (11). The clinical symptoms of gastrointestinal and mesenteric lymphangiomas vary from asymptomatic to acute abdominal symptoms such as obstruction or bleeding, according to the size and the localization of the tumour (10,12,13). The treatment is mainly surgical and consists of enucleation when feasible. Segmental intestinal resection is achieved when the cyst adheres intimately to the bowel (13, 14). The clinical features of intra-abdominal lymphangiomas are diverse, ranging from an asymptomatic abdominal tumour to symptoms of an acute abdomen (15). Therefore, a mass may be discovered incidentally during examination for an unrelated illness.

CASE REPORT

A 62-year-old female was admitted to our hospital for close examination and treatment due to unexplained intermittent abdominal pain especially in the left abdominal area, abdominal discomfort, nausea and vomiting. She had no history of abdominal surgery. The symptoms occurred over the past 6 months.

The physical findings were non-specific. The laboratory findings were within the reference ranges, and the tumour markers (AFP, CEA, CA 19-9, CA 15-3, CA125, TPS) were negative. Magnetic resonance imaging (MRI) of the abdomen and pelvis was performed (figure 1a,b). The patient did not present any other multicentric lymphatic



1a 1b

Figure 1a: Sagittal T2-weighted MR image shows a high signal intensity multilocular lesion in the recto-uterine pouch.
Figure 1b: Axial T1-weighted image shows low signal intensity within the lymphangioma.

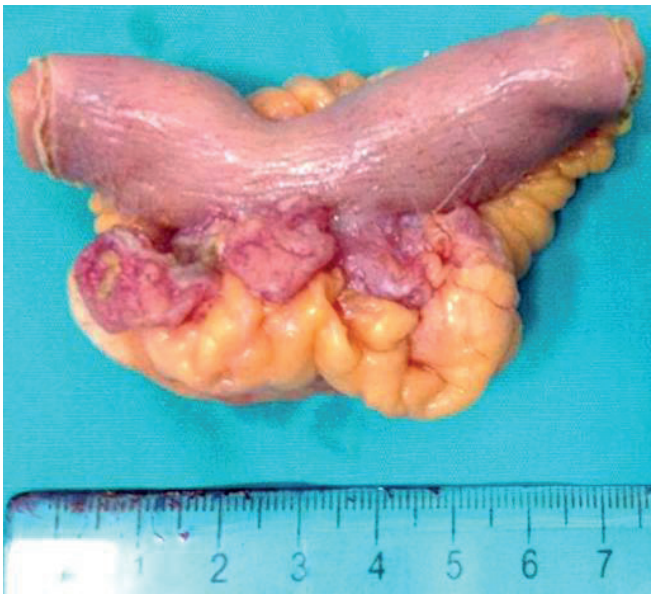


Figure 2: Resected segment of the small bowel with the infiltrating tumour mass

or vascular malformation. Her family and medical history were unremarkable.

Laparotomy was performed, and the tumour was excised completely with small-bowel resection and end-to-end anastomosis. During the laparotomy, a yellowish cystic tumour with a soft consistency was found in the mesentery of the ileum (figure 2). The mesenteric mass was lobulated, cystic and measured approximately 20 mm × 15 mm × 13 mm. The mass consisted of multiple well-defined locules measuring between 4 mm-10 mm, some were filled with a milky fluid with a volume of approximately 100 mL. There was no communication between the cystic mass and the lumen of the small bowel. The tumour was not adherent to the wall of the intestine or adjacent organs. No ascites was seen in the peritoneal cavity, nor was there any dilatation or inflammatory change of the intestines or mesentery.

Microscopic examination revealed that the cystic walls comprised of smooth muscle were lined with flat endothelial cells (figure 3). The cystic walls were generally thin. The stroma showed various sizes of small lymphatic spaces lined by a flat endothelium. Few subendothelial lymphoid

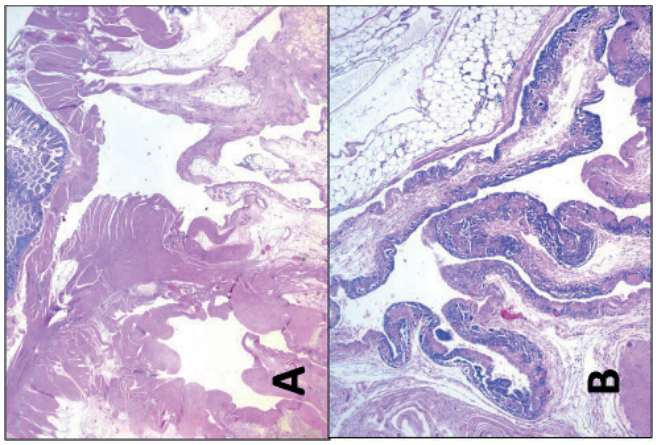


Figure 3. The intestinal wall revealed lymphatic malformation and presented irregular vascular channels with propagation into the mesenteric fat (A, H&E, 20x) containing a scattered collection of smooth muscle in the walls of the larger vascular channels accompanied by a dense perivascular lymphoid infiltration (B, H&E, 50x).

follicles were observed, supporting the diagnosis of cystic lymphangioma.

The procedure was tolerated well by the patient, without any clinically significant postoperative complications. The postoperative period was uneventful, and the patient was discharged on the fifth postoperative day.

Regular check-ups demonstrated that the patient was completely symptom and disease free. One year after surgery, an abdominal CT revealed no signs of disease recurrence.

DISCUSSION

As many as 90% of lymphangiomas present in children younger than 3 years, and the sex ratio is roughly equal in childhood (7). These tumour masses are extremely rare in adults. These cases account for less than 1 in 20,000 to 1 in 250,000 hospital admissions (16,17), and they are more frequently observed in men than women with a M/F ratio of 3:1 (18).

This condition is probably a developmental anomaly of the lymphatic system, explained by its primary occurrence in children, with over 80% diagnosed in the first few years of life (19). This theory would explain why lymphangiomas occur primarily in children. However, it is suggested that abdominal trauma, lymphatic obstruction, inflammatory processes, surgery, or radiation therapy may lead to the secondary formation of such a tumour (16,17).

A review of the English language literature for the 50-year period from 1960 to 2009 revealed only 19 reports of small bowel lymphangiomas (20,21). A total of 40 patients are reported, with a wide age range at presentation from 5 to 75 years, and an equal gender distribution has been observed. Of the cases in which a specific anatomical small bowel location was noted, 24 were within the jejunum and 5 in the ileum (20). Hanagiri and colleagues also documented an additional 33 patients from the Japanese literature between 1967 and 1990. In addition to the 19 English literature reports, 8 were from the Far East, including 5 Japanese and 3 Taiwanese manuscripts, suggesting that there may be a Japanese/Taiwanese predisposition to small bowel lymphangiomas. In the Japanese language literature, although the age distribution was comparable, there were twice as many males as females with an equal distribution between ileum and jejunum (20).

Lymphangiomas are traditionally classified into three histologic types: capillary (simple), cavernous, and cystic (7). The capillary type usually originates in the skin and consists of uniform small thin-walled lymphatic spaces. The cavernous type is composed of various sizes of dilated lymphatic spaces associated with lymphoid stroma and shows a connection with the adjacent normal lymphatic spaces. The cystic type consists of dilated lymphatic spaces of various sizes associated with collagen and smooth-muscle bundles in the stroma but lacks connection to the adjacent normal lymphatic spaces. Cystic lymphangioma findings are similar to cavernous lymphangioma findings in that dilated lymphatic spaces of variable size are seen in both (19).

Patients with mesenteric lymphangiomas are usually asymptomatic and the mass is discovered only incidentally during examination or surgery for an unrelated illness until the tumours enlarge. Abdominal pain, the presence of a palpable mass and distention seem to be the most common symptoms, but the clinical presentation varies.

The mass is usually discovered only incidentally during examination or surgery for an unrelated illness; however, some patients may have acute clinical symptoms caused by compression of the adjacent structures or by complications such as infection, perforation, torsion and rupture (22). However, mesenteric lymphangiomas may cause complications such as infiltration of the intestine, or involvement of the main branch of the mesenteric arteries or adjacent organs that necessitate segmental resection of the intestine (23,24,25)

The reason for reporting this case is to emphasize the fact that the primary treatment for lymphangiomas is radical surgical excision, even when asymptomatic, because of its potential to grow remarkably and invade adjacent structures or develop complications; they also have a risk of sarcoma transformation upon irradiation.

Other submucosal tumours, such as leiomyoma, lipoma or other cystic lesions, such as enteric duplication cysts as well as carcinoid tumours or mucocele, should be considered as the differential diagnoses. Preoperative investigations, including CT and MRI, give information about anatomical position, size and extent of the cysts, but they are insufficient in establishing an accurate preoperative diagnosis (26). Sometimes radical resection might be technically impossible. If radical surgery is not technically possible, injection of bleomycin or OK-432 into the tumour has been reported to be effective (27).

Mesenteric lymphangiomas are very rare, but they can cause acute abdominal symptoms that require emergent

surgery. Therefore, they should be included in the differential diagnosis of the acute abdominal condition. Although benign in nature, mesenteric lymphangiomas may cause significant morbidity or mortality due to their large size and critical location when they compress the adjacent structures.

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TEN MARATHONS IN TEN DAYS: EFFECTS ON BIOCHEMICAL PARAMETERS AND REDOX BALANCE – CASE REPORT

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DESET MARATONA ZA DESET DANA: UTICAJ NA BIOHEMIJSKE PARAMETRE I REDOKS RAVNOTEŽU – PRIKAZ SLUČAJA

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ABSTRACT

Production of free radicals and oxidative damage during physical activity is a topic that is intensively studied and paid a lot of attention, first of all in professional sports. Marathon is categorized as extremely demanding sports discipline, as it induces high energy consumption and also requires special mental self-control. We presented cases of two athletes of different age, who have been on dissimilar level of sports readiness, and also had various approach to physical activity and exercise. During 10 days they ran out 10 marathons, partly on a flat terrain, and partly on hilly, which produced different level of effort in conquering the terrain. Also, both athletes had complex supplementation scheme in order to prevent electrolyte imbalance and excessive production of free radicals. Blood samples were taken in the morning and immediately after the end of the marathon. Measured oxidative stress biomarkers changed without a noticeable pattern, but these changes did not vary greatly among themselves. Catalase activity in both marathon runners was higher after marathon almost after every race for 10 days. On the other hand, amount of reduced glutathione was lower after marathon in both athletes in the same manner. Based on the obtained results we can conclude that adequate supplementation could have crucial role in prevention of oxidative damage.

Keywords: *marathon; physical activity; oxidative stress; reactive oxygen species; supplementation*

SAŽETAK

Stvaranje slobodnih radikala i oksidaciono oštećenje je tema kojoj se posvećuje dosta pažnje i koja se intenzivno istražuje, pre svega kod profesionalnih sportista. Maraton se svrstava u izuzetno zahtevne sportske discipline koja podrazumeva veliku energetsku potrošnju i specifičnu mentalnu samokontrolu. Prikazali smo slučaj dvojice maratonaca različite starosne dobi, različite fizičke spremnosti i sa različitim prisupom fizičkoj aktivnosti i vežbanju. Tokom 10 dana istrčali su 10 maratona, delom na ravnom, a delom na brdovitom terenu, što je uzrokovalo različito opterećenje tokom savladavanja pojedinih deonica. Takođe, trkači su imali kompleksnu shemu suplementacije u cilju prevencije elektrolitnog disbalansa i prekomerne produkcije slobodnih radikala. Uzorci krvi su uzimani ujutru i odmah nakon završetka maratona. Promne vrednosti merenih biomarkera oksidacionog stresa nisu imale bilo kakav obrazac, ali ove promene nisu značajno varirale između maratonaca. Aktivnost katalaze kod oba maratonca je bila znatno viša nakon maratona gotovo tokom svih 10 dana. Sa druge strane, količina redukovanog glutationa je bila niža nakon maraton kod oba trkača. Na osnovu dobijenih rezultata može da se zaključi da adekvatna suplementacija može da ima presudnu ulogu u prevenciji oksidacionog oštećenja.

Ključne reči: *maraton; fizička aktivnost; oksidacioni stres; reaktivne vrste kiseonika; suplementacija*

ABBREVIATIONS

ATP – adenosine triphosphate
CoQ10 – Coenzyme Q10
CARB – protein carbonyls
CAT – catalase
GSH – reduced glutathione
MUM – mountain ultra marathon
NO – nitric oxide
RONS – reactive oxygen and nitrogen species
ROS – reactive oxygen species
TAC – total antioxidant capacity
TBARS – thiobarbituric acid reactive substances

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INTRODUCTION

Intense physical activity lasting over prolonged period of time presents great challenge even for well-trained athletes. Marathon is categorized as extremely demanding sports discipline, as it induces high energy consumption and also requires special mental self-control. However, as one of the oldest and most demanding sports disciplines, marathon is very popular both between professional athletes and for people who practice sports occasionally. Despite this fact, we do not have enough data on influence of trainings and marathon competitions on redox balance in athlete's body, its effect on production and elimination of free radicals and elements of antioxidative system, and as result of this possible tissue damage caused by free radicals.

Oxidative stress is actually misbalance between production and elimination of free radicals. Numerous external and internal factors causes increased production of reactive type of oxygen and nitrogen (RONS), amongst others, physical activity is also accounted here. In early 80-ties causal relationship between physical activity and producing of RONS was noticed (1). Increased demand for ATP during aerobe physical activities can initially overwhelm electronic transport chain located on inner membrane of mitochondria and causes increased production of superoxide anion radicals (2). Taking into consideration that such increased production of free radicals is mainly of moderate extent, it is higher probability that physical activity and RONS produced in mitochondria's of skeletal muscles play role in signal paths, rather than directly inducing oxidative stress (3). Increased RONS production during moderate and repeated physical activity changes gene expression, which can cause genesis of mitochondria and increasing its capacity (4). However, increased oxygen consumption during intense oxidative phosphorylation occurring while practicing physical activity, as well as releasing catecholamine directly causes production of RONS, as well as increased phagocyte activity caused by muscle damage during physical activity (5). In addition to skeleton muscles, liver also plays very important role in body response to acute and repeated physical activity, as well as in the process of body adapting to increased production of free radicals and inflammation (6).

Sports training means body exposure to repeated periods of physical endeavor with aim to evolve mechanisms of adaptation to changes occurring in body during physical activity hormesis rule (7). However, physical endeavor of high intensity causes multiple changes which overstretch mechanisms of adaptation, eventually causing oxidative stress and inflammation and resulting in deterioration of physical parameters (8). Results of research on the topic of marathon effect on free radicals production and oxidative stress are contradictory and non-conclusive (9); while in other research has been shown that ultra-marathon does not cause significant changes in redox balance (10).

Taking into consideration all above facts, aim of this research is following of biochemical parameters and biomarkers of oxidative stress and elements of anti-oxidative system in two marathon runners, of different age and different previous sports exposure and experience, who ran 10 marathons in 10 consecutive days, with previously established program for diet, supplements, recovery and rest.

CASE REPORT

We followed two athletes (S1 and S2) of different age (S1 – 22 years, S2 – 44 years), who have been on dissimilar level of sports readiness, and also had various approach to physical activity and exercise (S1 is professional triathlon sportsman, while S2 is non-professional marathon runner). Both participants were healthy, without any anatomic specifications of the body. During 10 days they ran out 10 marathons, partly on a flat terrain, and partly on hilly, where the altitude varied from 342 to 1161 meters above sea level, which produced different level of effort in conquering the terrain.

Both athletes were monitored daily for 10 days, in following areas: body weight, arterial tension, maximal and average heart rate, calories consumption, as well as supplements and recovery plans. Waking up occurred every day at 8:00 am, followed by morning measurement (body weight and arterial tension) and taking a blood sample at 8.30, breakfast at 9:00, with taking supplements during breakfast. Second blood sample was taken right after the end of the marathon. Marathon started at 12:00, and participants took rehydration during marathon. Immediately after finishing marathon second measurement of body weight and arterial tension was conducted, as well as second blood drawing. Recovery cocktail was taken around 17:00, and dinner and supplements were taken at 18:00. Massage and recovery was around 20:00, together with supplements. Going to bed was between 21:30 and 22:00.

Over the course of these 10 days of marathon races, runners on average imported 250 g of proteins, 500 g of carbohydrates, 60 g of fats and 7 g of fibers, which totals about 4000 kcal, while the average energy consumption per marathon amounted to 2289 kcal. Supplements before the start of marathon meant taking of 333.3 mg Ca^{2+}, 133.3 mg of Mg^{2+}, 8.3 mg of Zn^{2+}, 1000 mg of Omega-3 fatty acids, 100 mg of CoQ10 and 400 IU of vitamin E. Solution

Table 1. Reference values for observed laboratory parameters in the general population

Glucose	4.1-6.4 mmol/L
Urea	2-9 mmol/L
Creatinine	Female: 45-90 μmol/L Male: 50-105 μmol/L
Protein	66-83 g/L
Albumin	35-52 g/L
Globulin	25-33 g/L
Potassium	3.6-5.5 mmol/L
Sodium	135-155 mmol/L

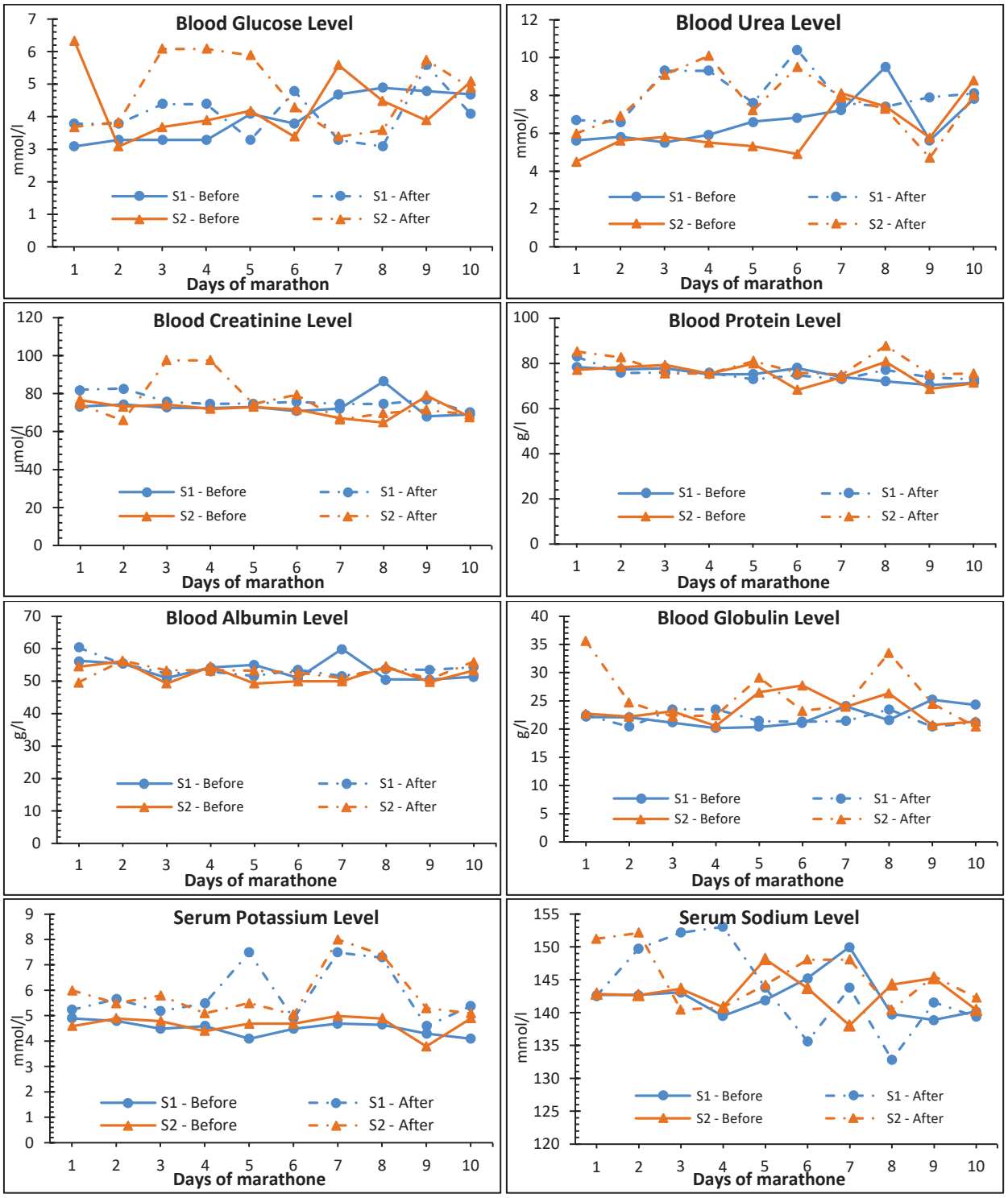

Figure 1

for rehydration during marathon race contained amino acid solution (20 ml), 5 g of glutabolic, 20 g of dextrose, 5 g of potassium chloride, 1000 mg of vitamin C dissolved in 1000 ml of water. Recovery cocktail contained 45 g of whey proteins, 44 g of carbohydrates and complex of vitamin B. Supplements at the end of the day meant 1000 mg of Omega-3 fatty acids, 44 g of carbohydrates, 333.3 mg of Ca^{2+}, 133.3 mg of Mg^{2+}, 8.3 mg of Zn^{2+} and 3 tablets of vitamin B complex.

Values of blood glucose, as well as urea and creatinine, varied mainly within physiological range (Figure 1, Table 1). Similarly, blood protein, albumin and globulin levels were maintained within normal values (Figure 1, Table 1). In both runners serum potassium levels were higher after marathon compared to values before, but in the 7th and 8th day these values were considerably above the upper limit value (Figure 1, Table 1). Serum sodium levels were changed without a certain rule, but mostly within the

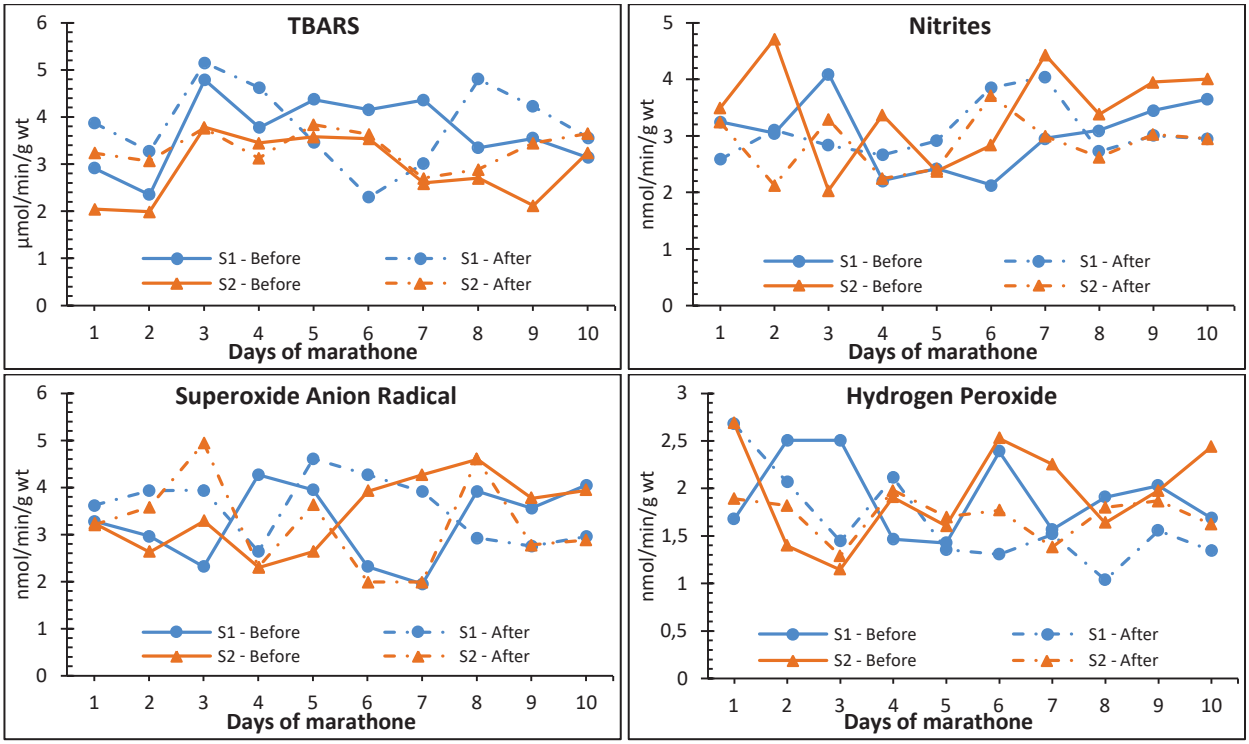

Figure 2

physiological range (Figure 1, Table 1). All mentioned biochemical parameters were measured using standard biochemical procedures.

Measured oxidative stress biomarkers (Level of lipid peroxidation measured by thiobarbituric acid assay – TBARS, Nitrites - as a measure of released nitric oxide (NO), Superoxide anion radical, and Hydrogen peroxide) changed without a noticeable pattern, but these changes did not vary greatly among themselves (Figure 2). All above mentioned oxidative stress biomarkers were determined spectrophotometrically using the previously described methods (11-14).

Catalase activity in both marathon runners was higher after marathon almost after every race for 10 days (Figure 3). On the other hand, amount of reduced glutathione was lower after marathon in both athletes in the same manner

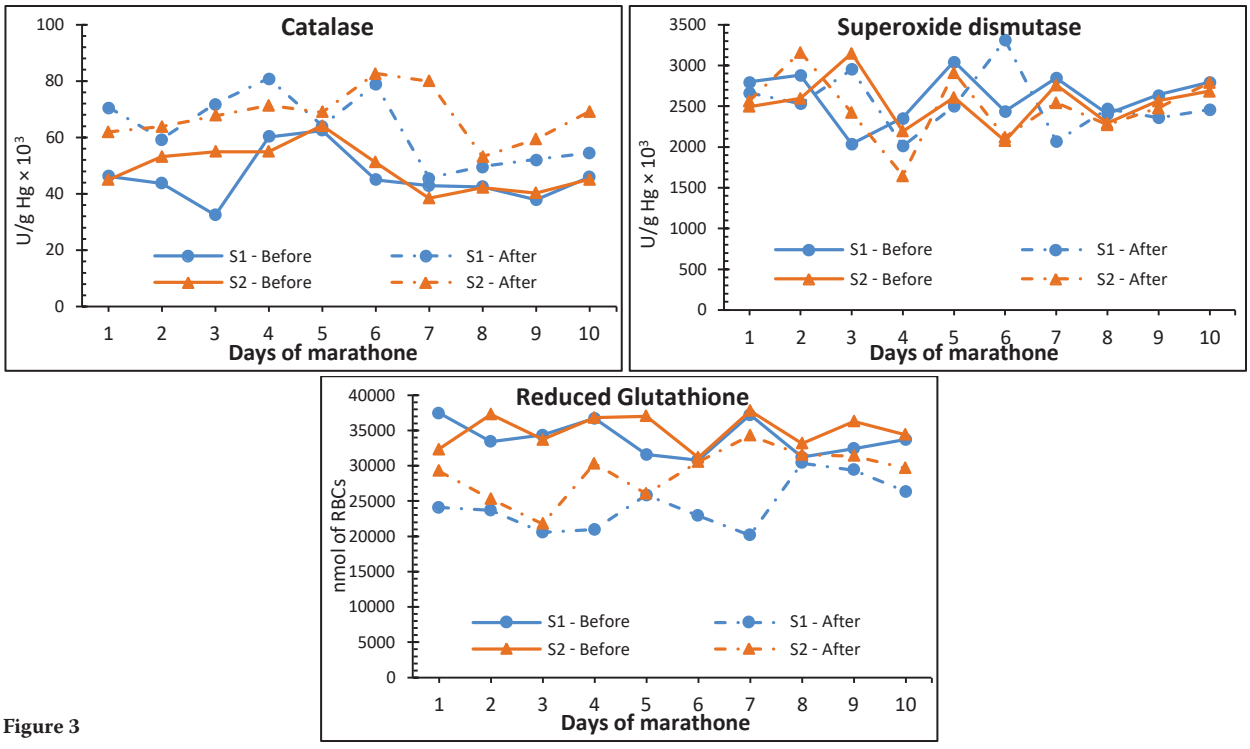

Figure 3

(Figure 3). Activity of superoxide dismutase did not exhibit any regular pattern of changes (Figure 3). Similarly to oxidative stress biomarkers, values of described antioxidative elements were determined by spectrophotometry (15-18).

DISCUSSION

Kratz and coworkers in their study followed 37 marathon runners during the 2001 Boston Marathon and showed the statistically significant increase of in the values for glucose, albumin, total protein, creatinine and some other biochemical parameters, which were not the subject of this investigation, 4 hours after marathon (19). Namely, authors of mentioned study took blood samples day before marathon, and 4 hours and 24 hours after marathon. 24 hours after marathon values of creatinine were still higher compared to values before marathon. On the other hand, there were statistically insignificant increase in values of sodium and potassium. Traiperm and colleagues followed fifty adolescent marathon runners, and blood samples were taken before, at the end and 24 h after a marathon to investigate parameters of metabolism, liver and kidney function (20). There was a statistically significant increase in the levels of blood glucose and creatinine immediately after the race. Bearing in mind that in this investigation glucose and creatinine level were generally maintained within the physiological values, these discrepancies could be the consequence of different amount of carbohydrate in rehydration beverages. Gratze and coworkers in their study showed unchanged values of serum potassium before and after marathon (21).

In a study performed by Mrakic-Sposta and colleagues it was assessed the effect of mountain ultra marathon (MUM) on production of ROS and oxidative damage in forty-six experienced ultra-marathon runners (10). It was shown that this extreme physical effort has induced increase in production of free radicals, antioxidative defense and oxidative damage. These results differ from the results of this research, and this difference could be the consequence of various race regimes, as well as probably different supplementation protocols. Namely, MUM represents an extreme physical effort, much more demanding than the regime in this research, while there was no specific supplementation protocol and any control of use of vitamin/minerals supplements, herbs and medications. Samaras and coauthors showed results regarding the effects of different supplementation regimes on redox balance and endothelial function in ultra-marathon runners for a two-months-period (22). In group of athletes with supplementation enriched with whey protein bar containing carbohydrates and protein in a specific ratio (1:1) and tomato juice, measured oxidative stress biomarkers (total antioxidant capacity - TAC, GSH, TBARS and protein carbonyls - CARB) were significantly lower. Similarly to the results of this study, Ypatios and colleagues pointed out the reduction of GSH during MUM race of 103 km (23), while TBARS, TAC, CARB, CAT activity in erythrocytes, as other measured parameters of oxidative stress, were widely varied in the respondents, so there were no statistically significant differences in the changes. Bearing in mind the results of this study and other mentioned investigations it is indicative that it is not possible to define a redox status change pattern during physical activity, above all extreme physical efforts. One of the reasons for the nonconformity of research results that deal with this topic is a large number of factors that can affect redox balance, but supplementation with antioxidants is probably one of the key issues (24).

CONCLUSION

Taking into account the results of this case presentation, and other studies dealing with similar topics, it can be concluded that redox balance during physical activity depends on many factors. Namely, increased production of free radicals during physical activity is undisputed, but oxidative damage could be prevented. One of the most important links in extreme physical efforts is adequate supplementation, among other things, the use of appropriate antioxidants at the right time. Some future investigations should reveal what are the most appropriate supplements in certain types of sports, and how to adapt them to each athlete individually in accordance with their constitution and needs, in order to achieve as good results as possible with the least damage.

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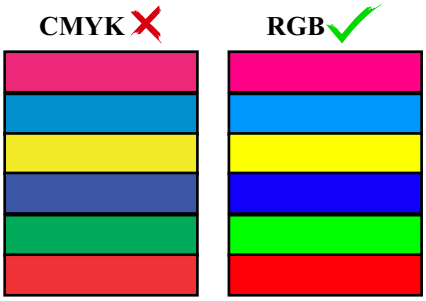

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