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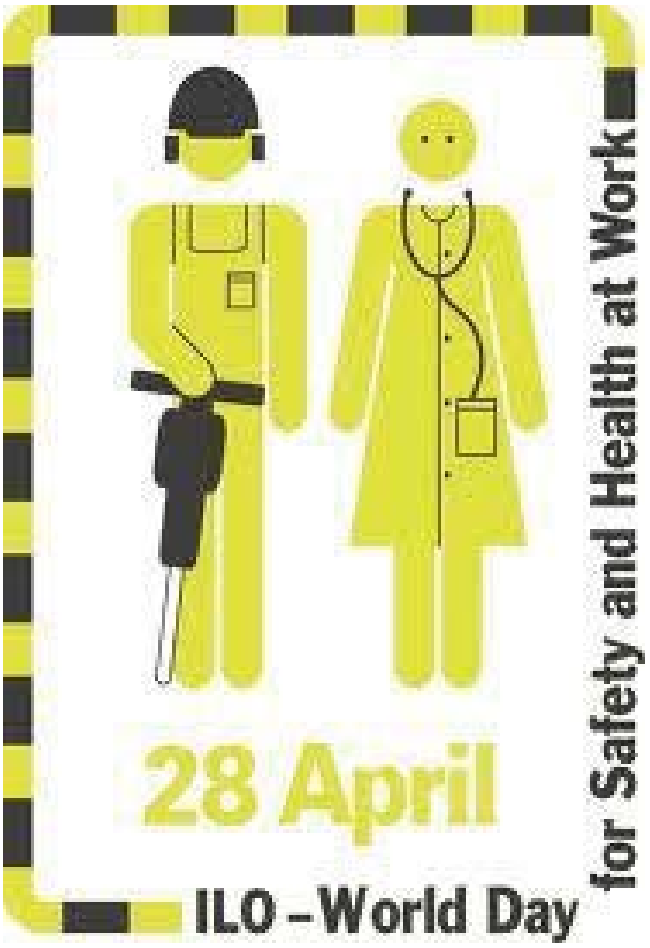

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SADRŽAJ / CONTENTS

The International Labour Organization (ILO) marks the World Day for Safety and Health at Work on the 28 April to promote the prevention of occupational accidents and diseases globally. Investment in prevention has led to a significant decrease in occupational accidents and diseases and consenquently can save millions of lives and prevent enormous human suffering. The theme for the World Day for Safety and Health at Work in 2013 is: The Prevention of Occupational Diseases (see Editorial, p. 343–5).

Međunarodna organizacija rada (*International Labour Organization* - ILO) obeležava 28. april kao Svetski dan bezbednosti i zdravlja na radu, sa ciljem da se unapredi prevencija profesionalnih akcidenata i oboljenja na globalnom nivou. Ulaganje u prevenciju značajno smanjuje pojavu profesionalnih bolesti i nesreća na radu i, posledično, može da spasi milione života i spreči ogromne ljudske patnje.
Tema ovogodišnjeg Svetskog dana bezbednosti i zdravlja na radu jeste: Prevencija profesionalnih bolesti (vidi Uvodnik, str. 343–5).

E D I T O R I A L / U V O D N I K

The World Day for Safety and Health at Work

Svetski dan bezbednosti i zdravlja na radu

Branka Djurović

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The International Labour Organization (ILO) celebrates 28th April as the World Day for Safety and Health at Work. This day was established by the ILO, at the initiative of the World Trade Union, 2003 in memory of workers died at work all over the world with the aim to improve the safety and health development at work.

ILO data show numerous serious problems even nowadays. Still, each year 2.3 million people in the world die from diseases related to work, an average of 6,300 people each day. Of these, the injuries are cause of 321,000 death, and illness (occupational or work-related) is cause of death of 2.2 million poeple, which amounts to more than 5,500 a day. However, only 15% of workers in the world have provided regular medical care [1].

Although technologically developed EU has not spared these problems. According to the European Agency for Safety and Health at Work (EU-OSHA) in the EU 5,580 people die every year in accidents at work and 159,500 people die from work-related diseases. EU-OSHA estimates that every three and a half minutes somebody in the EU dies from work-related causes. Nevertheless, during last decade, the number of occupational accidents resulting in death permanently decreased, although unevenly by year and country [1, 3, 5].

Costs of occupational diseases treatment are very high, not only for the worker and his family but society, in whole. The ILO estimates that economic losses amounting to 4% of the world's gross national product. Because the inadequate prevention is the main cause of occupational diseases worldwide, ILO dedicated this year to its improvement and suggests to all countries to take concrete actions [1, 4].

ILO has adopted a number of strategic general documents for the improvement of safety and health, as well as a number of specific ones related to working conditions. To ensure the application of the same, ILO adopted an Action Plan for the period 2010–2016. This Plan provides the activities of all the relevant subjects for the improvement of safety and health at work from the governmental bodies and agencies, through employers, inspections, to the workers themselves. A special at-

Međunarodna organizacija rada (*International Labour Organization* – ILO) obeležava 28. april kao Svetski dan bezbednosti i zdravlja na radu. Ovaj dan ustanovljen je 2003. godine na inicijativu Svetske trgovačke unije, 2003. godine u znak sećanja na radnike postradale na radnim mestima širom sveta, sa ciljem da podstakne razvoj bezbednosti i zdravlja na radu.

Podaci ILO ukazuju na postojanje značajnih problema u oblasti zaštite zdravlja na radu. I danas 2,3 miliona ljudi u svetu godišnje umre zbog oboljenja u vezi sa radom, što je u proseku 6 300 osoba svakog dana. Od toga, zbog povreda na radu umire 321 000, a zbog bolesti (profesionalnih ili u vezi sa radom) 2,2 miliona ljudi, što iznosi više od 5 500 smrtnih slučajeva dnevno. Uprkos ovim podacima, samo 15% radnika u svetu ima obezbeđen redovan medicinski nadzor [1].

Iako tehnološki razvijena, Evropska unija (EU) nije pošteđena ovih problema. Prema podacima Evropske agencije za bezbednost i zdravlje na radu (EU-OSHA) u zemljama EU godišnje umire 5 580 osoba u nesrećama na radu i njih 159 500 od bolesti povezanih sa radom. EU-OSHA procenjuje da svaka tri i po minuta neko u EU umre od uzroka povezanih sa radom. Tokom poslednje dekade broj nesreća na radu sa smrtnim ishodom, ipak se, stalno mada neujednačeno po godinama i zemljama, smanjivao [1, 3, 5].

Troškovi profesionalnih bolesti su visoki ne samo za obolelog i njegovu porodicu, već i za čitavo društvo. ILO procenjuje da ovi troškovi iznose 4% bruto nacionalnog dohotka. Pošto je neadekvatna prevencija glavni razlog nastanka profesinalnih oboljenja, ILO je tekuću godinu posvetila poboljšanju prevencije i preporučila svim zemljama da preduzmu konkretne mere [1, 4].

ILO je usvojila čitav niz strateških dokumenata opšteg tipa za unapređenje bezbednosti i zdravlja na radu, kao i veći broj specifičnih propisa za pojedine štetnosti. Da bi obezbedila primenu istih, usvojila je i Akcioni plan za period 2010–2016. Ovim planom predviđene su aktivnosti svih relevantnih činilaca za unapređenje bezbednosti i zdravlja na radu – od državnih tela i agencija, preko poslodavaca, do samih radnika. Posebna pažnja poklonjena je malim i srednjim preduze-

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tention is paid to small and medium enterprises as the units with the most problems. The Action Plan was stressed that each country has to adapt activities according to their own situation, taking into account the specific problems [1, 2, 3].

World Health Organization (WHO) joined the efforts, and adopted a global action plan for health and safety for the period 2008–2017 on the meeting held on May 2007. This plan was unanimously adopted by the 193 member states of the WHO, became an unified framework for the planning, implementation and evaluation of basic activities for the protection and promotion of health in the workplace [6].

Serbia has joined the celebration of the World Day for Safety and Health at Work after Government Decision to establish Day of Safety and Health at Work in Serbia ("RS Official Gazette", No. 17/10).

In Serbia the number of fatal injuries and reduced the number of collective harm in 2011 were by 20% and 18%, respectively lower than in 2010. This trend continued in 2012. Statistics shows that in Serbia in the workplace annually died about 40 people, and about 1,000 of them are severely injured. The highest number of accidents happen because of non-compliance with safety regulations and failure to use protective equipement. According to the available data, about 50% of accidents happen, as well as in the EU, in the construction industry.

During recent years, Serbia made efforts to establish new or correct the existing legal framework in this area. Law on Safety and Health at Work was adopted in 2005, followed by the adoption of nearly twenty-laws in line with EU directives, first of all with the requirements of the Directive 89/391 EEC. One of the most important is certainly the Regulations on the Procedure for the Risk Asessment in the Workplace and Work Environment. The essence of the change is that employers are responsible for ensuring safety and health at work, or for its main part–risk assessment and preventive measures in the workplace.

In accordance with all these documents National Strategy on Health and Safety at Work 2009–2012 ("Off. Gazette of RS", no. 32/2009) was established to ensure the implementation of its decisions.

According to the Law on Safety and Health at Work, Department for Safety and Health at Work was organized, with the aim to develop cooperation with international organizations and harmonize regulations and practices with EU. It is believed that the best way to achieve these goals is the improvement of work culture, primarily through education of relevant subjects. The department also organizes professional examinations for the performance of safety and health at work and issues licenses to legal entities and entrepreneurs to perform these tasks.

Still, a lot of problems have to be solved.

A particular problem in Serbia is irregular and incomplete implementation of adopted legislation. It is estimated that the causes are on several levels: general-social problem, such as unregulated relations between employers, especially small and medium enterprises, with employees. Work "off the books", the employment of inadequate education and avoidance of employers to provide full security and safety for

ćima kao sredinama sa najviše problema u ovoj oblasti. Akcionim planom naglašeno je da svaka zemlja mora da prilagodi aktivnosti prema sopstvenoj situaciji uvažavajući specifičnosti sopstvenih problema [1, 2, 3].

Naporima se pridružila i Svetska zdravstvena organizacija koja je na skupštini održanoj maja 2007. usvojila Globalni plan aktivnosti za zdravlje na radu, za period 2008–2017 godine. Ovaj plan usvojen je jednoglasno od strane 193 države članice, čime je postavljen jedinstveni okvir za planiranje, realizaciju i evaluaciju osnovnih aktivnosti za zaštitu i promociju zdravlja na radnom mestu [6].

Srbija se pridružila obeležavanju Svetskog dana bezbednosti i zdravlja na radu 2010, odlukom Vlade o ustanovljavanju Dana bezbednosti i zdravlja na radu u Republici Srbiji („Službeni glasnik RS“, broj 17/10).

Podaci iz naše zemlje pokazuju da je broj smrtonosnih povreda na radu 2011. godini bio 20% manji nego 2010, a za 18% bio je smanjen i broj kolektivnih povreda. Ta tendencija nastavljena je i 2012. godine. Statistika pokazuje da u Srbiji na radnom mestu godišnje strada oko 40 radnika, a oko 1 000 njih se teško povredi. Najveći broj povreda na radu dešava se zbog nepoštovanja bezbednosnih propisa i nekorišćenja zaštitne opreme. Prema raspoloživim podacima oko 50% povreda na radu dešava se u građevinarstvu, kao i u zemljama EU [7].

Poslednjih godina u Srbiji se ulažu veliki napori da se koriguje postojeći zakonski okvir u ovoj oblasti. Zakon o bezbednosti i zdravlju na radu donet 2005. godine pratilo je donošenje skoro dvadeset podzakonskih akata usklađenih sa Direktivama EU, pre svih sa zahtevima Direktive 89/391 EEC o uvođenju mera za podsticanje i poboljšanju bezbednosti i zdravlja zaposlenih na radu. Jedan od najznačajnijih donetih pravilnika svakako je Pravilnik o načinu i postupku procene rizika na radnom mestu i u radnoj okolini. Suština promena je da su poslodavci odgovorni za sistem bezbednosti i zdravlja na radu, odnosno za njegov osnovni deo – procenu rizika i preventivne mere na radnim mestima.

U skladu sa navedenim dokumentima doneta je Nacionalna strategija o bezbednosti i zdravlju na radu za period 2009–2012 (Sl. list RS, br. 32/2009), kojom je trebalo obezbediti sprovođenje donetih odluka.

Prema odredbama Zakona o bezbednosti i zdravlju na radu osnovana je i Uprava za bezbednost i zdravlje na radu sa osnovnim zadatkom da razvija saradnju sa međunarodnim organizacijama i harmonizuje propise i praksu sa direktivama EU. Smatra se da je najbolji put za postizanje ovih ciljeva podizanje kulture rada, pre svega kroz obuku svih zainetresovanih strana. Uprava, takođe, organizuje polaganje stručnih ispita za obavljanje poslova bezbednosti i zdravlja na radu i izdaje licence pravnim licima i preduzetnicima za obavljanje ovih poslova.

U skladu sa korigovanim propisima potrebno je u narednom periodu rešiti veliki broj problema.

Poseban problem u Srbiji predstavljaju neredovna i nekompletna primena donetih zakonskih propisa. Ocenjuje se da se uzroci nalaze na više nivoa: opšti – društveni problemi, kao što su neregulisani odnosi između poslodavaca, prvenstveno malih i srednjih preduzeća, sa zaposlenima, rad „na crno“, zapošljavanje radnika neadekvatnog obrazovanja i izbegavanje poslodavaca da pruže potpunu bezbednost na radu zarad sops-

the sake of their own profits. Many employers still consider invest in health and safety at work as a cost rather than an investment. Many workers are not insured against industrial accidents and occupational diseases and as a result injuries are often unreported. In addition, records of accidents at work and occupational diseases are not carried on the whole territory of Serbia, so that collected data on occupational diseases and injuries, do not give a true picture of the situation in the country. The data that Serbia has a significantly lower number of workplace injuries and occupational diseases than EU countries are not considered real.

EU Directives are not completely followed, particularly in the area of education. Therefore, it will be very important part of the National Strategy of Health and Safety at Work for the next period 2013–2017 [7].

A list of occupational diseases in Serbia is incomplete. With only 56 diseases, it is well below the European and the international average. In EU countries, the list contains up to 250 diseases, and on the list of the ILO there are 106 of them. Therefore, during last year only 119 workers received a disability pension due to illness or injury caused by poor working conditions, even though 42,000 workers requested it. A proposal for a new list of occupational diseases involves the existence of "open work" that would include a provision that the occupational disease may be other diseases that are not explicitly mentioned in the list, but which can be proved to have caused hazards in the workplace [7].

Occupational medicine in Serbia has collapsed – from 860 occupational medicine specialists in 2000 to 240 nowadays. Most of specialists chose to work in the capacity of the chosen physician, following the changes in the financing of health services. As a consequence, preventive measures are neglected.

Bearing in mind that the goal of this year's World Day for Safety and Health at Work is to improve prevention, it is necessary to make a system solution that would eliminate the above shortcomings. The money spent on health and safety at work should not be seen as the cost but the best investment for the economy of each country and society as a whole.

tvenog profita. Mnogi poslodavci i dalje doživljavaju ulaganje u bezbednost i zdravlje na radu samo kao rashod.

Mnogi radnici nisu osigurani od povreda na radu i profesionalnih bolesti, zbog čega se one često i ne prijavljuju. Osim toga, evidencija o povredama na radu i profesionalnim bolestima se ne vodi na celoj teritoriji Srbije, tako da prikupljeni podaci o profesionalnim bolestima ne daju realnu sliku stanja u zemlji, te se podaci da u Srbiji ima značajno manji broj povreda na radu i profesionalnih bolesti negó u zemljama EU ne mogu smatrati realnim.

Direktive EU u praksi se ne poštuju u potpunosti, naročito u oblasti obuke zaposlenih. Stoga, prema Strategiji bezbednosti i zdravlja na radu Srbije za naredni period 2013–2017, ona će zauzimati veoma važno mesto [7].

Lista profesionalnih bolesti u Srbiji je nepotpuna. Na njoj se nalazi samo 56 oboljenja, što je mnogo manje od evropskog i međunarodnog proseka. U zemljama EU ova lista sadrži i do 250 oboljenja, a na spisku Međunarodne organizacije rada nalazi se njih 106. Kod nas je samo 119 radnika prošle godine dobilo invalidsku penziju zbog oboljenja i povreda nastalih usled loših uslova rada, iako je razmatrano čak 42 000 zahteva. Predlog nove liste profesionalnih bolesti uključuje i postojanje „otvorenog dela“ koji bi podrazumevao odredbu da se profesionalnim oboljenjem mogu smatrati i druge bolesti koje eksplicitno nisu navedene na listi, a za koje se može dokazati da su izazvane štetnostima na radnom mestu [7].

Služba medicine rada u Srbiji je urušena – od 860 specijalista medicina rada, koliko ih je bilo početkom 2000. godine, do 240 lekara koji trenutno rade. Većina lekara je izabrala da radi u svojstvu izabranog lekara, posle promena u finansiranju Službe medicine rada, zbog čega je preventivni rad zanemaren.

Imajući u vidu da je cilj ovogodišnjeg Svetskog dana bezbednosti i zdravlja na radu poboljšanje prevencije, neophodno je doneti sistemska rešenja, kojima bi se otklonili navedeni nedostaci. Rešenja su poznata, a novac potrošen na bezbednost i zdravlje na radu ne sme biti posmatran kao trošak, već kao najbolja investicija za privredu svake zemlje i društvo u celini.

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ORIGINAL ARTICLE

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Receptor activator of nuclear factor kappa B (RANK) as a determinant of peri-implantitis

Receptor aktivatora nuklearnog faktora kapa B kao činilac periimplantitisa

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Abstract

Background/Aim. Peri-implantitis presents inflammatory process that affects soft and hard supporting tissues of osseointegrated implant based on inflammatory osteoclastogenesis. The aim of this study was to investigate whether receptor activator of nuclear factor kappa B (RANK) concentrations in peri-implant crevicular fluid could be associated with clinical parameters that reflect inflammatory nature of peri-implantitis. **Methods.** The study included 67 patients, 22 with diagnosed peri-implantitis, 22 persons with healthy peri-implant tissues and 23 patients with periodontitis. Clinical parameters from each patient were recorded and samples of peri-implant/gingival crevicular fluid were collected for the enzyme-linked immunosorbent assay (ELISA) analysis. **Results.** RANK concentration was significantly increased in samples from the patients with peri-implantitis when compared to healthy implants ($p < 0.0001$), where the average levels were 9 times higher. At the same time RANK concentration was significantly higher in peri-implantitis than in periodontitis sites ($p < 0.0001$). In implant patients pocket depths and bleeding on probing values were positively associated with high RANK concentrations ($p < 0.0001$). **Conclusion.** These results revealed association of increased RANK concentration in samples of peri-implant/gingival crevicular fluid with peri-implant inflammation and suggests that RANK could be a pathologic determinant of peri-implantitis, thereby a potential parameter in assessment of peri-implant tissue inflammation and a potential target in designing treatment strategies.

Key words:
receptor activator of nuclear factor-kappa b; sensitivity and specificity; dental implantation, endosseus; periodontitis.

Apstrakt

Uvod/Cilj. Periimplantitis predstavlja inflamatorni proces koji zahvata meko i tvrdo potporno tkivo osteointegrisanog implantata, i zasnovan je na inflamatornoj osteoklastogenezi. Cilj studije bio je da se utvrdi povezanost koncentracije receptora aktivatora nuklearnog faktora kapa-B (RANK), kao glavnog receptora osteoklastnog metabolizma, sa kliničkim parametrima periimplantitisa. **Metode.** Studija je uključila 67 sistemski zdravih pacijenata (22 sa periimplantitisom, 22 sa zdravim implantatima i 23 sa periodontopatijom). Pacijentima su mereni klinički parametri i uziman je uzorak periimplantne/gingivalne tečnosti za određivanje koncentracije RANK-a ELISA metodom. **Rezultati.** Koncentracija RANK-a bila je značajno povišena kod periimplantitisa u odnosu na zdrave implantate ($p < 0,0001$), gde je srednja vrednost koncentracije bila 9 puta veća. Istovremeno, RANK je bio značajno viši kod periimplantitisa nego kod parodontopatije ($p < 0,0001$). U grupi sa implantatima dubina periodontalnog džepa i krvarenje na probu bili su pozitivno udruženi sa visokim vrednostima RANK-a ($p < 0,0001$). **Zaključak.** Rezultati istraživanja pokazuju udruženost povišenosti koncentracije RANK-a sa periimplantnom inflamacijom i navodi na zaključak da bi RANK mogao da bude patološka determinanta periimplantitisa, a time i potencijalni parametar za praćenje inflamacije periimplantnog tkiva i potencijalni cilj za pravljenje terapijskih strategija.

Ključne reči:
receptor, aktivator nuklearnog faktora-kappa-b; osetljivost i specifičnost; stomatološka enosalna implantacija; periodontitis.

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Introduction

Peri-implantitis represents an inflammatory process that affects soft and hard supporting tissues of an osseointegrated implant, where the infection and excessive biomechanical forces are recognized as main etiologic factors [1, 2]. After induction, the peri-implantitis pathogenesis results from the interplay between specific subgingival microorganisms and inflammatory and immune responses, acting in the same way and using the same effector mechanisms as evidenced in periodontal disease (periodontitis) [3, 4]. *Aggregatibacter actinomycetemcomitans*, a gram-negative facultative capnophilic bacteria, is identified as the major etiological pathogen of localized juvenile periodontitis (LJP) until recently known as localized aggressive periodontitis and rapidly progressing periodontitis [5]. Data from experimental model of (NOD)-SCID mice reconstituted with human peripheral blood leukocytes from patients with periodontitis and challenged with *A. actinomycetemcomitans* clearly showed that activated human CD4 T cells are essential effectors of alveolar bone destruction [6]. Maintenance, formation, and remodeling of alveolar bone is an outcome of balanced activity of final effector cells, bone-resorbing osteoclasts and bone-producing osteoblasts. Osteoclastogenesis with consequential bone loss represents the hallmark of peri-implantitis, distinguishing it from previous developmental stage, peri-mucositis, where the process is limited only on soft tissues [7, 8]. In course of such inflammatory bone resorption, receptor activator of nuclear factor kappa B (RANK) and his ligand (RANKL) have been recognized as key regulatory factors in osteoclasts metabolism [9–11], particularly in periodontal disease. Receptor activator of nuclear factor kappa B also known as the osteoclasts differentiation factor receptor is a 11A member of the tumor necrosis factor (TNF) superfamily. The human RANK is a transmembrane receptor of 616 amino-acids expressed primarily on the cells of the monocyte/macrophage lineage including preosteoclasts and osteoclasts, B- and T- lymphocytes, dendritic cells and fibroblasts [12, 13]. Since RANK is localized on the surfaces of preosteoclasts and osteoclasts its ligation by a specific ligand, RANKL, leads to differentiation and maturation of progenitor cells simultaneously with osteoclasts activity enhancement [14–16]. The key signal for this mechanism is the achievement of critical concentrations of pro-inflammatory cytokines whose gene transcription is regulated by nuclear factor kappa B (NF-κB) [17].

Regulation of RANK/RANKL interaction is performed by a receptor-like molecule named osteoprotegerin (OPG) which binds RANKL with high affinity and thereby blocks RANKL/RANK interaction with a consequential inhibition of osteoclasts activity [18, 19]. RANKL could be found in soluble form or expressed by osteoblasts, stromal cells, fibroblasts, B-cells and T-cells [20, 17] under different stimulation such as pro-resorptive hormones (such as parathormone, epinephrine, 17β-estradiol and glucocorticoides), cytokines (such as IL-1, IL-6, IL-8, IL-11, IL-17, TNFα and IFNγ) and bacterial lipopolysaccharide (LPS) [18, 21].

Clinical and radiological parameters of peri-implantitis are conventional tools for determining diagnosis and status in established tissue impairment, but are insensitive for early diagnosis and as a prognostic factors. Peri-implant crevicular fluid (PICF) was found to be reliable in reflecting surrounding tissues status since the volume and composition directly depends on their condition [22]. Considering that, a number of researches were conducted on the topic of RANKL and OPG evaluation in PICF and gingival crevicular fluid (GCF) at different statuses of supporting tissues [23, 24]. However, these results on RANK and its role in peri-implantitis are still non-existent. The aim of this study was to investigate whether RANK concentrations in PICF could be associated with clinical parameters that reflect inflammatory nature of peri-implantitis.

Methods

This was the cross-sectional study conducted in the Clinic of Periodontology and Oral Medicine, School of Dentistry, Belgrade, Serbia, Clinic for Maxillofacial, Oral Surgery and Implantology, Military Medical Academy and Institute for Medical Research, Military Medical Academy from June 2009 until February 2011. The study included 67 patients divided into 3 groups: peri-implantitis (n = 22), healthy implants (n = 22) and periodontitis (n = 23). Peri-implantitis was accepted in the presence of clinical signs (Figure 1) including: peri-implant pocket depth (PPD) ≥ 5 mm or in the presence of gingival recession relative clinical attachment level (rCAL) ≥ 4 mm, with positive bleeding on probing (BOP) and recorded radiographic bone loss involving ≥ 2 threads compared to radiography taken at the time of prosthetic replacement. Intraoral radiographies were performed for radiological evidence of bone loss using paralleling technique, where implant threads were used as referent points. Only peri-implantitis after at least 2 years of loading and without previous peri-implantitis treating were included. As healthy peri-implant tissues were accepted implants without any clinical signs of inflammation including the absence of subjective difficulties, BOP = 0 and PPD ≤ 3mm. Implants included in the study were delayed loaded endosseal implants with the purity level of 2/ASTM (American Society

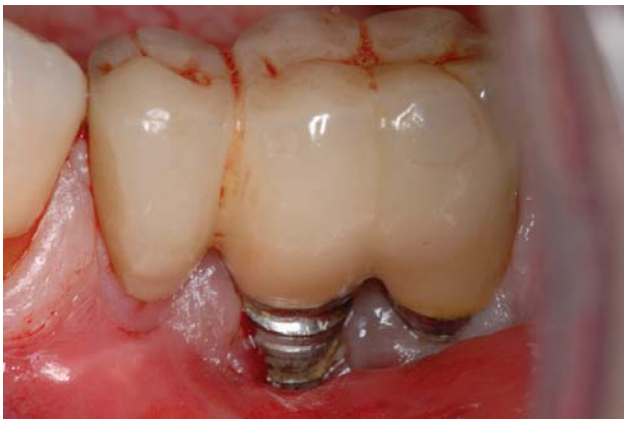

Fig. 1 – Clinical signs of peri-implantitis presented by positive bleeding on probing and a clinically visible loss of soft and hard peri-implant tissues

for Testing and Materials) (99.98%) and a sand-blasted, large-grit, acid (SLA) etched surface inserted. Implants were 4.5 mm in diameter, 3.5 mm long with 4 threads. As periodontitis were accepted the patients with diagnosed severe generalized chronic periodontitis accordingly to the classification of periodontal disease [25].

All the patients were systemically healthy adult non-smokers, and exclusion criteria were received peri-implant/periodontal treatment in the preceding 1 year, usage of antibiotics and anti-inflammatory agents within the preceding 3 months, menstruation, pregnancy and lactation in female patients. The study protocol was approved by the Ethics Committees of both two institutions (Ethics Committee School of Dentistry and Ethics Committee Military Medical Academy), patients were informed on the study protocol and they were obligated to give written consent before procedures.

Clinical examination

The following clinical parameters were measured in 6 points: mesio-bucal, medio-bucal, disto-bucal, mesio-lingual, medio-lingual and disto-lingual (Figure 2): PPD and packet depth (PD) by BOP: presence (1) or absence (0) of bleeding for up to 15 sec after probing, and visible plaque accumulation (PI): presence (1) or absence (0) of plaque along the mucosal margin [26]. All measurements were performed by the one same trained and calibrated examiner using the same type of the graduated periodontal probe (North Carolina–Hu-Friedy, Chicago, IL, USA). Intra-examiner calibration was performed twice, before and during the study, by assessing PPD and with a degree of agreement within ± 1 mm higher than 85%. The implant/tooth site with the deepest probing depth was chosen as a representative for sampling; in case of similar probing depths the anterior point was chosen as a step toward higher precision.

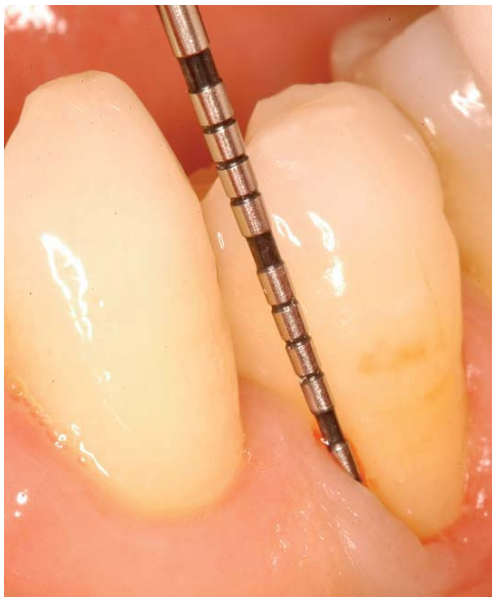

Fig. 2 – Measurement of periodontal pocket depth using a graduated periodontal probe at the tooth with positive bleeding on probing and with a clinically visible loss of soft and hard periodontal tissues

The peri-implant crevicular fluid (PICF) and gingival crevicular fluid (GCF)sampling

PICP and GCF samples were obtained from the patients using the filter paper technique 24 h after the examination. After removing the supragingival biofilm with sterile cotton rolls, the sampling place was isolated with cotton rolls and gently air dried 1 min before sampling in the aim to eliminate any potential contamination with saliva. A paper strip of standard length and height (Periopaper, Pro Flow, Amityville, NY, USA) was inserted into the peri-implant and gingival/periodontal sulcus/pocket until mild resistance was felt and left in place for 30 s. Strips that were visually contaminated with blood or saliva were discarded. Sampled fluid volume was measured with calibrated Periotron 6000 (Interstate Drug Exchange, Amityville, NY, USA). After measurement strips were inserted in microcentrifuge plastic tubes with 100 μL of sterile phosphatebuffered saline. The sampling time method which includes a total amount of RANK in picograms (pg) per site during 30 s was chosen because the method was supported by previous studies as convenient for related researches [27]. Following 10 s of vortexing, eluates were centrifuged 5 min at 3000 g to remove plaque and cellular elements, after that the strips were removed. The samples were stored at −70°C until enzyme-linked immunosorbent assay (ELISA) analysis.

Determination of RANK using ELISA

Concentrations of RANK in PICF and GCF were assessed using a commercially available ELISA kit (R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's recommendations. A calibration curve was plotted by regression analysis and the optical density of the sample was used to estimate the concentration of RANK. The intensity of the color was measured using spectrophotometry (450/620 nm, ELISA processor II, Boehring, Germany). The obtained values of RANK were calculated from picomoles into picograms, and the concentration was expressed as RANK (pg) per sample / PICF volume (mL).

Statistical analysis

Analysis of the obtained data was performed using statistical software (SAS Enterprise Guide 4.1, SAS Institute Inc., 2008). After descriptive statistical analysis, data were examined by the Shapiro-Wilk and Kolmogorov-Smirnov test in order to test the normality assumption. Since normality was not achieved for each clinical parameter, further analysis was based upon non-parametric tests. In some cases „Exact" option was applied in order to obtain more precise *p* values. The Wilcoxon test was done to assess the difference in mean for each clinical parameter within groups, whereas another pair wise comparison was done by the Kruskal–Wallis test. The significance level established for all analyses was 5% ($p < 0.05$).

Results

The study population of 67 subjects included 30 females and 37 males, the average age of 38.8 ± 7.73 years (23–60 years).

The volume of collected PICF/GCF was similar in samples of all the investigated groups (Table 1). Mean score values of PPD, BOP and plaque accumulation index (PI) were as expected significantly elevated in the peri-implantitis and periodontitis groups comparing to the controls, but did not differ among each other. Finally, mean RANK levels were highest in the samples of the peri-implantits group and lowest in the control group. Score values of BOP, PI, PPD, volume of collected PICF/GCF and RANK concentrations did not correlate with gender and age of the investigated subjects (Table 2).

The score values of all the clinical parameters were significantly increased in inflamed sites, either in peri-implantitis or periodontitis groups, as expected considering group's characteristics. When we divided patients with peri-implantits according to RANK levels detected in their PICF, mean score levels of peri-implant pocket depth and positive bleeding on probing were significantly higher in those patients that had RANK levels above 1,000 pg/mL, comparing to the group of those patient with lower RANK concentration (Table 4). These findings point out strong association of crucial clinical signs, PPD and BOP with high local RANK concentration.

Table 1

Descriptive statistics of RANK concentration and the measured clinical parameters among the groups

Group of patients	PICF/GCF (uL)	RANK (pg/mL)	BOP (score)	PI (score)	PPD/PD (score)
Control	0.44 ± 0.19	255.36 ± 240.31	0.00 ± 0.00	0.81 ± 0.90	1.72 ± 0.45
Peri-implantitis	0.61 ± 0.23	1514.49 ± 888.01	6.00 ± 0.00	5.04 ± 1.81	5.72 ± 0.88
Periodontitis	0.55 ± 0.39	421.79 ± 266.93	5.30 ± 1.11	5.08 ± 0.79	6.34 ± 1.52

PICF/GCF – peri-implant crevicular fluid/gingival crevicular fluid; RANK – receptor activator of nuclear factor kappa-B; BOP – bleeding on probing; PI – plaque accumulation index; PPD/PD – peri-implant pocket depth/pocket depth

Table 2

Correlation of gender and age with the measured parameters

Patients	PICF/GCF (uL)	RANK (pg/ml)	BOP (score)	PI (score)	PPD/PD (score)
Gender (M/F)	R = -0.049 (p = 0.742)	R = -0.041 (p = 0.786)	R = -0.037 (p = 0.807)	R = 0.015 (p = 0.922)	R = 0,22889 (p = 0,1217)
Age (years)	R = -0.024 (p = 0.631)	R = -0.014 (p = 0.802)	R = -0.042 (p = 0.112)	R = 0.045 (p = 0.622)	R = 0,117 (p = 0,1217)

PICF/GCF – peri-implant crevicular fluid/gingival crevicular fluid; RANK – receptor activator of nuclear factor kappa-B; BOP – bleeding on probing; PI – plaque accumulation index; PPD/PD –peri-implant pocket depth/pocket depth; M – male; F – female

The concentration of RANK was significantly higher in the peri-implantitis than in the control group with healthy implants ($p < 0.0001$). By comparing RANK concentration between the peri-implantitis and the periodontitis group it is observed that RANK concentration was significantly increased in peri-implantitis sites ($p < 0.0001$) (Table 3).

Discussion

A variety of studies were dedicated to resolution of the multifactorial pathogenesis of peri-implantitis, aiming to improve the success of one of the main therapeutic solutions in contemporary dentistry. Still, numerous efforts to identify any

Table 3

Analysis of the differences of clinical and biochemical parameters between the groups (Wilcoxon test)

Groups of patients	RANK (pg/mL)	BOP (score)	PI (score)	PPD/PD (score)
The peri-implantits (P-I) *vs* control (C)	$p < 0.0001$ P-I > C	$p < 0.0001$ P-I > C	$p = 0.033$ P-I > C	$p < 0.0001$ P-I > C
The peri-implantitis (P-I) *vs* periodontitis (P-D)	$p < 0.0001$ P-I > P-D	$p = 0.061$	$p = 0.109$	$p = 0.177$
The periodontitis (P-D) *vs* control (C)	$p = 0.061$	$p < 0.0001$ P-D > C	$p < 0.0001$ P-D > C	$p < 0.0001$ P-D > C

RANK – receptor activator of nuclear factor kappa-B; BOP – bleeding on probing; PI – plaque accumulation index; PPD/PD – peri-implant pocket depth/pocket depth

Table 4

Clinical parameters analysis in implant sites according to RANK concentration

Clinical parameters	RANK < 1,000 pg/mL	RANK > 1,000 pg/mL	p
PPD (mm)	5.78 ± 1.02	3.57 ± 0.52	< 0001
BOP (score)	6.00 ± 0.00	3.73 ± 0.27	< .0001
PI (score)	5.12 ± 0.42	4.98 ± 0.40	= *0.419*

RANK – receptor activator of nuclear factor kappa-B; PPD –peri-implant pocket depth; BOP – bleeding on probing; PI – plaque accumulation index

reliable determinant and disease predictor are far from a usable parameter. To authors' knowledge this is the first study investigating RANK in patients suffering from peri-implantitis, hence direct comparison was limited. In this study, RANK values were assessed as a potential parameter, peri-implantitis for concerning its key role as a receptor mediated bone loss.

The mean RANK concetrations were several times higher in the PICF/GCF samples of peri-implantitis group comparing to the control group of patients with healthy implants, that had no signs of gingival inflammation. The highest individual concentration in healthy implants was lower than the lowest one in the peri-implantitis, indicating clear association of high RANK concentrations with peri-implant inflammation. Furthermore, the significant difference in RANK concentration was evidenced between the peri-implantitis and the periodontitis group, with highest levels in the peri-implantitis group.

From these findings, it could be concluded that a locally increased RANK concentration provided a potential base for more intensive inflammation comparing to periodontitis [28–35].

Our results additionally confirmed the association of clinical parameters as indicators of inflammation (probing depths and BOP) with high RANK values.

RANK is known to activate a cascade of intracellular signaling pathways resulting in rapid nuclear translocation and transcription of the genes coding pro-inflammatory cytokines [35]. The process is based on recruitment of TNF receptor-associated factor (TRAF) proteins that regulate transduction of signals from RANK with consequential activation of NF-κB as well as activation of mitogen-activated protein kinase pathway, where these two are recognized as crucial in regulation of expression and transcription of the genes coding pro-inflammatory cytokines. Moreover, RANK poses specific biological feature to induce osteoclastogenesis ligand independently by self-assembling reported by Kanazawa and Kudo [29], and additionally supported by Otero et al. [30]. They reported spontaneous osteoclastogenesis based on RANK activation that was driven by IkB kinase β (IKKβ) activated by proinflammatory cytokines TNFα and IL-1. In regard to these facts, increase in RANK could proportionally augment osteoclastogenesis independently of his ligand, usually considered as the main factor in inflammatory bone loss, therewith could present a pathologic pattern of enhanced inflammatory response specific to peri-implant inflammation [36–38].

Scores of clinical parameters were in general significantly higher in inflamed sites as expected considering group characteristics, but it was observed that the lowest value of PI in the peri-implantitis group was higher than the highest value in the healthy implants, and findings of increased PI are in correspondence with the previous findings of Duarte et al. [24]. By considering dental plaque as a possible source of of LPS [31, 32] increased PI values in peri-implantitis could suggest increase in RANKL expression in response to LPS stimulation described by Choi et al. [37]. Moreover the association of increased concentration of RANKL with peri-implantitis and their severity is already reported [24]. Local osteoclastogenesis could be also enhanced by augmentation of RANK/RANKL complexes, which are directly proportional to concentration of ligand and receptors found to be increased in peri-implantitis.

Regarding two proposed mechanisms, RANK increase could be a powerful enhancer of peri-implant inflammation by increasing transcriptions of genes coding pro-inflammatory mediators (such as IL-1, IL-2, IL-6, IL-8, IL-12, TNFα, cyclooxygenase-2 and nitric oxid synthase) [36] with consequential elevation of entire cytokine concentration. Local cytokine increment induced by locally produced and expressed RANK could result in osteoclasts differentiation and upregulation of their activity, the same as under increased RANKL expression [17], implicating that RANK could create a vicious circle with its increase.

On the other side, as documented in experimental models and in human samples of patients with periodontitis, activated local CD4 T lymphocytes are principal regulatory cells in alveolar bone destruction. In periodontal disease, gingival production of inflammatory mediators is under strong influence of locally activated Th-17 and Th-1 lymphocytes. Furthermore, Takahashi et al. [38] anticipated that locally activated IL-17 producing T-lymphocytes may be a primary source of RANKL in perodontitis. If we consider that peri-implantits and periodontits could be generated by similar mechanisms, increased RANK levels could have intense influence upon local T-lymphocytes, upregulating their functions, and *vice versa*, resulting in high osteoclasting activity and bone destruction.

This is the first study suggesting that an increased concentration of RANK could be related to peri-implantitis; thereby the special emphasis of forthcoming researches should be on the RANK biology, since the numerous studies were focused on RANKL and OPG [33, 34].

Conclusion

Taking into consideration the obtained results, increased concentration of RANK could be a parameter useful for diagnosis and monitoring peri-implantitis. On the other side, dissolving RANK functioning could provide new therapeutic strategies by bringing new target for therapeutic acting.

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Influence of peritoneal dialysis solution biocompatibility on long-term survival of patients on continuous ambulatory peritoneal dialysis and the technique itself

Uticaj biokompatibilnosti rastvora za peritoneumsku dijalizu na višegodišnje preživljavanje bolesnika na kontinuiranoj ambulantnoj peritoneumskoj dijalizi i same metode lečenja

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Abstract

Background/Aim. Morbidity and mortality of continous ambulatory peritoneal dialysis (CAPD) patients is still very high. The aim of the study was to evaluate the effects of peritoneal dialysis (PD) solutions (standard *vs* biocompatible) on long-term patients' and the techique survival. **Methods.** A total of 42 stable patients on CAPD participated in this cross-sectional study. They were prospectively followed-up during the twelve years. Patients with severe anemia (Hb < 10 g/L) and malignant disease ware excluded. Twenty one (50%) patients were treated with the standard PD solutions (CAPDP-1) while the other 21 (50%) were treated with biocompatible PD solutions [(lower level of glucose degradation products, lower concentration of Ca^{2+} and neutral pH (CAPDP-2)]. All patients were analyzed for a presence of vascular calcification, nutrition status, and parameters of inflammation after 2.5 ± 0.6 years of starting CAPD, and these variables considered in the analysis as risk factors. **Results.** The patients from the group CAPDP-2 compared to those from the group CAPDP-1 had lower level of high-sensitivity C-reactive protein (hs-CRP) ($p = 0.003$), and better nutritional status as confirmed by the mid-arm circumference ($p = 0.015$), and mid-arm muscle circumference ($p = 0.002$) and subjective global assessment ($p = 0.000$). Also, they had lower vascular calcifications as confirmed by intima media thickness (IMT) ($p = 0.003$), degree of carotid narrowing ($p = 0.001$) and calcified plaques of common carotid arteries (CCA) ($p = 0.008$). Kaplan-Meier analysis confirmed better survival of patients from the group CAPDP-2 than those from the group CAPDP-1 (1-, 5-, and 10-year patients survival rate was: 100%, 61.9% and 14.3% for the group CAPDP-1, and 100%, 85.7%, and 52.4% for the group CAPDP-2, respectively; $p = 0.0345$). The 1-, 5-, and 10-year technique survival rate was: 100%, 71.4%, and 38.1% for the group CAPDP-1, and 100%, 85.7%, and 76.2% for the group CAPDP-2, respectively; ($p = 0.0719$). Duration of dialysis, serum triglyceride and cardiovascular score (quantitative scoring system consisting of: ejection fraction (EF) of left ventricle < 50%; IMT > 1 mm; carotid narrowing degree > 50%, presence of carotid plaques in both common carotide, ischaemic heart disease, cerebrovascular event and peripheral vascular disease with or without amputation) were independent predictors of overall patient survival. Duration of dialysis was only independent predictor of overall technique survival. **Conclusion.** Although patients treated with biocompatible solutions showed significantly better survival, the role of biocompatibility of CAPD solutions in patients and technique survival have to be confirmed. Namely, multivariate analysis confirmed that duration of dialysis, serum triglyceride and cardiovascular score significantly predicted overall CAPD patients survival, while only duration of dialysis was found to be independent predictor of overall techique survival.

Key words:
peritoneal dialysis, continuous ambulatory; survival analysis; dialysis solutions; morbidity; mortality; risk factors.

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Apstrakt

Uvod/Cilj. Morbiditet i mortalitet bolesnika na kontinuiranoj ambulantnoj peritoneumskoj dijalizi (KAPD) i dalje je neprihvatljivo visok. Cilj rada bio je da se proceni uticaj vrste dijaliznih rasvora (bioinkompatibilni *vs* biokompatibilni) na višegodišnje preživljavanje bolesnika i same tehnike KAPD. **Metode.** Ovom studijom preseka sa delimično prospektivnim praćenjem ishoda lečenja obuhvaćeno je ukupno 42 nasumice izabrana, stabilna bolesnika (26 muškaraca i 16 žena) lečena primenom metode KAPD tokom poslednjih 12 godina. Isključeni su bolesnici sa teškom anemijom (Hb <10 g/L) i malignom bolešću. Pri tome, 21 (50%) bolesnika kontinuirano je lečeno bioinkompatibilnim rastvorom za KAPD (kiseli standardni rastvor – ANDY-disc; grupa KAPDB-1), dok je preostalih 21 bolesnik sve vreme bilo na biokompatibilnijem rastvoru za KAPD (neutralni rastvor sa znatno manjom koncentracijom degradacionih produkata glukoze, 1.25 mmol/L Ca i 40 mmol/L laktata – Gambrosol Trio; grupa KAPDB-2). Svim bolesnicima određeni su odabrani parametri hronične inflamacije, malnutricije i ateroskleroze zajedno sa transportnim karakteristikama peritoneumske membrane i rezidualnom bubrežnom funkcijom nakon 2,5 ± 0,6 god od započinjanja KAPD. Svi dobijeni rezultati analizirani su kao potencijalni faktori rizika. **Rezultati.** Grupa KAPDB-2 u odnosu na KAPDB-1 imala je statistički značajno niže vrednosti serumskog hs-CRP (p = 0,003) i bolje parametre nutritivnog statusa izražene kroz obim nadlaktice (p = 0,015), obim mišića nadlaktice (p = 0,002) i subjektivnu globalnu procenu (p = 0,000) kao i u manjoj meri prisutnu aterosklerozu potvrđeno debljinom intimomedijalnog kompleksa (IMT) (p = 0,003), stepenom suženja karotida (p = 0,001) i prisustvom kalcifikovanih ateromatoznih plakova na karotidnim arterijama (p = 0,008). Kaplan-Meier-ova kriva preživljavanja potvrdila je značajno duže preživljavanje bolesnika u grupi KAPD-2 u odnosu na KAPDB-1 (1-, 5-, i 10-godišnje preživljavanje bolesnika iznosilo je redom: 100%, 61.9% i 14.3% u KAPDB-1, a 100%, 85,7% i 52,4% u KAPDB-2 grupi; p = 0,0345). Stopa 1-, 5-, i 10-godišnjeg preživljavanja metode iznosila je: 100%, 71,4% i 38,1% u KAPDB-1, a 100%, 85,7% i 76,2% u KAPDB-2 grupi (p = 0,0719). Kao nezavisni prediktori opšteg preživljavanja bolesnika na KAPD izdvojili su se: dijalizni staž, nivo serumskih triglicerida i skor kardiovaskularnog morbiditeta (kvantitativni sistem zbrajanja prisutnih sledećih parametara: ejekciona frakcija (EF) leve komore < 50%; IMT >1 mm; suženje lumena karotida > 50%; kalcifikovani ateromatozni plakovi na obe karotide; ishemijska bolest srca; cerebrovaskularni događaj i periferna vaskularna bolest sa ili bez gangrene). Kao nezavisan prediktor preživljavanja metode izdvojio se jedino dijalizni staž. **Zaključak.** Iako su bolesnici na KAPD sa biokompatibilnijim rastvorima pokazali statistički značajno bolje preživljavanje, ne možemo tvrditi da bioinkompatibilnost dijaliznih rastvora predstavlja značajan faktor rizika od preživljavanja bolesnika i same metode lečenja. Naime, multivarijantnom analizom kao prediktori opšteg preživljavanja bolesnika izdvojili su se samo dijalizni staž, nivo serumskih triglicerida i skor kardiovaskularnog morbiditeta, dok se za očuvanje peritoneumske membrane kao nezavisan faktor rizika prikazao samo dijalizni staž.

Ključne reči:
dijaliza, peritoneumska, ambulantna, kontinuirana; preživljavanje, analiza; rastvori, dijalizni; morbiditet; mortalitet; faktori rizika.

Introduction

Continuous ambulatory peritoneal dialysis (CAPD) has been a successful modality of renal replacement therapy for more than 30 years. CAPD, similarly to hemodyalisis (HD), has unsatisfactory mortality rate despite of all improvement of techniques that were described over the past decades [1]. The reason for high mortality is probably multifactorial: older age, co-morbidity, inflammation, malnutrition and atherosclerosis (MIA syndrome), decline in residual renal function (RRF) and increased peritoneal transport characteristics [2, 3]. Several reports in the literature suggest that racial and geographic difference may influence patients survival in dialysis populations [2]. Centre and patients characteristics may differ between study populations, and this may explain different literature reports. It is important to evaluate all predictors of patients and technique survival since correction of such risk factors may decrease morbidity and mortality and promote better quality of life in CAPD patients.

Annual morbidity rate of CAPD patients is more than 20% [4, 5], out of which 60% of patients die due to cardiovascular diseases (CVD) [6–8]. Progressive atherosclerosis significantly affect CV morbidity and mortality: 30%–60% of them suffer from calcification of hearth valves, while 70%–90% of patients suffer from calcification of coronary arteries [9–13]. Both prevalence and extent of calcification predicts CVD and total mortality in CAPD patients. It is also well known that MIA syndrome is an important predictors of mortality in PD patients [3, 14–16].

Recent developments in PD solution were aimed to improve their biocompatibility by changing buffers, osmotic agents and sterilization techniques, thereby reducing toxic effects on the immune system and functional deterioration of the peritoneal membrane [17]. Still, PD maintains a constant state of intraperitoneal inflammation which affects peritoneal membrane and has the potential to affect the efficiency of each PD dwell [18–21].

Currently, there are not many data on the effects of biocompatible solutions on survival. The long-term effects of pH neutral PD solutions that are low in glucose degradation products (GDP) are not clear. They seem to better preserve the peritoneal membrane and have less systemic effects than the conventional ones. Most of recent studies had a short follow-up (of only 6–12 months) for the confirmation of the effects of new biocompatible PD solutions on peritoneal transport, technique and patients survival [22].

The aim of the study was to evaluate a potential influence of biocompatibility of dialysis solutions on long-term CAPD patients and the techique survival.

Methods

This single-center cross-sectional study with prospective follow-up of the outcomes was performed in the Military Medical Academy Belgrade, where patients were treated by CAPD according to the mode of insurance: biocompatible PD solutions were covered by military insurance while patients with civil insurance were treated with bioincompatible PD solutions from the first PD start. The patients with military insurance were rarely officers (n = 5) but the members of their families (spouse, offspring). Pre-end stage renal disease (ESRD) treatment was not dependent on the type of insurance and those who had military insurance had all other access to medical care except of more expensive biocompatible CAPD solutions once when they reached ESRD.

The study included 42 stable randomly selected CAPD patients from both groups (26 men and 16 women) during the twelve years. Those with severe anemia (Hb < 10 g/L), history of or current systemic inflammatory disease or immunomodulatory therapy and malignant disease were excluded. Twenty one (50%) patients were treated with the standard bioincompatible PD solutions [conventional glucose-based, lactate-buffered solutions – Stay safe, ANDY-disc; Fresenius Medical Care, (the CAPDP-1 group)] while the remaining 21 (50%) of the patients were treated with biocompatible PD solutions [lower level of glucose degradation products (GDPs), lower concentration of Ca^{2+} and neutral pH – Fresenius Medical Care Stay Safe balance; Gambrosol Trio, (the CAPDP-2 group)]. There was no switch-over between modalities. There were no significant differences in prescription of statins, aspirin, erythropoietin, vitamin D and iron between the groups from starting CAPD until the time of analysis.

After 2.5 ± 0.6 years of CAPD starting, all the patients underwent echocardiography and B-mode ultrasonography of common carotid arteries CCA together with assessments of nutrition status, residual renal function, peritoneal solute transport and some biochemical parameters of systemic and local inflammation, and these variables were considered in the analysis of risk factors.

Data including age, gender and underlying renal disease were analyzed at the moment of starting CAPD. Data including residual renal function and peritoneal solute transport were observed after 2.5 ± 0.6 years of starting CAPD and were correlated with the presence of chronic inflammation, echocardiography data, B-mode ultrasonography of CCA data, parameters of malnutrition, peritoneal transport and cardiovascular score (CVS) which were determined after the same period of beginning on CAPD. The end-points of the study were patients death, transplantation, transfer to HD or the end of the study period in April 2009.

Residual renal function was estimated by measuring 24 h urine collection (residual diuresis) and serum level of a novel serum marker of the glomerular filtration rate – cystatin C by particle-enhanced nephelometric immunoassay (Dade-Behring's). Cystatin C in PD fluid was not measured**.** The normal average reference range of serum Cystatin C for patients without renal failure was 0.52–0.90 mg/L for women, and 0.56–0.98 mg/L for men.

High-sensitivity C-reactive protein (Hs-CRP) as acute-phase parameters of systemic inflammation, was measured by using the Tina-quant CRP (Latex) highly sensitive assay (Roche Diagnostics GmBH, Mannheim, Germany). The lower limit of detection for hs-CRP 0.01 mg/L. CRP values less than 5 mg/L was considered normal. A fasting venous blood samples were taken from the subjects before the morning exchange after a 12 h our fasting.

Effluent concentration of CA-125 as a marker of mesothelial cell mass and pro-inflammatory cytokine interleukin (IL)-6 as a marker of local inflammation were measured in overnight effluent in both groups of CAPD patients. Dialysate samples were taken immediately after the dwell. The effluent Ca-125 concentration was measured using an electrochemoluminescence immunoassay (CECLIA) (Lecsys 2010; Roche Diagnostics, Heidelberg, Germany), the sensitivity of which was 0.60 U/mL. The effluent CA-125 concentrations greater than 35 U /mL were considered as a good values.

Peritoneal level of IL-6 was determined by specific commercial ELISA kits (Biosource, Camarillo, CA, USA). The lowest threshold of detectability for IL-6 was 2 pg/mL.

The nutritional status of patients was assessed by measurement of serum albumin, total cholesterol and triglycerides, body mass index (BMI), anthropometric parameters and by subjective global assessments (SGA). Body mass index was calculated by the equation published elsewhere [23].

Anthropometric measurements included mid-arm circumference (MAC), triceps skinfold (TSF), and a calculated estimate of the mid-arm muscle circumference (MAMC) according to NKF K/DOQI Guidelines [23, 24]. SGA was based on methodology described by Kalantar-Zadeh et al. [24]. The data were weighed and the patients were classified in terms of three major SGA scores: 1 = well nourished, 2 = moderate malnutrition or 3 = severe malnutrition.

Peritoneal solute transport was investigated by peritoneal equilibration test (PET) and by measuring 24-h peritoneal ultrafiltration (UF, ml) using standard method described by Twardowski [25].

Echocardiography measurements were made by a single experienced cardiologist according to the recommendations of the American Society of Echocardiography [26] with Aspen-ACUSON device equipped with a 2.5 MHz probe. Cardiac valvular calcification was defined as bright echoes of >1 mm on one or more cusps of the aortic valve, mitral valve or mitral annulus.

B-mode ultrasonography of CCA was performed by using the ALOCA SSD 2000 system equipment with 7.5 MHz linear transducers. A trained sonographer evaluated intima-media thickness (IMT, mm), carotid narrowing degree (%) and the presence of carotid plaques in both CCAs 4 cm from the bulbs, within carotid bulbs and the first 2 cm of the internal and external carotid arteries. Plaques were defined as echogenic structures showing protrusion into the lumen with focal widening that was 50% greater than the IMT of adjancent sites. Highly echogenic plaques producing bright

white echoes with shadowing were considered to be calcifications. Such plaques were defined as representing arterial intimal calcification pattern.

Cardiovascular score included: ejection fraction (EF) of left ventricle < 40%; IMT > 1 mm; carotid narrowing degree > 50%, the presence of carotid plaques in both common carotide, ischemic heart disease, cerebrovascular event and peripheral vascular disease with or without amputation. The cardiovascular morbidity score for each patient was defined as the number of these domains affected, varying from score 0 to score 7.

Patients outcome included a reason of death: cardiovascular diseases (ishemic heart disease, cerebrovascular disease and peripherial vascular disease) and noncardiovascular diseases (peritonitis, multiorgan failure).

Patients survival analysis included data from the start of CAPD until the end of the follow-up period in April 2009 or date of death, censored at the time of renal transplantation and transfer to hemodialysis.

Technique survival analysis included data from the start of CAPD until the date of transfer to HD or at the end of a follow-up period in April 2009, censored at the time of renal transplantation and date of patients death.

Statistical calculations were performed using the SPSS software program. Data were expressed as percentages for discrete factors, and mean values for continuous variables. Medians were used for continues variables without normal distribution. Student's *t*-test (parametric data) and Kruscal Wallis test or Mann-Whitney (non-parametric data) were used to compare the subgroups. The χ^2 test was used to compare the nominal variables between different subgroups. Acturial survival rates were determined by the Kaplan-Meier method. A log-rank test was used to compare the patient and technique survival between the subgroups. The Cox proportional hazards model was used to identify the factors predicting patient mortality and technique survival. The Cox model for multivariate analysis was constructed by those factors significant at univariate analysis. In all the comparisons, a p value < 0.05 was considered statistically significant.

Results

In this paper we analyzed the patients divided in two groups according to the type of insurance. Even so, the selection bias was avoided since there were no significant differences between the groups in age, gender, underlying renal disease, residual renal function, ultrafiltration and peritoneal transport characteristics (Table 1). In addition, there were no differences between groups in comorbidity and previous medication [(including erythropoietin stimulating agents, angiotensin-converting enzyme (ACE) inhibitors, iron and vitamin D, social status and monthly income (data not shown)].

Table 1

General characteristics of the examined patients at the moment of analysis [age, gender and underlying renal disease at the moment of starting continuous ambulatory peritoneal dialysis (CAPD)]; other 2.5 ± 0.6 yearsfollowingstarting CAPD)

The observed parameters	The examined groups		*p*-value
	CAPDP-1	CAPDP-2	
Number of patients	21	21	/
Average age (years), $\bar{x}$ ± SD	60.5 ± 13.7	65.8 ± 12.2	NS
Gender, n (%)			
male	11 (52.4)	15 (71.4)	NS
female	10 (47.6)	6 (28.6)	
Cause of CRF, n (%)			
diabetic nephropathy	7 (33.3)	8 (38.1)	
chronic GN	2 (9.5)	2 (9.5)	
nephroangiosclerosis	8 (38.1)	10 (47.6)	NS
BEN	2 (9.5)	0 (0)	
nephrolithiasis	1 (4.8)	1 (4.8)	
unknown	1 (4.8)	0 (0)	
Residual diuresis (L/day), $\bar{x}$ ± SD	0.64 ± 0.72	0.65 ± 0.59	NS
Cystatin C (mg/L), $\bar{x}$ ± SD	6.23 ± 1.62	5.36 ± 1.31	NS
Peritoneal transport, n (%)			
low	3 (14.3)	5 (23.8)	
low average	9 (42.9)	12 (57.1)	NS
high average	7 (33.3)	3 (14.3)	
high	2 (9.5)	1 (4.8)	
Ultrafiltration volume (mL/24h), $\bar{x}$ ± SD	938.1 ± 563.0	892.2 ± 598.7	NS

CAPDP-1 – the group of patients treated by bioincompatible peritoneal dialysis solutions;
CAPDP-2 – the group of patients treated by biocompatible peritoneal dialysis solutions; CRF – chronic renal failure;
GN – glomerulonephritis; BEN – Balkan endemic nephropathy; rGFR – residual glomerular filtration rate; NS – not significant

At the moment of analysis (after 2.5 ± 0.6 years of starting CAPD) inflammatory markers in the serum and in peritoneal effluent were analyzed (Table 2). The mean value of serum hs-CRP was significantly lower in the CAPDP-2 than in the CAPDP-1 group, while there were no significant differences between the groups concerning the effluent level of IL-6 and CA-125.

Nutritional parameters are presented in Table 3. There were no significant differences between the groups in total serum cholesterol, triglyceride, albumin and BMI. By comparing mid-arm circumference, mid-arm muscle circumfer-

Table 2

Biochemical markers of inflammation for the examined patients at the moment of analysis (2.5 ± 0.6 years following starting continuous ambulatory peritoneal dialysis – CAPD)

The observed parameters	The examined groups		*p*-value
	CAPDP-1	CAPDP-2	
Markers of systemic inflammation			
hs-CRP (mg/L), $\bar{x}$ ± SD (median)	6.3 ± 4.5 (5.31)	3.7 ± 2.6 (3.53)	0.003
Markers of local inflammation			
effluent IL-6 (pg/mL), $\bar{x}$ ± SD (median)	135.6 ± 114.1 (84.8)	117.3 ± 79.8 (80.0)	NS
effluent CA-125 (U/mL), $\bar{x}$ ± SD (median)	30.3 ± 21.8 (24.04)	42.7 ± 32.4 (31.6)	NS

CAPDP-1– the group of patients treated by bioincompatible peritoneal dialysis solutions; CAPDP-2 – the group of patients treated by biocompatible peritoneal dialysis solutions; hs-CRP – high-sensitivity C-reactive protein; NS – not significant

Table 3

Nutritional parameters for the examined groups of patients at the time of analysis (2.5 ± 0.6 years folloving starting continuous ambulatory peritoneal dialysis – CAPD)

The observed parameters	The examined groups		*p*-value
	CAPDP-1	CAPDP-2	
Serum albumin (g/L), $\bar{x}$ ± SD (median)	30.2 ± 4.1 (30.0)	30.2 ± 3.7 (30.0)	NS
Serum total cholesterol (mmol/L), $\bar{x}$ ± SD (median)	6.1 ± 1.4 (5.83)	5.4 ± 1.3 (5.41)	NS
Serum triglycerides (mmol/L), $\bar{x}$ ± SD (median)	2.4 ± 1.3 (2.1)	2.4 ± 1.6 (1.92)	NS
Body mass index (kg/m^2), $\bar{x}$ ± SD (median)	24.8 ± 4.0 (25.05)	24.6 ± 1.9 (24.32)	NS
MAC (cm), $\bar{x}$ ± SD (median)	27.9 ± 4.0 (27.0)	28.4 ± 2.4 (29.5)	0.015
MAMC (cm), $\bar{x}$ ± SD (median)	22.7 ± 2.4 (22.3)	23.1 ± 2.9 (24.2)	0.002
Subjective global assessment, n (%)			
well nourished	6 (28.6)	18 (85.7)	
mildly malnourished	10 (47.6)	3 (14.3)	0.000
moderate to severe malnutrition	5 (23.8)	0 (0)	

CAPDP-1 – the group of patients treated by bioincompatible peritoneal dialysis solutions; CAPDP – the group of patients treated by biocompatible peritoneal dialysis solutions; MAC – mid-arm circumference; MAMC – mid-arm muscle circumference; NS – not significant

ence and subjective global assessment it was confirmed that the patients from the CAPDP-1 group had significantly worse nutritional status than those from the CAPDP-2 group.

Cardiovascular scores are presented in Table 4. Both groups of the patients had mean EF, in the normal range. Although the patients on CAPDP-1 solutions had higher frequency of valvular calcification, the difference between groups did not reach statistical significance. Significant differences between groups were observed in prevalence of left ventricular hypertrophy (LVH), CVS, IMT, the degree of carotid narrowing and calcified plaques of CCA.

Clinical outcome is shown in Table 5. At the end of follow-up, 57.1% of the patients in the CAPDP-1 group and 47.7% in the CAPDP-2 group died. The most frequent causes of death were cardiovascular diseases in both groups without a statistical significance.

Patients and technique survival rates are shown in Figures 1 and 2. By Kaplan-Meier analysis, it was revealed that patients who underwent CAPD by bioincompatible PD solutions had significantly lower survival than those on CAPD by more biocompatible solutions. The median duration of treatment (from the start of CAPD to the end of follow up period)

Table 4

Markers of cardiovascular morbidity for the examined patients at the moment of analysis (2.5 ± 0.6 years following starting CAPD)

The observed parameters	The examined groups		*p* value
	CAPDP-1	CAPDP-2	
IMT (mm), $\bar{x}$ ± SD	1.6 ± 0.5	1.2 ± 0.3	$p = 0.005$
Carotid narrowing degree (%), $\bar{x}$ ± SD	32.4 ± 16.5	12.9 ± 14.9	$p = 0.000$
Presence of calcified plaques, n (%)	20 (95.2)	13 (61.9)	$p = 0.003$
Ejection fraction (%), $\bar{x}$ ± SD	57.1 ± 7.1	59.9 ± 3.6	NS
Presence of LVH, n (%)	19 (90.5)	13 (61.9)	$p = 0.039$
Presence of valvular calcification, n (%)	15 (71.4)	9 (42.9)	NS
CVS, n (%)	1 (4.8)	1 (4.8)	
0			
1	0 (0)	4 (19.0)	
2	3 (14.3)	7 (33.3)	
3	5 (23.8)	6 (28.6)	$p = 0.012$
4	10 (47.6)	3 (14.3)	
5	2 (9.5)	0 (0)	
6	0 (0)	0 (0)	
7	0 (0)	0 (0)	

CAPDP-1 – the group of patients treated by bioincompatible peritoneal dialysis solutions; CAPDP-2 – the group of patients treated by biocompatible peritoneal dialysis solutions; IMT – intima-media thickness; LVH – left ventricular hypertrophy; CVS – cardiovascular score (see the text); NS – not significant

Table 5

Clinical outcomes for the examined patients

The observed parameters	The examined groups		*p* value
	CAPDP-1	CAPDP-2	
Follow-up duration (months), median	78	128	
Clinical outcomes, n (%)			
remained alive on CAPD	3 (14.3)	7 (33.3)	
transplanted	1 (4.8)	0 (0)	NS
transferred to HD and stayed alive	5 (23.8)	4 (19.0)	
died	12 (57.1)	10 (47.7)	
Cardiovascular causes of death, n (%)	7 (58.3)	8 (80.0)	
ishemic heart disease	6 (50.0)	4 (40.0)	
cerebrovascular disease	1 (8.3)	2 (20.0)	NS
peripheral vascular disease	0 (0)	2 (20.0)	
Non-cardiovascular causes of death, n (%)	5 (41.7)	2 (20.0)	
peritonitis	2 (16.7)	1 (10.0)	NS
multiorgan failure	3 (25.0)	1 (10.0)	

CAPDP-1– the group of patients treated by bioincompatible peritoneal dialysis solutions; CAPDP-2 – the group of patients treated by biocompatible peritoneal dialysis solutions; HD – hemodialysis; NS – not significant

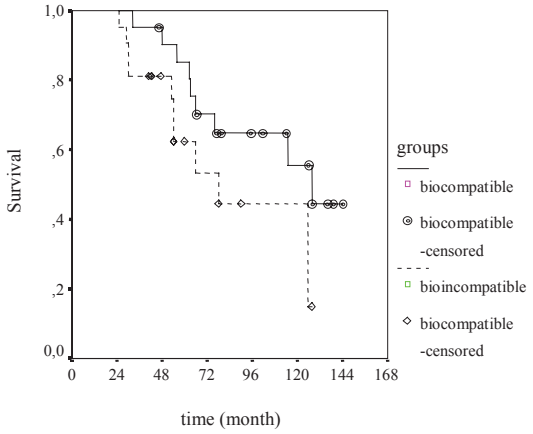

Fig. 1 – Kaplan-Meier survival curves for peritoneal dialysis patients treated by biocompatible and bioincompatible peritoneal dialysis solutions (Log- Rank; *p* = 0.0345).

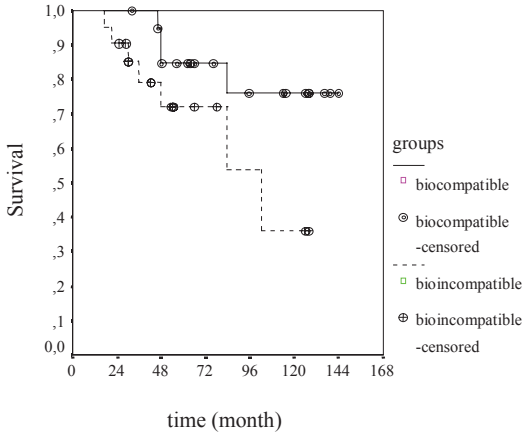

Fig. 2 – Kaplan-Meier survival curves of technique survival using biocompatible and bioincompatible peritoneal dialyis solutions (Log-Rank; *p* = 0.0719).

for the CAPDP-1 group was 78 months (range 30 to 128 months) while the median duration of the treatment for the CAPDP-2 group was 128 months (range 32 to 144 months).

Technique survival rate was not different between the groups (Figure 2). The median technique survival for the CAPDP-1 group was 102 months (range 30 to 128 months) while the median technique survival for the CAPDP-2 group was not affected (more than 50% of the patients in the CAPDP-2 group had functional peritoneal membrane and catheter at the end of the follow-up).

Predictors of patients survival are shown in Table 6. By Cox proportional hazards analysis, duration of dialysis, serum triglyceride and cardiovascular score were found to be independent predictors of overall patient survival.

Predictors of technique survival are shown in Table 7. By Cox proportional hazards analysis, only duration of dialysis was found to be an independent predictor of overall technique survival.

Discussion

In the present study, we compare the long-term effects of conventional glucose-based PD solutions and a new, neutral bicarbonate-/lactate-based PD solutions with lower level of glucose degradation products and lower concentration of Ca^{2+} on some markers of MIA syndrome and CAPD patients and the techique survival. This study showed a statistically significantly higher chronic inflammation, malnutrition and cardiovascular morbidity rate in patients treated by bioincompatible than patients treated by biocompatible dialysis solution. Mortality data revealed a similar 2-year-survival in both groups. However, with time patients who underwent CAPD by bioincompatible PD solutions had significantly worse survival than those on CAPD with biocompatible solutions. Although there was a trend toward better technique survival in the patients on CAPD by biocompatible solutions, a significant difference between the CAPDP groups was not confirmed by our study. Cox proportional hazards analysis confirmed that the duration of dialysis, serum triglyceride and cardiovascular morbidity score were independent predictors of overall patient survival, while the duration of dialysis was only independent predictor of overall technique survival.

Inspite big technical improvements during the last 20 years, morbidity and mortality rate of patients undergoing CAPD is still very high. Recent studies suggest that, during

Table 6

Univariate and multivariate Cox regression model on patient survival for the overall group of patients

Parameters	Univariate		Multivariate	
	RR (95%CI)	Significance	RR (95%CI)	Significance
PD solutions biocompatibility	0.403 (0.167–0.976)	0.044*	0.761 (0.283–2.048)	0.588
Gender	1.703 (0.715–4.056)	0.229	/	/
Average age	0.998 (0.973–1.023)	0.865	/	/
Duration of dialysis	0.568 (0.415–0.776)	0.000*	0.457 (0.307–0.680)	0.000*
C-reactive protein	1.105 (0.987–1.238)	0.083	/	/
Serum cholesterol	1.318 (0.942–1.842)	0.107	/	/
Serum triglycerides	1.382 (1.035–1.844)	0.028*	1.450 (1.067–1.969)	0.018*
Serum albumin	0.946 (0.850–1.053)	0.312	/	/
Cystatin C	1.103 (0.806–1.509)	0.539	/	/
Residual diuresis	1.012 (0.537–1.907)	0.971	/	/
Effluent IL6	0.997 (0.992–1.003)	0.327	/	/
Effluent CA-125	1.012 (0.997–1.028)	0.110	/	/
Peritoneal transport	1.006 (0.724–1.399)	0.969	/	/
Ultrafiltration volumen	1.000 (0.999–1.001)	0.889	/	/
Eject fraction	0.994 (0.940–1.050)	0.828	/	/
Presence of LVH	0.738 (0.297–1.838)	0.514	/	/
Presence of VC	1.993 (0.829–4.794)	0.123	/	/
IMT	0.434 (0.138–1.366)	0.153	/	/
Carotid narrowing degree	1.013 (0.994–1.033)	0.189	/	/
Presence of calcified plaques	0.959 (0.350–2.627)	0.935	/	/
BMI	0.963 (0.765–1.213)	0.748	/	/
MAC	0.909 (0.739–1.116)	0.361	/	/
MAMC	0.989 (0.804–1.217)	0.916	/	/
SGA	1.491 (0.985–2.259)	0.059	/	/
CVS	1.448 (1.058–1.981)	0.021*	2.095 (1.362–3.223)	0.001*

CI – confidence interval; RR – relative risk;* statistically significant; LVH – left ventricular hypertrophy; VC – valvular calcification; IMT – intima-media thickness; BMI – body mass index; MAC – mid-arm circumference; MAMC – mid-arm muscle circumference; CVS – cardiovascular score

Table 7

Univariate and multivariate Cox regression model on techique survival for overall group of patients

Parameters	Univariate		Multivariate	
	RR (95%CI)	Significance	RR (95%CI)	Significance
PD solutions biocompatibility	0.342 (0.099–1.184)	0.090	/	/
Gender	0.902 (0.237–3.432)	0.880	/	/
Average age	0.985 (0.952–1.020)	0.409	/	/
Duration of dialysis	0.598 (0.404–0.886)	0.010*	0.598 (0.404–0.886)	0.010*
C-reactive protein	1.095 (0.949–1.264)	0.216	/	/
Serum cholesterol	1.005 (0.631–1.598)	0.985	/	/
Serum triglycerides	0.921 (0.579–1.465)	0.727	/	/
Serum albumin	0.969 (0.828–1.135)	0.699	/	/
Serum cystatin C	1.193 (0.770–1.848)	0.431	/	/
Residual diuresis	0.920 (0.368–2.299)	0.859	/	/
Effluent IL6	0.971 (0.829–1.139)	0.720	/	/
Effluent Ca125	0.999 (0.991–1.006)	0.726	/	/
Peritoneal transport	0.821 (0.592–1.138)	0.236	/	/
Ultrafiltration volumen	0.993 (0.969–1.019)	0.601	/	/
Eject fraction	0.984 (0.918–1.056)	0.663	/	/
Presence of LVH	0.826 (0.241–2.830)	0.761	/	/
Presence of VC	0.819 (0.233–2.881)	0.756	/	/
IMT	2.023 (0.546–7.498)	0.292	/	/
Carotid narrowing degree	0.996 (0.967–1.026)	0.791	/	/
Presence of calcified plaques	1.269 (0.335–4.802)	0.726	/	/
BMI	1.248 (0.941–1.653)	0.124	/	/
MAC	1.317 (0.459–3.775)	0.608	/	/
MAMC	1.224 (0.908–1.650)	0.185	/	/
SGA	1.141 (0.568–2.293)	0.712	/	/
CVS	0.860 (0.581–1.273)	0.451	/	/

CI – confidence interval; RR – relative risk;* statistically significant; LVH – left ventricular hypertrophy; VC – valvular calcification; IMT – intima media thickness; BMI – body mass index; MAC – mid-arm circumference; MAMC – mid-arm muscle circumference; CVS – cardiovascular score

the first 2 years of follow-up, the survival rate of patients with chronic kidney disease who begin PD is the same as or better than those who begin HD. However, the majority of these studies show higher mortality rates in PD during the second year and thereafter [27, 28]. Different risk factors were reported to be important for outcome of patient on CAPD: age and race, underlying disease (diabetes), residual renal function, MIA syndrome and peritoneal membrane characteristics.

In general, age and the presence of diabetes at the beginning of the treatment are the main factors associated with coronary artery calcifications and mortality in dialysis patients [13, 28, 29]. Chow et al. [30] reported that diabetes mellitus was the strongest risk factor for sudden death after accounting for other cardiovascular and relevant risk factors. In our study, the age and the prevalence of diabetes mellitus were similar in both groups.

Residual renal function (RRF) during the first years of PD is an important factor of PD adequacy, contributing of 20%–50% a total solute clearance. In a recent reanalysis of the CANUSA study, there is clear evidence indicating higher contribution of RRF to the clinical outcomes of PD patients than peritoneal clearance. Namely, patients with RRF had better survival than those without [31]. Williams et al. [32] and Haag-Weber et al. [33] showed urine volume higher in patients treated with the new biocompatible PD solutions. Szeto et al. [34] analyzed the effect of the biocompatible PD solution (balance) in 25 randomized patients and found out the beneficial effect of those solutions on membrane characteristics and CRP; however, there were no differences between conventional and bicompatible solution concerning daily ultrafiltration and urine volume. Our patients had preserved RRF at the start of CAPD without a significant difference between the groups during the treatment (data not showen). Still, the contribution of a diminished ultrafiltration and subclinical fluide overload remains unexplored and may influence RRF in patients treated with biocompatible solutions [35].

Chronic inflammation may also play a major role in high cardiovascular mortality rate in CAPD patients [36]. Approximately 30%–50% of non-dialysis, hemodialysis and peritoneal dialysis patients had a state of chronic inflammation as defined by increased biochemical markers of the acute-phase response, including CRP or proinflammatory cytokines [37]. Components of dialysis solutions, especially GDPs, damage peritoneal cells and may trigger an inflammatory response. The use of a more biocompatible, neutral pH PD solution with a low concentration of GDPs was shown to result in significant reduction of intraperitoneal inflammation [32, 38, 39]. However, the study by Pejek et al. [40] showed no difference between a conventional solution (Dianeal) and a more biocompatible solution (Physioneal) in effluent macrophage inflammatory activation after a timed overnight dwell. Also, the systemic levels of IL-6 and hs-CRP did not differ between the two solutions [33, 40]. In our groups of patients, mean value of serum hs-CRP was significantly lower in the patients who underwent CAPD by biocompatible PD solutions than in the patients on CAPD treated with bioincompatible solutions, while parameters of local inflammation were similar between the CAPD groups. These findings are in agreement with our previous results that confirmed no difference in cytokines levels in patients treated with different PD solutions [41].

Protein energy malnutrition and muscle wasting are present in many patients with chronic renal failure and significantly influence patients outcome. This may be a consequence of uremia *per se* or related to co-morbid conditions [42]. Also, many studies report that inflammation may be an important cause of malnutrition [43]. Qureshi et al. [44] showed elevated serum CRP not only associated with hypoalbuminemia, but also more commonly with in malnourished patients as assessed by SGA of nutritional status. Zheng et al. [45] observed that GDPs in the PD solution are probably involved in the suppression of appetite and that the degree of inhibition is proportional to pH and glucose concentration. All our patients had similar values of serum total cholesterol, triglycerides, albumin and BMI. However, the mean values of mid-arm circumference, mid-arm muscle circumference and subjective global assessment were significantly better in the patients treated with biocompatible solutions than the patients on CAPD with bioincompatible solutions. This means that chronic peritoneal dialysis with bioincompatible solutions may influence muscle wasting.

The results of studies that evaluated the effects of novel more biocompatible solutions on peritoneal ultrafiltration (UF) rate and peritoneal solute transport are conflicting [46, 47]. These studies did not show that biocompatibility of PD solutions had significant influence on peritoneal UF rate and solute transport characteristics in a selected group of patients.

Cardiovascular complications are the major causes of morbidity and mortality in PD patients mainly due to cardiovascular calcifications and progressive atherosclerosis [4–13]. The present study shows a high overall cardiovascular morbidity rate in both groups of patients with statistically significant differences in the presence of LVH, all parameters of peripheral vascular disease and cardiovascular score between the groups. Since there were no differences between the groups in the incidence of diabetes, hypertension, ultrafiltration volume and medication, it is possible that biocompatible PD solutions might have beneficial effects on cardiovascular morbidity. Still, there may be numerous additional factors that may influence cardiovascular parameters including subclinical overhydration and others not included in this study.

In Western countries, cardiovascular disease is a leading cause of mortality in dialysis patients [6, 48, 49]. Lee et al. [50] reported that infectious disease was the leading cause of mortality for dialysis patients and caused significantly more mortality in HD than in PD patients. In our study, the most frequent causes of death were cardiovascular diseases in both groups without statistically significant difference.

Our study presents a better patients survival rate using biocompatible PD solutions and similar or worse long-term patients survival rate using bioincompatible PD solutions than in several reports [2, 51–56]. One of the explanations of better results in our study could be a small number of the selected groups and elimination of patients with severe comor-

bidity (see excluding criteria in the section Methods) and using evidently better biocompatible CAPD solutions.

This study also presents the better overall technique survival rate than in several reports [2, 48, 51–56]. Thus, 1-, 2-, and 3-year technique survival rates were 86.0%, 73.6% and 60.5%, respectively, for Korean, and 89.%, 65.9% and 51.9%, respectively, for Swedish patients [2]. In our patients, 1-, 2- and 3-year technique survival rates were: in the CAPDP-1 group 100%, 90.5%, 80.9%, respectively, and in the CAPDP-2 group 100% for all the three periods. Even so, the difference between the groups did not reach a statistical significance and one of the explanations could be the small number of patients in both groups. Long-term technique survival on CAPD by bioincompatible PD solutions was addressed by many authors, but there were no studies to confirm the effects of new biocompatible PD solutions on the technique and patients survival after a follow-up period of more than two years [22]. In our study we analyzed the effects of new, neutral PD solutions on the technique and patients survival after a follow-up period of up to 12 years.

By Cox proportional hazards analysis we showed duration of dialysis, serum triglyceride and cardiovascular score to be independent predictors of overall patients survival. Only duration of dialysis was found to be independent predictor of overall technique survival.

The present study has to be interpreted in the light of several weak points. The study population included patients with military insurance and those with civil one. Although there were no significant differences between them, one may raise the question about selection bias. Cross-sectional analysis of potentional risk factors does not provide more dynamic data that may change with the time on CAPD. A small number of patients may influence statistical significance and we believe that inclusion of a higher number of patients may contribute to the final conclusion. Apart from those presented, there may be more parameters not included in this study that could reveal the effects of biocompatibility of PD solution on parameters of MIA syndrome and patients and technique survival.

Conclusion

Patients undergoing CAPD have high cardiovascular morbidity. Chronic inflammation revealed by hs-CRP, protein energy malnutrition and peripheral atherosclerosis had higher prevalence in those treated by bioincompatible PD solutions. Patients survival after a 2-year-follow-up is significantly better if patients treated by biocompatible solutions. No difference in the technique survival is observed between the groups of our patients at any point of time.

Although patients treated with biocompatible solutions showed a significantly better survival, Cox regression analysis did not confirm that biocompatibility of PD solutions was independent predictor of patients and the technique survival.

In our setting, duration of dialysis, serum triglyceride and cardiovascular score significantly predicted an overall CAPD patients survival, while duration of dialysis was found to be the only independent predictor of overall technique survival. Further well designed and controlled studies on higher number of patients are needed to highlight the role of biocompatibility in outcome of patients on chronic peritoneal dialysis.

Disclosure

The authors declare that no financial conflict of interest exists. The authors alone are responsible for the content and writing the paper.

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Uticaj stavova roditelja na habilitaciju dece sa oštećenim sluhom

The effect of parental attitudes on habilitation of hearing impaired children

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Apstrakt

Uvod/Cilj. Habilitacija dece sa oštećenim sluhom je veoma kompleksan proces i zahteva timski pristup. Dužina habilitacionog perioda, kao i sami efekti su individualni i zavise od mnogo faktora. Cilj svakog habilitacionog procesa je poboljšanje kvaliteta života pojedincu u najvećoj mogućoj meri, bez obzira na to da li je ugrađen kohlearni implantant ili primenjen neki drugi vid pojačanja sluha. U dugogodišnjoj praksi pokazalo se da je uticaj roditelja i njihovih stavova u habilitacionom procesu veliki. Cilj našeg istraživanja bio je da se ispita koliki je taj uticaj, kako bi se obuka roditelja sprovodila tako da pomognu svojoj deci da maksimalno iskoriste svoj potencijal. **Metode.** U istraživanju su korišćeni: polustrukturisani intervju, skala roditeljskih stavova (the Parental Attitudes Scale – PAD) i paket notingamske rane procene (Nottingham Early Estimates – NEAP). Učesnici u ovoj studiji bili su roditelji sa decom od četiri do 15 godina. Status je uzet na početku i tokom procesa habilitacije i svi su aktivno učestvovali najmanje tri meseca. U statističoj analizi primenjene su deskriptivne i inferencijalne statističke tehnike. **Rezultati.** Utvrđena je značajna razlika za određene roditeljske stavove. Oni pokazuju da je bliska saradnja i kvalitetan interaktivan odnos stručnjaka sa roditeljima ove dece preduslov za uspešnu habilitaciju. **Zaključak.** Rezultati ovog istraživanja pokazuju da stavovi roditelja značajno utiču na habilitacioni proces dece sa oštećenim sluhom i govornim poremećajima. Pokazalo se da su oni naročito važni za decu sa većim oštećenjem sluha. Takođe, primećeno je da u našem društvu o slušno oštećenoj deci uglavnom brinu majke, što ukazuje da je neophodno uključivanje oba roditelja u habilitaciju dece sa oštećenim sluhom.

Ključne reči:
rehabilitacija; stavovi; roditelji; sluh, poremećaj; deca; deca, predškolska; adolescencija; kvalitet života.

Abstract

Background/Aim. Habilitation of children with hearing loss is a very complex process and requires a team work. Habilitation period length, as well as the effects themselves are individual and depend on many factors. The goal of any habilitation process is to improve the quality of life of each individual to the maximal extent possible, regardless of whether embedded cochlear implant, or other forms of amplification applied. A long-standing practice has shown that the influence of parents and their attitudes in the habilitation process is great. The aim of this study was to examine the extent of this influence in order to educate the parents so to help their children maximize their potential. **Methods.** The instruments used in this study were: semi-structured interview, the Parental Attitudes Scale (PAD), Package Nottingham Early Estimates (NEAP). The participants in this study were the parents with children aged 4–15 years. The extent of hearing loss in the children was recorded at the beginning and during the habilitation process and all were actively involved at least three months. For statistical analysis of this study the descriptive and inferential statistical techniques were applied. **Results.** The results of our study show significant differences in certain parental attitudes. A close cooperation of the parents and quality experts interactions with the parents are a prerequisite for a successful habilitation. **Conclusion.** The result of this research show that the process of habilitation of children with hearing and speech disorders is significantly affected by the parent attitudes. Parental attitudes were proved to be especially important for children with greater hearing loss. It was also noted that in our society mainly mothers are concerned with hearing-damaged children, which indicates that the educational process should be extend to both parents.

Key words:
rehabilitation; attitude; parents; hearing disorders; child; child preschool; adolescent; quality of life.

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Uvod

Porodica je socijalna sredina u kojoj odrasta gotovo svaka osoba. To je najvažnija društvena grupa u čijoj blizini je osoba rođena i za koju je vezana tokom celog svog života. Prema mišljenju Ogryzko - Wiewiorske [1], porodica je oaza za mnoge ljude, takođe, vrsta male domovine koja garantuje bezbednost, emocionalnu podršku, i pomaže da se zadrži dobro stanje duha neke osobe. Obično se smatra osnovom društvene zajednice. Ona utiče na ličnost osobe, njen život i napredak. Porodica je mesto gde dete doživljava svoje prve socijalne kontakte i počinje učenje i razumevanje društvenih vrednosti, a koje kasnije postaju osnova za stvaranje ličnog sistema normi i običaja. Porodična kuća predstavlja prirodan ambijent za čoveka koji mu omogućava da doživi i oseti svoju subjektivnost i dostojanstvo. Za dete porodica je najprirodnije i najefikasnije prvo obrazovno okruženje, koje utiče na njega tokom celog života. Prema mišljenju Dąbrowske [2], najjače vaspitno delovanje porodice ispoljava se u ranim periodima života deteta, u njegovom detinjstvu, kada ono nije, ili je pod veoma slabim uticajem društvene sredine.

Za svaku osobu porodica i vaspitno okruženje koje ona pruža je drugačije od ostalog vaspitnog okruženja uglavnom zbog prisutnih bioloških veza i emotivne bliskosti. Porodica daje detetu snagu i bezbednost, uslove odgovarajuće za njegov rast i razvoj, koje dete prenosi u društveni život. Saznanje i sposobnost učenja su individualni, tako da imaju jedinstven uticaj na fizički, psihološki, socijalni i moralni napredak i, kao rezultat, na formiranje ličnosti deteta [3].

Stavovi roditelja dece sa oštećenim sluhom su različiti. Ne mogu svi roditelji prihvatiti invalidnost svog deteta. Saznanje da dete ima oštećenje sluha može izazvati veliku emocionalnu neravnotežu. Postoje tri koraka da se prihvati činjenica da dete ima gubitak sluha: prvo, stanje emocionalne krize i konstruktivno prihvatanje. Uspešna habilitacija kod ove dece, kao i mogućnost komunikacije i uključivanje u socijalnu sredinu u velikoj meri zavise od stavova njihovih roditelja [4].

Cilj ove studije bio je da se ispitaju stavovi roditelja koji su važni za uspeh habilitacionog procesa za koji roditelje treba obučiti tako da pomognu svojoj deci da maksimalno iskoriste svoje potencijale.

Metode

Deca koja su obuhvaćena ovom studijom (n = 30) bila su približnih govornih sposobnosti na početku, iako različitog uzrasta. U grupi dece sa slušnim aparatima, oštećenje sluha bilo je od 75 do 90 dB, a u grupi dece sa ugrađenim kohlearnim 90 dB ili više. Oni su i ranije bili uključeni u habilitacioni proces, ali bez dobrih rezultata. Ukupno 18 dece sa podešenim slušnim aparatom, neposredno pred ispitivanje ispoljilo je povećanu potrebu da čuju nego deca sa kohlearnim implantatom (n = 12), od kojih je četvoro bilo uzrasta preko 10 godina. U 3-mesečnom istraživanju, svi su imali isti svakodnevni tretman, kao i njihovi roditelji, tako da su oni imali pristup svim segmentima njihovog napretka. Na početku studije urađeni su testovi slušanja: profil standardnih veština slušanja – (*Listening Skills Standard Profile* – LIP).

Sledeći korak u istraživanju bio je intervjuisanje roditelja, a zatim popunjavanje upitnika o stavovima prema detetu sa oštećenjem sluha. Zatim su njihova deca testirana baterijom testova za procenu komunikacije i kognitivne sposobnosti. Posle toga sprovođen je svakodnevni habilitacioni proces sledeća tri meseca. Po završetku, deca su ponovo testirana istom baterijom testova za procenu komunikacije i kognitivnih sposobnosti.

Polustrukturisani intervju je prilagođen što je moguće sličnijim porodičnim karakteristikama: starost, obrazovanje, socijalno okruženje (homogeni skup). Namerno nije uslovljavano koji od roditelja da učestvuje u studiji, ostavljano je da roditelji izaberu sami, prema dotadašnjem angažovanju oko deteta.

Skala roditeljskih stavova (*Parental Attitudes Scale* – PAD) je prevod standardizovanog upitnika od 46 pitanja, koji je popunjavao roditelj sa većim učešćem u habilitacionom procesu deteta [5].

Ova skala nastala je posmatranjem dece oštećenog sluha u njihovoj prirodnoj, porodičnoj atmosferi, praćenjem njihovog napretka u procesu habilitacije. Kvalitet roditeljskih stavova meri se različitim instrumentima. Skalom roditeljskih stavova ispituje se emocionalna klima za decu: anksioznost, frustracije, bol, sreća, uzbuđenje, dosada, odbacivanje, bes, komfor, bezbednost, konfuzija, nesigurnost, prezir, ravnodušnost, očaj, usamljenost, umor, tuga kao i prihvatanje-odbacivanje, dominacija-tolerancija, zadovoljstvo-nezadovoljstvo.

Proces habilitacije je sadržao: program za podsticanje razvoja govornojezičkih sposobnosti, slušni program obuke za podsticanje kognitivnog razvoja, program za podsticanje društveno-emocionalnog razvoja, psihološko savetovanje, grupni rad sa decom i grupni rad sa roditeljima.

Uspešnost habilitacije procenjivana je uz pomoć testova za ranu procenu: Paket notingamske rane procene (*Pack Nottingham's early estimates* – NEAP) Nikolopoulos et al. [6]; NEAP modifikovan za decu oštećenog sluha (*NEAP modified for children with hearing amplification*); Rana procena veštine slušanja (*Infant Listening Skills* – ILIPI *Asseaament*); Uobičajene veštine slušanja (*Listening Skills Standard* – LIP *Profile*); *Categories of Auditory Performance* (CAP); Procena razumljivosti govora (*Speech intelligibility* – SIR); Skala za procenu govora (*Speech Scale* – MUSSO).

Svi testovi primenjeni u cilju procene uspešnosti habilitacije dece oštećenog sluha, intervjuisanje njihovih roditelja, sprovođenje ankete, kao i obrada podataka, vršeni su od strane stručnog tima: specijaliste medicinske rahabilitacije sluha i govora i surdoaudiologa koji su sprovodili habilitacioni proces, uz poštovanje etičkih standarda Odbora za eksperimente na ljudima.

Ispitanici su bili roditelji sa decom uzrasta 4–15 godina. U ovoj studiji učestvovalo je 30 dece: 12 sa ugrađenim kohlearnim implantima i 18 sa slušnim pojačivačima. Svi su bili uključeni u svakodnevni habilitacioni proces koji je trajao najmanje tri meseca.

Za obradu podataka primenjene su opisne i inferencijalne statističke tehnike.

Rezultati

Rezultati koji su dobijeni stepenom slaganja roditelja na skali od -4 do +4 iz PAD upoređeni su sa rezultatima koje su deca pokazala nakon tromesečne rehabilitacije, po oceni stručnjaka koji su sprovodili habilitacioni program sa njima, koristeći standardizovanu petofaznu procenu. Od 46 pitanja u PAD skali devet pitanja je pokazalo značajnu razliku. U tabeli 1 prikazane su vrednosti *t*-testa i statistička značajnost.

Tabela 1

Devet pitanja od ukupno 46 na skali roditeljskih stavova (*Parental Attitudes Scale* – PAD) koja su pokazala statističku značajnost

Pitanja	Dobar	Loš	*t*-test	*p*
Podrška/Briga za dobrobit deteta				
1. Ja osećam da moje dete zna šta je najbolje za njega	1,44	-0,42	2,093	0,046
2. Mislim da bi deca trebalo da budu mnogo srećnija nego što je moje dete	2,83	0,75	2,176	0,038
Razumevanje potrebe za komunikacijom				
1. Ne mislim da je prezauzetost dobar stil života za dete	1,00	3,42	-2,374	0,025
2. Pokušavam da sklonim svoje dete od situacije koje mogu da budu previše uzbudljive	2,83	0,33	2,325	0,028
Prihvatanje detetovog stanja				
1. Za mene je veoma iritirajuće kada druge žene govore o svojoj deci	1,22	3,33	-2,223	0,034
2. Moje dete me često uznemiri	0,5	3,08	-2,738	0,011
Manje dominantnosti i više razumevanja za dete				
1. Mislim da moje dete treba da se ponaša u skladu sa svim mojim zahtevima	-0,33	2	-2,653	0,013
2. Ponekad se pitam, hoće li moje dete ikada odrasti				
Manje zamorenosti i briga o svakodnevnim poslovima	-0,33	2,42	-2,592	0,015
1. Kada završim svoj radni dan potrebno mi je da budem neko vreme udaljen od svog deteta	1,89	3,5	-2,325	0,028

Diskusija

U prikazanoj studiji bolje rezultate pokazala su deca čiji su roditelji pružali veću podršku i briga u odnosu na druge, imali više razumevanja za njihove potrebe za komunikacijom, odmah prihvatili njihovu invalidnost, bili manje dominantni i imali više razumevanja za njihove potrebe, manje bili opterećeni svakodnevnim obavezama i brigama. Najlošije rezultate pokazala su deca koja su postala svesna činjenice da ne ispunjavaju očekivanja roditelja i da su za njih izvor razočarenja. Utvrđeno je da u slučaju kada roditelji imaju negativan stav prema oštećenju sluha, deca ne razmišljaju o izražavanju ikakve pozitivne emocije. Istraživanje je pokazalo da je odbijanje invaliditeta češće od prihvatanja. Ispostavilo se da uspešnost habilitacije slušno oštećene dece u velikoj meri zavisi od načina i tretiranja u najranijem periodu njihovog života, u kome se započinje i njihov emocionalni i socijalni razvoj. Uspešnost habilitacionog procesa ima uticaja i na moralni i na lični razvoj, što se ogledalo i u vaspitno-obrazovnoj komponenti.

U našem istraživanju bili su zastupljeni svi tipovi roditeljskih stavova koje je opisala. Borzyszkowa [7]: adekvatni roditelji (svesni invaliditeta i njihovi zahtevi su adekvatni mogućnostima deteta); previše ljubazni roditelji (invalidnost se smatra nepravdom sudbine, što rezultira preteranom pažnjom i nenametanjem zahteva; prestrogi roditelji (činjenica invaliditeta je odbijena i zahtevi roditelja su previše visoki u poređenju sa mogućnostima deteta) i neutralni roditelji (svesni invalidnosti, ali ovo deluje destabilizujuće na njih tako da ne veruju da je bilo koja rehabilitacija moguća).

Istraživanje je, takođe, ukazalo na zanimljivu činjenicu da su 90% ispitanika u intervjuu bile majke. Ovo ukazuje da u našem društvu brigu o deci sa oštećenim sluhom obično vode majke i da su one, često, same u borbi da obezbede svojoj deci bolji život. To nameće potrebu da se menja svest našeg društva o slušno oštećenoj deci i da se uključe oba roditelja u proces habilitacije njihove dece.

U literaturi postoji lista vaspitnih ciljeva porodice, koji su neophodni za pravilan razvoj deteta, a koji pomažu da dete zadovolji svoje potrebe. Zastupljene su sledeće oblasti: fizičko zdravlje i napredak (obezbeđivanje uslova za pravilan fizički razvoj i negovanje sposobnosti dobrog upravljanja vremenom); psihologija i emocije (to se odnosi na pravilno izražavanje osećanja, empatiju, saradnju, intelektualni napredak, što se postiže organizovanjem situacije koja obogaćuje znanje deteta, obezbeđenje obrazovnih mogućnosti i razvoj ličnih interesa i životne aspiracije); kulturno nasleđe (to može da se uradi uvođenjem deteta u svet vrednosti, u život zajednice, u politički život i gajenjem patriotizma); rad (ovde se kroz nastavu detetu razvijaju odgovornost i sposobnost za rad u timu); nezavisnost (dete se priprema za samostalan život u porodici i u društvu) [8].

Navedene vrste vaspitnih zadataka sadrže sve oblasti razvoja deteta: fizičku (biološku), psihološku (emocionalnu), intelektualnu, socijalnu, kulturnu i duhovnu. Dete je integrisana osoba i to definiše okvir u kojem bi trebalo da se odvija vaspitni proces. Trebalo bi obratiti pažnju na odnos porodice i njen doživljaj invalidnog deteta. Ovaj problem zahteva višekomponentni pristup, jer je specifično vaspitno okruženje u

kome oštećenje određuje i menja vrstu i kvalitet obrazovnog i društvenog uticaja.

Socijalna podrška je „pre svega pomoć koja je dostupna od strane pojedinca ili grupe u problematičnim, stresnim ili odlučujućim situacijama. Pruža se uglavnom kada se osoba suočava sa različitim životnim zadacima".

Postoji nekoliko tipova podrške [9]. Emotivna podrška se svodi na slanje verbalnih i neverbalnih delova informacija kao što su: „mi te volimo", „ne predajemo se", „ne dajemo se", od roditelja na dete. Procena podrške se svodi na slanje informacija kao što su: „Ti si veoma važan", „Samo napred".

Podrška deteta sa oštećenim sluhom treba da uključi komponente koje su od vitalnog značaja u procesu razvoja govora i izgovoranja. Pojam „oštećenje sluha" podrazumeva sva moguća oštećenja slušnog sistema, nezavisno od vrste, težine, mesta i uzroka oštećenja. Oštećenje sluha je „svako oštećenje slušnog organa ili nemogućnost neke osobe da obradi čujuće senzacije na nivou mentalnih informacija" [10].

Širom sveta teorijska i eksperimentalna istraživanja dokazuju da je prvi period detetovog života, uključujući i veštine slušanja, najefikasnije vreme za njegov razvoj. Ovo je period od mimikrije i formiranja navika, a nesumnjivo i period razvoja govora i jezika [11]. Članovi porodice moraju da prihvate dete oštećenog sluha, moraju da shvate problem i po potrebi formiraju poslove koji bi mogli poslužiti da poboljšaju detetov razvoj.

Habilitacija bi trebalo da postane deo svakodnevnog života roditelja, i ako je to slučaj, onda to takođe pokazuje uslove dobrog mentalnog okruženja za celu porodicu. To je zato što je, veoma često, sluh ovakvog deteta ograničen, za razliku od roditelja koji nemaju taj problem, i nije im teško da budu „dobri roditelji" [2].

Model „saosećajan roditelj", takođe se suočava sa problemima, u situacijama izražavanja simpatije i empatije koje zahtevaju kontakt između roditelja i deteta [4, 11].

Treba napomenuti da je proces rehabilitacije dece oštećenog sluha uspešniji, ako i roditelji i govorni terapeut primenjuju vaspitni proces. Czerkawska i Sward [12], u istraživanju na uzorku od 31 deteta, uzrasta 7–15 god. sa teškim ili najtežim oštećenjem sluha, ukazuju da oni pohađaju redovnu školu, i da su najvažniji uticaj na njihov obrazovni uspeh imali stavovi njihovih roditelja. Ovi roditelji su počeli ranu rehabilitaciju dece i od tada su neprestano bili u kontaktu sa specijalistima za govor i sluh i poštovali su sve instrukcije. Uprkos činjenici da su mnoga urođena ili rano stečena oštećenja sluha trajna, moguće je smanjiti posledice gubitka sluha ako se na vreme počne i sprovodi odgovarajući slušni tretman [13]. U slučaju dece oštećenog sluha, roditelji su tu da pomognu svojoj deci da pravilno funkcionišu u društvu i da im pomognu da pređu barijeru komunikacije, i da ih osposobe da razgovaraju i kontaktiraju sa svojim čujućim vršnjacima. Vidljiv efekat prelaska prepreka je njihov početak uklapanja u pravila školske integracije, a kasnije posao i društveni život [14]. Završni zadatak podrške deci oštećenog sluha u njihovim porodicama je brza priprema za polazak u redovnu školu, tako da im se omogući puna adaptacija na uslove života u integraciji sa ljudima koji čuju [15–17]. Veliki broj dece sa teškim oštećenjem sluha, čiji roditelji rade sistematski sa njima na razvoju njihovog govora i jezika, pod kontrolom stručnjaka, u mogućnosti su da pohađaju redovne škole zajedno sa svojim čujućim vršnjacima [11].

Konačno, smatramo da podrška deteta sa oštećenim sluhom u njegovom porodičnom okruženju, mora biti prilično sistematska. Samo sistematska, dugoročna i adekvatna podrška porodice jednog slušno oštećenog deteta može povećati efikasnost vaspitno-rehabilitacionog procesa, a to može povećati intelektualni potencijal deteta.

Zaključak

Uticaj roditelja u procesu habilitacije deteta oštećenog sluha je veliki i dominantan faktor u dobijanju traženog efekta. Potrebno je da roditelji budu detaljno obavešteni o ozbiljnosti oštećenja, načinu rešavanja, uticaju procesa habilitacije na ličnost deteta. Neophodno je da se roditelji uključe rano, kako bi na vreme ušli u proces obrazovanja i da im se omogući da sprovedu lečenje u kući. Rešavanje ovog problema zahteva timsku saradnju stručnjaka različitih specijalnosti. Dobra saradnja tima stručnjaka sa roditeljima doprinosi da oni doživljavaju različite aspekte i prihvataju invaliditet svoga deteta, da učestvuju u procesu rehabilitacije i maksimalno pomognu da se iskoristi potencijal njihovog deteta.

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Hepcidin and iron metabolism disorders in patients with chronic kidney disease

Hepcidin i poremećaji metabolizma gvožđa kod bolesnika sa hroničnom bubrežnom bolešću

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Abstract

Bacground/Aim. Hepcidin may play a pathogenetic role in iron metobolism disorders. The aim of this study was to determine the correlation between hepcidin concentration and parameters of iron metabolism in patients with different stage of chronic kidney disease (CKD). **Methods.** The study involved 104 patients with CKD: 64 on hemodialysis (HD) and 40 patients in pre-dialysis stadium (pre-HD) with adequate erythropoetin therapy and iron supplementation. The HD group was divided in four subgroups according to the level of serum ferritin (up to 100; 100–199; 200–499 and over 500 ng/mL). Parameters of anemia, iron status, inflamation and hepcidin level were evaluated. **Results.** The HD patients had a significantly lower eritrocyte count, erythrocytes indexes, hemoglobin and transferrin saturation and significantly higher iron, ferritin, hepcidin and total iron binding capacity (TIBC). The HD subgroups up to 199 ng/mL of serum feritin had lower high-sensitivity C-reactive protein (hsCRP), iron and higher unbuffered iron binding capacity (UIBC), transferrin saturation and TIBC compared to the HD subgroups over 200 ng/mL. The lowest and the highest ferritin subgroups had the highest hepcidin level and it showed significant correlation with ferritin. **Conclusion.** Hepcidin may serve as a marker for better diagnosing and monitoring anemia and iron metabolism disorders in CKD.

Key words:
iron; ferritins; anemia; kidney failure, chronic.

Apstrakt

Uvod/Cilj. Hepcidin može imati patogenetsku ulogu u poremećajima metabolizma gvožđa. Cilj ovog istraživanja bio je da se utvrdi povezanost koncentracije hepcidina i parametara metabolizma gvožđa kod bolesnika u različitim fazama hroničnog bubrežnog oboljenja (CKD). **Metode.** Studija je obuhvatila 104 bolesnika sa CKD: 64 na hemodijalizi (HD) i 40 bolesnika u zadnjoj fazi bubrežne bolesti u predijaliznom stadijumu sa adekvatnom eritropoetinskom terapijom i suplementima gvožđa. Grupa HD bila je podeljenja u četiri podgrupe prema nivou serumskog feritina (do 100; 100–199; 200–499 i preko 500 ng/mL). Određivani su parametri anemije, statusa gvožđa, inflamacije i hepcidina. **Rezultati.** Bolesnici HD grupe imali su znatno niži broj eritrocita, eritrocitne indekse, hemaglobin i saturaciju transferina i znatno veće vrednosti gvožđa, feritina, hepcidina i totalni kapacitet vezivnog gvožđa (TIBC). HD podgrupe sa vrednostima feritina do 199 ng/mL imale su niži visokosenzitivni C-reaktivni protein (hsCRP) i nivo gvožđa i visok slobodni kapacitet vezivanja gvožđa (UIBC) u odnosu na HD podgrupe za preko 200 ng/mL feritina u serumu. Podgrupe sa najvišim i najnižim vrednostima feritina imale su najveće vrednosti hepcidina što je bilo u značajnoj korelaciji sa vrednostima feritina. **Zaključak.** Hepcidin može poslužiti kao marker za bolju dijagnozu i praćenje anemije i poremećaje metabolizma gvožđa u CKD.

Ključne reči:
gvožđe; feritin; anemija; bubreg, hronična insuficijencija.

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Introduction

Anemia is a major complication of chronic uremia in the pre-dialysis period and during maintenance dialysis. Anemia develops from the moderate stage of chronic kidney disease (CKD), worsens with the progression of renal failure and is not, or is only incompletely, improved by maintenance dialysis [1, 2].

Iron deficiency can occur in all hemodialysis patients as a result of continuing blood losses and increased iron utilization as a result of erythropoiesis-stimulating protein therapy [3].

Hepcidin is a systemic key regulator of iron homeostasis found on the surface of macrofages and enterocyte that induces internalization and degradation of ferroportin [4, 5]. Thus, hepcidin inhibits the release of iron from macrofages reducing the iron absorption in the bowels. In addition, hepcidin may directly prevent proliferation and erythroid-progenitor survival (synthesis) [6]. Increased iron stores and inflammation induce hepcidin production, whereas hypoxia, anemia, iron deficiency, increased erythropoiesis and recombinant human erythropoietin (rHuEPO) attenuate hepcidin synthesis [7–11].

Hepcidin may play a pathogenetic role in iron metabolism disorders, as well as rHuEPO resistance. However, the molecular hypoxic or anemic regulation mechanisms are still unclear. Several studies have shown that erythropoesis induction is sufficient to reduce hepcidin synthesis, and not hypoxia or anemia [9, 12–14]. The erythropoiesis is increased by rHuEPO, and iron should be mobilized from the storages in order to meet the demands of the bone marrow. A significant reduction in circulating hepcidin level caused by rHuEPO therapy may explain the increased iron release. The connection between hepcidin synthesis and erythropoiesis points to the erythrocytes and liver regulator existence [7, 9, 15].

The aim of this study was to determine the correlation between hepcidin concentration and parameters of iron metabolism in patients with different degree of CKD.

Methods

The study was performed at the Clinic of Nephrology, Clinical Center Niš and Clinical-Biochemical Laboratory of the Military Hospital in Niš. A complete patient history was noted for all the investigated patients. The study involved 104 pateints with CKD divided into two groups: the hemodialysis (HD) group and pre-dialysis stadium group (pre-HD) comprised 64 patients who were dialyzed three times per week for 4 hours *via* polysulfone dialyzers (F6 and F7 HPS Fresenius Medical Care, Bad Homburg, Germany), using the bicarbonate dialysis solutions and standard heparinization. All the HD patients were on rHuEPO and oral iron therapy [European Best Practice Guidelines (EBPG)] and if they had absolute (ferritin < 100 ng/mL) or functional [ferritin >100 ng/mL, transferrin saturation (TSAT) < 20%] iron deficiency [16], we initiated the IV Venofer (Lek Ljubljana) (iron sucrose) protocol[17]. Pre-HD stadium was defined as 3 [glomerular filtration rate (GRF) 30–59 mL/min/1.73m^2] and 4 (GFR 15–29 mL/min/1.73m^2) stadium of CKD by the National Kidney Foundation[17]. Pre-HD group consisted of 40 patients who were in the stadium with adequate erythropoetin therapy and iron oral supplementation. According to the EBPG for studying anemia in patients with CKD, iron deficiency is described as the main cause of erythropoiesis stimulating agents treatment resistance, whether there is absolute (ferritin < 100 ng/mL, transferrin saturation < 20%) or functional (ferritin > 100 ng/mL and transferrin saturation < 20%) iron deficiency. That is why the HD group was divided in four subgroups according to the level of serum ferritin (ferritin concentration up to 100 ng/mL; from 100–199 ng/mL; from 200–499 ng/mL and over 500 ng/mL).

The exclusion criteria were: less than 18 years old, evidence of acute infection or trauma in the last four weeks, history of parenteral iron injection in the last 14 days, history of blood transfusion in the last one month, hemoglobinopathy, malignancy, recent overt blood loss, and post-transplant status. All the patients showed no signs of infection or hepatitis B and C.

Blood was extracted using the closed vacuum system for all the patients. Tubes with EDTA anticoagulant were used for the hematological parameters, whereas for the biochemical parameters, the tubes were without anticoagulant. After sampling, blood was put into a centrifuge and separated from the serums out of which the following biochemical and hematological parameters were evaluated: the overall blood count red blood cells (RBC); hemoglobin (Hb); hematocrit (HCT); median cell volume (MCV); median concentration of hemoglobin (MCH); median cell hemoglobin concentration (MCHC) were determined on hematological autoanalyzer ADVIA 120 Simens ex Bayer. Iron, total iron binding capacity (TIBC), unbuffered iron binding capacity (UIBC), transferrin saturation, albumin and high-sensitivity C-reactive protein (hsCRP) were determined on a biochemical analyzer (Dimension, Dade Behring), while ferritin was assayed by using a commercially available immunohistochemical test (Cobas e 411 Rosch).

Hepcidin was determined using the commercial ELISA test (DRG, Marburg, Germany). The measure range of the assay is 0.9–140 ng/mL. The analytical low level of sensitivity of the DRG ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the Zero Standard (SO) and was found to be 0.9 ng/mL.

The research was approved by institutional review boards of Faculty of Medicine, University in Niš and institutional Ethics Committee's number 01-4097-1/06.07.2011. Inform consent was obtained from all the participants.

Statistical analysis was performed using the standard descriptive methods (mean ± SD), and corresponding analytical tests. Levene's Test for Equality of Variances was performed to determine the equality of variances, and appropriate independent samples, while the Student's *t*-test was used to compare the means. The intergroup variability was determined using the ANOVA test and *post hoc* analysis, and the Mann-Whitney test was used as a non-parametric test. The correlation between the results was tested with the Pearson´s Correlation Coefficient.

Results

In the HD group, 42 male and 22 female patients were analyzed unlike the pre-HD group, in which 34 male and 6 female patients were analyzed. Clinical characteristics and parameters of anemia of investigated groups with CKD are shown in Table 1. Baseline characteristics of CKD patients did not show statistically significant difference between the HD and pre-HD group, but the patients on hemodialysis had a significantly lower number of RBC, Hb concentration and HCT, MCV, MCH values ($p < 0.01$) and transferrin saturation ($p < 0.05$) compared to the pre-HD group. Higher iron concentrations and TIBC ($p < 0.05$) were found in the HD patients group (Table 1).

Patient division on the basis of ferritin levels in the patients with CKD on hemodialysis is shown in Tables 2 and 3.

Hematological anemia parameters did not show any significant differences in the subgroups of HD group patients with various ferritin value intervals (Table 2).

The ANOVA analysis showed the existence of significant intergroup differences in iron, transferrin saturation, UIBC, TIBC, hsCRP and hepcidin values among the tested patients groups. *Post hoc* analysis revealed that the patients with ferritin levels < 100 ng/mL and 100–199 ng/mL had significantly lower hsCRP as well as significantly higher UIBC, and transferrin saturation levels compared to the groups with ferritin 200–499 ng/mL and > 500 ng/mL. The patients with ferritin levels < 100 ng/mL and 100–199 ng/mL had significantly lower iron and significantly higher TIBC levels compared to the group with ferritin > 500 ng/mL. The HD patients group with lowest < 100 and highest > 500

Table 1

The clinical characteristics and parameters of anemia in the patients with chronic kidney disease (CKD)

Parameters	Groups of patients	
	HD (n= 64)	pre-HD (n = 40)
Age (years), $\bar{x} \pm SD$	62.6 ± 6	65.1 ± 4.7
CKD history (years), $\bar{x} \pm SD$	5.78 ± 4	8 ± 4.7
Hemodialysis history (years), $\bar{x} \pm SD$	6.97 ± 6.15	–
GRF (mL/min/1.73m^2), $\bar{x} \pm SD$	7.85 ± 4.2	36.7 ± 3.8
Hypertension n, (%)	56 (88)	23 (61)
Smoking, n (%)	0/0	3/16
Systolic TA (mmHg), $\bar{x} \pm SD$	130 ± 7.9	132 ± 13.1
Diastolic TA (mmHg), $\bar{x} \pm SD$	80.3 ± 7.8	82.5 ± 8
RBC (T/L), $\bar{x} \pm SD$	3.08 ± 0.59	3.79 ± 0.20**
Hb (g/L), $\bar{x} \pm SD$	97.7 ± 20.19	112.15 ± 3.78**
HCT	29.57 ± 5.87	33.67 ± 1.21**
MCV	90.54 ± 2.03	94.58 ± 4.61**
MCH	30.28 ± 0.73	31.55 ± 1.75**
MCHC	330.8 ± 5.73	332.08 ± 15.77
Fe (µmol/L), $\bar{x} \pm SD$	20.56 ± 7.18	16.98 ± 2.03*
Transferrin saturation (%),$\bar{x} \pm SD$	19.34 ± 11.06	24.6 ± 4.8*
UIBC (µmol/L), $\bar{x} \pm SD$	39.64 ± 8.96	40.79 ± 5.68
TIBC (µmol/L), $\bar{x} \pm SD$	53.14 ± 21.92	41.03 ± 7.22*

*** $p < 0.05$; ** $p < 0.01$ *vs* hemodialysis (HD); RBC – red blood cells; Hb – hemoglobin; HCT – hematocrit; MCV – median cell volume; MCH – median concentration of hemoglobin; MCHC – median cell hemoglobin concentration; UIBC – unbuffered iron binding capacity, TIBC – total iron binding capacity; GRF – glomerular filtration rate**

Ferritin (Figure 1) and hepcidin (Figure 2) concentration were significantly higher ($p < 0.01$) in the HD group compared to the pre-HD group.

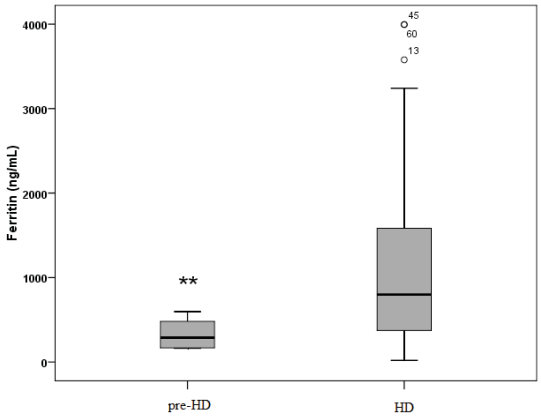

Fig. 1 – Ferritin concentration in the examined groups.

****$p < 0.01$ vs hemodialysis (HD), Mann-Whitney test; Boxplot summarizes the median, quartiles (25–75. percentiles), extreme values and outliers (o), error bars represents 95% confidence intervals (CI)**

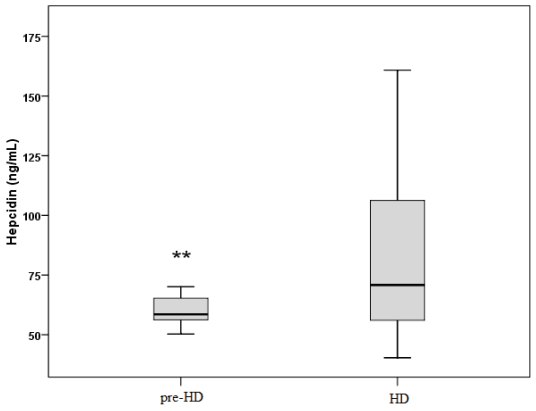

Fig. 2 – Hepcidin concentration in the examined groups

****$p < 0.01$ vs hemodialysis (HD); Mann-Whitney test; Boxplot summarizes the median, quartiles (25–75. percentiles) and extreme values, error bars represents 95% confidence intervals (CI)**

Table 2

The parameters of anemia according to the level of ferritin in the hemodialysis patients

Parameters	Ferritin (ng/mL)			
	< 100 (n = 12)	100–199 (n=12)	200–499 (n = 14)	> 500 (n = 26)
RBC (T/L)	3.32 ± 0.72	2.80 ± 0.53	3.28 ± 0.45	3.0 ± 0.58
Hb (g/L)	98.67 ± 26.99	89.42 ± 14.13	106.36 ± 17.37	96.42 ± 19.62
HCT (%)	30.69 ± 7.07	26.98 ± 4.29	31.68 ± 5.15	29.13 ± 6.05
MCV (fL)	92.63 ± 4.02	94.52 ± 4.08	94.89 ± 5.93	95.37 ± 4.28
MCH (pg/cell)	30.73 ± 1.85	31.58 ± 1.46	31.66 ± 2.15	31.86 ± 1.59
MCHC (g/dL)	323.0 ± 30.63	335.25 ± 8.15	332.86 ± 9.39	334.38 ± 9.45

The data are presented as mean ± SD; n – number of patients; RBC – red blood cells; Hb – hemoglobin; HCT – hematocrit; MCV – median cell volume; MCH – median concentration of hemoglobin; MCHC – median cell hemoglobin concentration

Table 3

Iron (Fe) status according to the level of ferritin in the hemodialysis patients

Variables	Ferritin (ng/mL)			
	< 100 (n = 12)	100–199 (n = 12)	200–499 (n = 14)	> 500 (n = 26)
Fe (μmol/L)	16.38 ± 3.26 [a]	16.54 ± 6.73 [a]	22.65 ± 6.97	23.23 ± 7.38
Transferin saturation (%)	29.38 ± 6.30 [b]	27.83 ± 7.45 [b]	16.56 ± 8.53	12.29 ± 9.47
UIBC (μmol/L)	48.42 ± 7.78 [b]	47.37 ± 8.38 [b]	39.16 ± 7.39	32.07 ± 8.25
TIBC (μmol/L)	58.10 ± 21.74 [a]	55.82 ± 18.74 [a]	46.90 ± 13.3	36.83 ± 6.94
Hepcidin (ng/mL)	92.51 ± 40.99 [c]	61.34 ± 12.97	65.19 ± 25.48	96.27 ± 29.1 [c]
hsCRP (mg/L)	3.73 ± 2.26 [b]	3.82 ± 1.90 [b]	6.02 ± 3.14	7.95 ± 2.11
Albumin (g/L)	20.26 ± 3.89[d]	30.74 ± 3.45	34.97 ± 3	27.15 ± 4.56

The data are presented as means ± SD; *Post hoc* Tukey HSD test: [a] $p < 0.05$ vs. >500; [b] $p < 0.05$ vs 200–499 and > 500; [c] $p < 0.05$ vs 100–199 and 200–499; [d] $p < 0.05$ vs all the rest; TIBC – total iron binding capacity; UIBC – unbuffered iron binding capacity; hsCRP – high-sensitivity C-reactive protein

ng/mL ferritin values had significantly higher hepcidin compared to 100–199 ng/mL and 200–499 ng/mL ferritin subgroups. The patients with ferritin levels < 100 ng/mL showed statistically significant lower albumin levels compared to the other groups of patients (Table 3).

Hepcidin showed a significant correlation with ferritin in both patient groups (HD – $r = 0.46$, $p < 0.01$; pre-HD – $r = 0.69$, $p < 0.01$), while hsCRP was in a significant correlation with hepcidin in HD patients only ($r = 0.565$, $p < 0.05$). In the HD patients albumin was significantly negatively correlated with hepcidin ($r = -0.487$, $p < 0.05$). In HD patients with chronic renal failure, bivariate analysis showed no significant correlation of hepcidin with any parameters of anemia. In pre-HD patients with chronic renal failure, hepcidin correlated inversely with RBC ($r = -0.81$, $p < 0.01$), MCV ($r = -0.738$, $p < 0.01$) and MCH ($r = -0.535$, $p < 0.05$) (Table 4).

Discussion

Determination of iron deficiency level in patients on hemodialysis is much more difficult than in normal population. In connection with the homeostasis of ferritin, three types of anemia have been identified in patients on hemodialysis (absolute, functional deficiency and reticuloendothelial blockade) even if there are still doubts in official markers and indicators that are currently used for identification [18].

Table 4

The correlation of hepcidin with iron (Fe) parameters in the hemodialysis (HD) patients

Parameters	Hepcidin	
	HD group	pre-HD group
Ferritin	0.467**	0.694**
hsCRP	0.565*	0.285
Albumin	-0.487*	0.015
% sat	0.156	-0.172
TIBC	-0.187	-0.165
UIBC	-0.181	0.012
Fe	0.062	-0.169
RBC	0.026	-0.811**
Hb	-0.063	0.317
HCT	0.015	0.254
MCV	0.085	-0.738**
MCH	-0.005	-0.535*
MCHC	-0.257	-0.216

*** – significant correlation at $p < 0.05$; ** – significant correlation at $p < 0.01$**
TIBC-total iron binding capacity; UIBC – unbuffered iron binding capacity; RBC –red blood cells; Hb – hemoglobin; HCT – hematocrit; MCV – median cell volume; MCH – median concentration of hemoglobin; MCHC – median cell hemoglobin concentration; hsCRP – high-sensitivity C-reactive protein

A routine monitoring of ferritin status in patients on hemodialysis is of vital importance in order to prevent the occurrence of iron deficiency and to avoid constantly increased value in assessing ferritin status. Insufficient iron supplies may lead to anemia as a result of iron defficiency [17], which in turn causes changes in the functioning of cardiovascular system (left ventricle hypertrophy, reduced ventricular hypertrophy ejection fraction and congestive heart disease), exhaustion and reduced quality of life [19–21]. Contrary to the above mentioned the correction of anemia leads to the improvement of heart morphology, reduction of the length of stay in hospital and improves the quality of life [22–23].

Well-known hematological parameters of anemic syndrome RBC, Hb, HCT, MCV and MCH are reduced in patients with hyperbaric oxigenation and in those on hemodialysis. However, in the HD dialysis group of patients increased iron, ferritin and hepcidin levels were observed, while transferrin saturation was significantly decreased. These data are consistent with a recent examination of De Dominico et al. [24] who confirmed the presence of inhibitory effects of hepcidin on iron levels. This relationship is explained by the mechanism of negative feedback because ferroportin loss from the surface of the cells causes a reduction of ferritin in plasma, which creates low transferrin saturation. In this way, less iron is transported to erythroblasts, leading to chronic anemia, which interferes with the production of hepcidin. On the other hand, iron is, trapped inside macrophages and ferritin [9].

A significant positive correlation between RBC number and hepcidin level was found in patients on pre-dialysis stage. This indicates the importance of monitoring hepcidin in patients on dialysis during the correction of anemic syndromes and disorders of iron metabolism, since the increased levels of transferrin and better fulfillment of erythrocytes does not reflect on an increase in their number. This may be a consequence of the proinflammatory state in the patients on hemodialysis. Inflammation can be caused by the dialysis itself, which leads to the increased concentrations of circulating cytokines such as interleukin-1 (IL-1) and IL-6, alpha-tumor necrosis factor (TNF-α) or γ-interferon [25–30] and hepcidin. They can directly affect the biological function of erythropoietin, which in turn causes the retention of iron in macrophages / monocytes, accompanied by reduced erythropoiesis of iron [15, 31].

Hepcidin synthesis is increased in iron overload conditions and during inflammation, while the decreased synthesis may be due to iron deficiency and anemia [9]. This is indicated by the positive correlation of hepcidin and ferritin in both groups, and hepcidin and hsCRP in patients with HD. Statistically higher levels of hsCRP were pointed to an inflammatory state in the HD group of patients and in the subgroups of patients with ferritin 200–400 ng/mL and the group with ferritin > 500 ng/mL in our study, which coincides with the findings of Ashby et al. [32]. This phenomenon can be explained by previous studies in cultures of human hepatocytes in which hepcidin is induced by IL-6 but not IL-1 or TNF-α [33]. Three different modes of regulation of hepcidin have been noticed: inflammatory, which depends on IL-6, regulation of iron levels (mainly determined by transferrin saturation) and suppression of hepcidin synthesis caused by hypoxia and anemia. It is believed that frequent use of iron may reduce the stimulation of hepcidin by creating a reduction in saturation transferin [34–36]. Pro-inflammatory state on the other hand can cause erythropoietin resistance [37]. Significantly higher levels of hepcidin in the group with ferritin levels > 500 ng/mL can be expected due to excessive amounts of iron, where the increased synthesis of hepcidin causes a negative feedback mechanism.

However, proinflammatory condition is not found in the group of patients with ferritin < 100 ng/mL, and there were statistically significantly higher levels of hepcidin. Judging by the significantly lower levels of albumin and negative correlation with hepcidin in these groups of patients, the reason is to be sought in the disorder of liver synthesis function. The research of Detivaud et al. [38] found a direct correlation of liver function with the level of hepcidin, while the research Małyszko et al. [39] showed a direct negative correlation of albumin and hepcidin. On the other hand, specific circulating binding proteins hepcidin are the α-2 macroglobulin and albumin [40], all of which can explain the increased levels of hepcidin in these patients.

Ferritin and transferrin saturation are irreplaceable markers in determining the iron status, which hepcidin is not comparable with. Hepcidin together with ferritin and transferrin saturation can give more insight in the evaluation of iron status in patients with chronic kidney failure and hemodialysis. No connection of hepcidin and transferrin saturation was found in this paper, but there was a direct correlation between hepcidin and ferritin. In addition, in the ferritin groups from 100 ng/mL to 499 ng/mL in the HD patients, a slight decrease in hepcidin was recorded. We think that it would be most appropriate to determine hepcidin in patients with chronic kidney failure and hemodialysis with the highest ferritin values. Since all the patients were on erythropoietin therapy, which leads to iron overload, the increased hepcidin values could indicate the appearance of erythropoietin resistance. This opinion requires further investigation. Hepcidin is certainly not the marker, at least for the time being, that would be used in clinical practice.

Conclusion

Hepcidin may have an important role as a marker in diagnosing and monitoring the iron metabolism disorders in CKD. It showed maximal values in the lowest and highest ferritin level group and was in linear correlation with ferritin in both patient groups. In this way, the determination of hepcidin may be of clinical importance in better anemia monitoring in both group of patients – pre-HD and HD.

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Quality assessment of total parenteral nutrition admixtures by the use of fractional factorial design

Analiza kvaliteta smeša za totalnu parenteralnu ishranu primenom delimičnog faktorijalnog dizajna

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Abstract

Background/Aim. Parenteral nutrition as a specific aspect of providing nutritients still remains a permanent topic of both theoretical and experimental research. Total parenteral nutrition (TPN) admixtures have complex contents making difficult to maintain their stability. The most critical parameter is the diameter of a lipid droplet, i.e. droplet size distribution. It is recommended that droplet size should not be more than 5 μm and that the presence of greater droplets should not exceede the value of 0.05%. Lipid droplets size is affected particularly by electrolyte addition, especially polyvalent cations. There is a danger of the added electrolytes interaction with lipid droplets which leads to their aggregation and negative effects upon the admixtures stability. The aim of this study was to assess the effect of added electrolyte and lipid phase quantity on the admixture stability. **Methods.** Electrolytes were added to the studied admixture of a defined basic formulation contents in accordance with recommendations from the literature. Droplets size measurements were performed using the method of laser diffraction with a laser particles analyzer. Effects of independent variables were calculated and evaluated using commercial software. Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations, as well as the quantity of fat phase were chosen as studied factors, i.e. independed variables. The system response, or dependent variable was the median of droplets size. Each of the factors was varied at two levels, higher (+1) and lower (-1), according to the 2^{5-2} fractional factorial design. **Results.** The study suggested the presence of relative uniformity of the results of all the measurements regardless of the quantity of added electrolytes and lipid phase. It was shown that undoubtedly there is the influence of 2-valent cations (calcium and magnesium) upon lipid droplets size, which is in a direct correlation with theoretical assumption. **Conclusion.** Within a 72-hour testing period there was no significant increase in droplet size, i.e. the studied admixtures remained stable considering droplet size median as the criterion of stability.

Key words:
parenteral nutrition; particle size; fat emulsions, intravenous; electrolytes; quality control.

Apstrakt

Uvod/Cilj. Parenteralna ishrana, kao specifičan vid nadoknade hranljivih materija, i dalje predstavlja stalnu temu teorijskog i eksperimentalnog izučavanja. Složeni sastav smeše za totalnu parenteralnu ishranu (TPI) otežava održanje njihove stabilnosti. Najkritičniji parameter je dijametar lipidnih kapi, odnosno raspodela veličina kapi. Postoji preporuka da veličina kapi ne bi trebalo da prelazi 5 μm i da zastupljenost većih kapi ne prelazi vrednost od 0,05%. Na veličinu lipidnih kapi poseban uticaj ima dodavanje elektrolita, naročito viševalentnih katjona. Postoji opasnost da dodati elektroliti interreaguju sa lipidnim kapima, što dovodi do njihovog spajanja i ima negativan uticaj na stabilnost smeša. Cilj ovog rada bio je da se istraži kako dodati elektroliti i količina lipidne faze utiču na stabilnost ovih smeša. **Metode.** Ispitivanoj smeši sa definisanim sastavom osnovne formulacije izrađenoj u bolničkoj apoteci, dodavani su elektroliti na osnovu preporuka iz literature. Merenje veličine kapi vršeno je metodom laserske difrakcije pomoću laserskog analizatora čestica. Uticaj nezavisno promenljivih je procenjen i izračunat primenom komercijalnog softvera. Kao nezavisno promenljive, u svojstvu ispitivanih faktora izabrani su koncentracije Na^+, K^+, Ca^{2+} i Mg^{2+}, kao i količina masne faze. Odgovor sistema, ili zavisno promenljiva veličina, bila je medijana veličine kapi. Svaki od faktora variran je na dva nivoa, gornji (+1) i donji (–1), odnosno primenjen je 2^{5-2} frakcioni fak-

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torijalni dizajn. **Rezultati.** Istraživanje je pokazalo da nezavisno od količine dodatih elektrolita i količine lipidne faze postoji relativna ujednačenost rezultata za sva merenja. Analiza pojedinačnih faktora ukazuje na nesumnjiv uticaj dvovalentnih katjona (kalcijuma i magnezijuma) na veličinu lipidnih kapi, što je u direktnoj korelaciji sa teoretskim postavkama. **Zaključak:** Tokom ispitivanog 72-časovnog perioda nije bilo značajnog povećanja veličine kapi, odnosno ispitivana smeša ostala je stabilna sa stanovišta medijane veličine kapi kao kriterijuma stabilnosti.

Ključne reči:
ishrana parenteralna; čestice, veličina; emulzije, masne; intravenske; elektroliti; kvalitet, kontrola.

Introduction

A major requirement to meet the safe and efficient use of total parenteral nutrition (TPN) admixtures concerns their stability, i.e. unchangeability over time. They are oil-in-water type emulsions (O/W) and containing more than 50 components (amino acids, carbohydrates, lipids, electrolytes, vitamins, oligoelements, insulin, heparin and water for injections). It is obviously difficult to obtain and maintain their quality, especially due to the fact that these components could interact, while these interactions are not visible.

The most critical parameter that could adversely affect the stability of TPN admixtures and endanger their suitability for the clinical use is the lipid droplet diameter. Numerous factors affect lipid droplet size, thus consequently, the stability of these emulsion systems. It is significant that lipid droplet size is particularly affected by electrolyte addition, especially by multivalent cations. However, while an admixture is required to contain electrolytes for the organism to maintain normal functioning, if high quantities of ions are added (which is the case in patients affected by metabolic disorder) it induces danger of their interaction with lipid droplets resulting in increasing of droplets size and their aggregation and imposing negative effect upon the stability of admixture [1].

It has to be emphasized here that the droplets of fat are usually not of the regular spherical shape differing from each other almost always in size, so that polydispersity is present in each sample. It is, therefore, more reasonable to refer to "droplet size distribution" than to "droplet size", these two parameters, however, being most often regarding as identical [2, 3]. It comes out that the most significant parameters to follow, except for the homogeneity, are the size stability, droplet size distribution, and even (uniform) distribution of individual droplets within the emulsion.

Attempts to introduce droplet (globule) size measurement technique into the emulsions quality analysis began in the 80s of the 20 century. It was then suggested the lipid droplet diameter to be 0.5–1 μm that is approximately equal to the diameter of endogenous lipids (chyllomicrons), while the cut-off droplet size to be 5 μm that was not a real requirement considering that there was no sophisticated measuring technique. It is not until 2004 that the United States Pharmacopeia (USP) introduced two criteria: globule size should not be more than 5 μm, and the presence of globules larger than that size should not exceed 0.05% [2, 4].

Emulsions are known to belong to the group of colloidal systems. Yet, the droplet size in emulsions could not be determined by means of the methods usually used to determine the droplet size in colloids. The reason for that being the fact that the droplets in emulsions are deformable and tend to aggregate into large droplets. This affects light scattering and light refraction and other phenomena using to determine the size of droplets [5].

It is well-known that there is no unique reliable method for measuring the size of emulsions droplets in a wide spectrum. Methods of measuring droplets, starting from classical to the most recent – modern ones, have been selectively used to cover a certain diapason of requirements and range of measuring [6, 7]. Any methods are specific in their own way, have their disadvantages and limitations and cover a certain range of the droplets size that could be determined by them. Besides, neither of the methods could determine a full droplets size distribution (starting from a few nm to many μm). The method of laser diffraction [8–10] is the most often used one. It is also the one used in this study.

The use of mathematical, statistical and other models make it possible to predict, that is to choose experimental settings. Although numerous, and titled as experimental design, the most often used are the methods of so-called factorial design. Factorial design is applied to determine the influence of certain factors on the system, giving the possibility to assess which of the factors exerts the most significant influence. Factorial design application provides a considerably high number of information on the studied system by a relatively low number of experiments [11, 12].

The aim of the study was to determine the impact of concentration and type of electrolytes, as well as a lipid phase on the size of lipid droplets by the use of experimental design as regard to the change of values ranging from the lowest to the highest ones, as required by the practice itself. The experimental design here helps understand the way the said factors influence the median value as the numerically and statistically significant characteristics of experimentally obtained values.

Methods

The studied TPN admixtures were prepared in the hospital pharmacy using the techniques of aseptic procedures in a laminar chamber. The study was performed in various time intervals: immediately after the preparation – 0 h, and after 12 h, both at the temperature of 25°C. Time of 12 h responds to the time of application TPN admixture to a patient. Next, the compounds were kept at the temperature spanning from 2°C to 8°C and analyzed after 72 hours.

Table 1 shows the basic formulation composition of a TPN compound, while Table 2 shows the independent variables, i.e. component values of various quantities (concen-

Table 1

The composition of a total parenteral nutrition (TPN) admixture

Components	TPN admixture quantity (mL)
Amino acids as Vamin 18* (nitrogen 18 g/L, amino acids 114g/L)	500
Glucose infundible 200 mg/mL**	1000
Intralipid 20%*	250 and 500

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Table 2

Real values of the independent variables

Independent variable	Lower level (−1)	Upper level (+1)
Na^+ concentration (mmoL), X_1 (Natrii chloridi inj. 100 mg/mL*)	50	150
K^+ concentration (mmoL), X_2 (Kalii chloridi inj. 74.5 mg/mL*)	75	100
Ca^{2+} concentration (mmoL), X_3 (Calcii chloridi inj. 100 mg/mL*)	10	20
Mg^{2+} concentration (mmoL), X_4 (Magnesii sulfatis inj. 250 mg/mL*)	10	20
Lipid phase quantity (g), X_5, (Intralipid 20%**)	50	100

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tration of the added electrolytes and the quantity of the lipid component).

The quantity of electrolytes added to the admixtures for TPN was determined on the basis of daily requirements by patients with the increased need for electrolytes (for example polytraumatized patients) in compliance with the guidelines from the literature [13].

The particle size was determined by a laser particle analyzer (Microtrac Fra 9200, Leeds & Northrup) which uses laser diffraction technique. Measurements were repeated three times for each sample. Visual analysis was used to observe flocculation and creaming of the emulsion after admixture [14].

As mentioned above, the most significant parameters to control, besides homogenity, are the stability of the size and droplet size distribution, as well as the uniformity of individual droplet size distribution within an emulsion. Considering numeric characteristics of random variables (a diameter of lipid droplets) it is common to define a certain characteristic size which indicates a kind of medium or orientational value around which any experimentally obtained value for the random variable are grouped. The median random variable was used in this study.

The median is a value defined as specifically divide a numerical series in two equal parts. One part includes any elements of the value equal or less than the median, while the other includes these of the equal or greater than the median. In that sense, in a sequence of values set by the size (”arranged sequence“), the median values are found exactly in the middle, thus they are also called ”50th percentile“ [15, 16].

In setting the experimental conditions, as well as in analyzing the results obtained by the experiment, to calculate factorial effects the analysis of variance (ANOVA) method was used, performed using the computer program Design Expert [12, 17]. The chosen factors, namely independent variables, were as follows: Na^+, K^+, Ca^{2+}, and Mg^{2+} concentrations, as well as the quantity of lipid phase. The system response, or dependent variable size was the median. The design, referred as 2^3, was chosen because according to the theory to obtain the response in the assessments like this one at least 8 experiments have to be performed. If each of the 5 factors would vary at 2 levels we could obtain a total factorial design type 2^5, thus in 32 experiments we would often obtain insignificant effects of higher order of magnitude.

Each of the factors ($X_1 – X_5$) was varied at two levels, namely higher (+1) and lower (-1), that is the fractional factorial design $2^{5–2}$ was applied. The responses were put into the mathematical model of the first-order polynomial including 5 variables and a constant member b_0 (further on referred to as constant) [12].

$y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5$

y - response (in this case the median of the droplet size),

b_0 - coefficient (Table 4). The constant b_0 is an average response for given in the any experiments, i.e. average effect of any factors; for the given experiment $b_0 = 1 / 5_i = {}_I\Sigma^5 y_i$,

b_1, b_2, b_3, b_4, b_5 - factorial effects,

X_1, X_2, X_3, X_4, X_5 – independent variables.

Results

There was no visual change in color, clarity, creaming or precipitates in the studied TPN during the study period.

To simplify factorial effects analysis, the parameters to verify are marked as coded (Table 3) presents experimental matrix of 2^{5-2} fractional factorial design; values of independent variables are presented in coded values. Table 3 also shows the values of median obtained by data processing of droplet sizes measured by a Microtrac Fra 9200.

The results indicate a relative uniform data for all the measurements regardless the quantity of electrolytes and the quantity of lipid phase in the studied compounds for TPN. There were no significant differences in droplets size distribution. Also, in all the measurements done the median values were not more than 0.6 μm. This fact implies that the com-

Table 3

Experimental matrix of 2^{5-2} experimental design; [values of independent variables are presented in coded values and system responses droplet sizes median (μm) after composing the preparation (0 h) and after 12 h and 72 h]

Number of samples	X_1	X_2	X_3	X_4	X_5	System responses y_1	y_2	y_3
1	−1	−1	−1	+1	+1	0.354	0.451	0.484
2	+1	−1	−1	+1	−1	0.241	0.429	0.524
3	−1	+1	−1	−1	+1	0.231	0.251	0.507
4	+1	+1	−1	−1	−1	0.292	0.250	0.479
5	−1	−1	+1	−1	−1	0.296	0.444	0.469
6	+1	−1	+1	−1	+1	0.494	0.485	0.445
7	−1	+1	+1	+1	−1	0.342	0.474	0.393
8	+1	+1	+1	+1	+1	0.231	0.515	0.417

Symbols in Table 3:
X_1 – concentration of Na^+; X_2 – concentration of K^+; X_3 – concentration of Ca^{2+}; X_4 – concentration of Mg^{2+}; X_5 – lipid phase quantity; −1 means minimum, +1 means maximum; y_1, y_2, y_3 – lipid droplets size median (μm) in 0 h, 12 h and 72 h

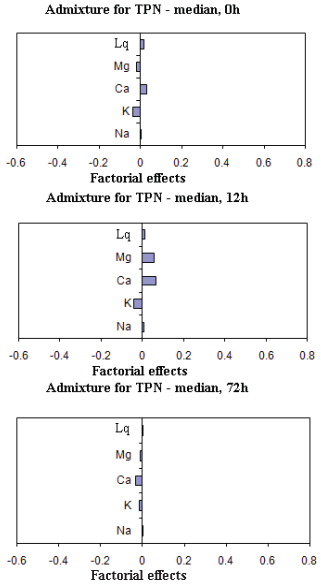
pounds within the whole period of testing, i.e. in a 72-hour period, were stable as regard to the droplet size median as the stability criterion.

Factorial effects analysis

Factorial analysis was done in order to assess which of the chosen factors significantly affect the median.

As displayed in the Table 4, it is obvious that the values of the regression coefficient during the time period in which the mixtures were analyzed had been the greatest when it is the question of the impact of calcium. These results are in direct correlation with theoretical postulations of the influence of polyvalent cations upon the droplets size increase [18]. However, immediately after the production of TPN mixture (0 h), the value of the regression coefficient shows that the impact of potassium is very similar to the value showing the influence of calcium.

Factorial effects can be presented graphically as Pareto chart (Figure 1).



Lq – lipid phase quantity

Fig. 1 – Pareto chart (median of droplets size)

Table 4

The calculated factorial effects and the applicable constants when droplet size median was followed up as a response

Parameters	0 h	12 h	72 h
Coefficient (b_0)	0.31000	0.4100	0.470
Independent variable		Factorial effects	
X_1	0.00437	0.0074	0.00143
X_2	-0.03600	-0.0400	-0.01600
X_3	0.03100	0.0670	-0.03400
X_4	-0.01800	0.0550	-0.01000
X_5	0.01700	0.0130	-0.00143

X_1 – concentration of Na^+; X_2 – concentration of K^+; X_3 – concentration of Ca^{2+}; X_4 – concentration of Mg^{2+}; X_5 – lipid phase quantity

Discussion

Since the beginning of using TPN admixtures, i.e. within the last 30 years, numerous studies on their stability [9, 19–21] have been performed. The majority of authors have suggested that the stability of each admixture has to be verified and that the obtained results cannot be generally accepted as well. Special attention has to be paid to limitations regarding the electrolyte addition, especially polyvalent cations.

Regardless the mentioned facts no clear guidelines have been defined for the maximal quantity of electrolytes that could be added. Due to the need to daily prepare TPN admixtures customized to the requirements of each patient it is necessary to study this problem in details.

Electrolytes addition to a TPN admixture, as mentioned above, disturbs its stability, that is to say it causes physicochemical changes. There are two kinds of interactions between electrolytes and fat globule surface: non-

specific and specific adsorption. Non-specific adsorption occurs when added monovalent cations Na^+ and K^+ are adsorbed at the surface of fat droplets. At high electrolyte concentrations, and above the critical flocculation concentration (CFC), electrostatic repulsive forces decrease their value thus, becoming equal to the Van der Waals attractive forces, and at a certain moment, the flocculaton process of an emulsion starts. Another adsorption kind, that, besides electrostatic interactions, involves the chemical interaction of ions and the surface of droplets is a so-called specific adsorption. It is the chemical process of adsorption occurring between polyvalent cations (Ca^{2+} and Mg^{2+}) and lipid droplets which forms a bridge with the anionic emulgator component of the two lipid droplets. It, thus, leads to the increase of the droplet size and the occurrence of various types of instability such as aggregation, coalescence, flocculation, phase separation, and emulsion phase inversion as well. In the terminal stage of destabilisation of the emulsion, a droplets size could range from 5 μm to 50 μm which is not allowed in parenteral emulsions. It has been shown that a higher concentration and valency of cations cause the destabilisation of the emulsion to a higher extent [21].

In planning the experiment, we started from the fact that the number of factors that could affect the stability of an emulsion for parenteral nutrition is high, thus it was necessary to choose adequate factors for the study. This choice was based on the previous experiences with this issue assessed both theoretically and practically. We also considered all the known results obtained in the earlier studies [12, 22]. In designing the research, as well as in the results processing, we considered the correlation between theoretical assumptions and the obtained results applicability in pharmacy and the clinical practice.

Since the levels of independent variables are marked by coded values, absolute values of regression coefficients directly give information about their influence upon the studied system. The higher absolute value of the regression coefficient, the higher the influence of the corresponding independent variable. If the regression coefficient has the sign of ”+“, or ”-”, the increase in the level of independent variable conditions increase, or decrease in the dependent variable, that is the studied system response.

Conclusion

The results of this study suggest that the assessed TPN admixture could be used within a 72-hour period (duration of the analysis). No significant increase in a droplet size was observed. Considering the droplet size median as the criterion of stability, the studied admixtures remained stable. Thus, this study also clarified how the changes in values of individual factors influence the system to respond. It could be concluded that the metod of the fractional factorial design is suitable for planning a trial and assessing the obtained results.

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Correlation between subjective and objective nasal breathing assessments in examinees with nasal septum deformities

Povezanost subjektivne i objektivne procene disajne funkcije nosa kod ispitanika sa deformitetom nosne pregrade

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Abstract

Background/Aim. Nasal obstruction is one of the most frequent disorders because of which patients see their Ear, Nose and Throath (ENT) doctors. Impaired nose breathing is a subjective symptom and it often does not coincide with clinical nose findings and functional tests of breathing function. Therefore, the aim of this study was to establish if there is an accordance between a subjective nose breathing assessment and objective methods (rhinomanometry and acoustic rhinometry) in assessing nose breathing function in patients with diverse nasal septum deformity degrees, as well as to establish an accordance between these two objective methods. **Methods.** This study involved the total of 90 examinees divided into three groups. The group I consisted of examinees with nasal septum deformities less than 10º. The group II consisted of examinees with nasal septum deformities ranged from 10º to 15º. The group III involved examinees with nasal septum deformities over 15º. Each examinee had subjectively graded his/her nasal breathing on the side of the nose septum deformity from 0 to 10, and afterwards the whole noses. Rhinomanometry and acoustic rhinometry were done on the side of the nasal septum deformities and after that on the other side of the nose using the Interacoustics SRE 2000 device. **Results.** In the groups II and III there was a positive correlation between a subjective nose breathing assessment and rhinomanometric values both on the side of the nasal septum deformities and the nose as a whole, ($p < 0.05$), and no correlation between these traits in the group I ($p > 0.05$). In none of the examined groups correlation was found between a subjective nose breathing assessment and rhinometric values, both minimum cross–sectional area (MCA) and volume (VOL), both on the side of the nasal septum deformities and the nose as a whole ($p > 0.05$). There was no correlation found between rhinomanometric and rhinometric MCA and VOL values in either on the sides of nasal septum deformities or the nose as a whole in any of the examined groups ($p > 0.05$). **Conclusion.** Rhinomanometry significantly correlates with the subjective nose breathing assessment and it can be used as a reliable and objective indicator of nose breathing in everyday clinical practice. Acoustic rhinometry, on the other hand, which does not correlate with a subjective nose breathing assessment could have a greater significance in a scientific sense than in clinical applying.

Key words:
nose; respiration disorders; nasal septum; rhinomanometry; rhinometry, acoustic.

Apstrakt

Uvod/Cilj. Nosna opstrukcija je jedna od najčešćih tegoba zbog koje se bolesnici javljaju otorinolaringologu. Otežano disanje na nos je subjektivan simptom i često se ne poklapa sa kliničkim nalazom u nosu i funkcionalnim testovima disajne funkcije. Upravo zbog toga cilj ovoga rada bio je da se utvrdi da li postoji podudarnost između subjektivne procene disanja na nos i objektivnih metoda (rinomanometrije i akustičke rinometrije) u proceni disajne funkcije nosa kod bolesnika sa različitim stepenom deformiteta nosne pregrade, kao i da li postoji podudarnost između ove dve objektivne metode međusobno. **Metode.** Istraživanje je obuhvatilo ukupno 90 ispitanika podeljenih u tri grupe. Grupu I činili su ispitanici sa deformitetom nosne pregrade manjim od 10º. U grupi II deformitet nosne pregrade iznosio je od 10º do 15º. U grupi III bili su ispitanici sa stepenom deformiteta nosne pregrade većim od 15º. Svaki ispitanik subjektivno je ocenio svoje disanje na nos na strani deformiteta nosne pregrade, a potom nosu kao celini, ocenom od 0 do 10. Rinomanomet-

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rija i akustička rinometrija, takođe, rađene su na strani deformiteta nosne pregrade, a potom i na drugoj strani nosa na aparatu Interacoustics SRE 2000. **Rezultati.** U grupama II i III nađena je pozitivna korelacija između subjektivne ocene disanja na nos i rinomanometrijskih vrednosti kako na strani deformiteta nosne pregrade, tako i u nosu kao celini, ($p < 0,05$), dok u grupi I nije nađena korelacija između ovih obeležja, ($p > 0,05$). Ni u jednoj grupi ispitanika nije nađena korelacija između subjektivne ocene disanja na nos i rinometrijskih vrednosti, kako vrednosti minimalnog poprečnog preseka nosa [*minimum cross-sectional area* (MCA)], tako i vrednosti volumena (VOL) ni na strani deformiteta nosne pregrade, ni u nosu kao celini, ($p > 0,05$). Nije nađena povezanost između rinomanometrijskih i rinometrijskih MCA i VOL vrednosti kako na strani deformiteta nosne pregrade, tako i u nosu kao celini, ni u jednoj grupi ispitanika ($p > 0,05$). **Zaključak.** Rinomanometrija u značajnoj meri koreliše sa subjektivnom ocenom disajne funkcije nosa i može se koristiti kao pouzdani objektivni pokazatelj disajne funkcije nosa u svakodnevnoj kliničkoj praksi. Akustička rinometrija, s druge strane, koja ne koreliše sa subjektivnom ocenom disanja na nos, veći značaj ima u naučnom smislu nego u kliničkoj primeni.

Ključne reči:
nos; disanje, poremećaji; nos, septum; rinomanometrija; rinometrija, akustička.

Introduction

Nasal obstruction is one of the most common disorders because of which patients are referred to otorhynolaryngologists. There are numerous factors causing it, but they can be divided into two basic groups: the first one being anatomic factor group leading to nasal obstruction, whereas the second one is the group characterized by changes in the mucus.

Nasal breathing is a subjective symptom and frequently does not coincide with clinical nasal findings [1]. This is the reason why the need for an objective assessment of breathing function arose, which could enable more precise diagnoses and indications for conservative, i.e. surgical treatment, as well as a more successful follow-up.

Rhinomanometry and acoustic rhinometry are most commonly used objective methods for assessment of nose breathing function. As rhinomanometry gives a dynamic nasal function assessment [2–5], acoustic rhinometry enables a static (anatomic) assessment of the nasal cavity condition [6, 7].

Although these two objective methods to assess nasal breathing function have been clinically applied for a relatively long time, rhinomanometry since the 50s of the twentieth century and acoustic rhinometry since the late 80s [8], contemporary authors still have opposite attitudes on their clinical applications. These opposite attitudes on the validity of clinical rhinomanometry and acoustic rhinometry derive from the reason why different authors have obtained different results on correspondence of subjective and objective nasal breathing function assessment by rhinomanometry and acoustic rhinometry. Also, certain authors have completely different results when nasal breathing function assessments are obtained by rhinomanometry and rhinometry.

The aim of this study was to establish whether there is a correspondence between a subjective nasal breathing function assessment and objective methods (rhinomanometry and acoustic rhinometry) in assessing nasal breathing functions in patients with different nasal septum deformity degrees as well as whether there is a correspondence between the two objective methods in nasal breathing function assessment.

Methods

The study involved a total of 90 examinees out of whom there were 26 female patients and 64 male patients. The average age of the examinees was 31.12 years. This study included otorhinolaryngological patiens with a rhinoscopically visual nasal septum deformities and no other otorhinolaryngological conditions and no lower respiratory tract ailments that could lead to a subjective assessment of breathing difficulties.

On the basis of nasal septum deformity degree, the examinees were divided into three groups (30 patients in each): the group I with nasal septum deformities less than 10º; the group II, with nasal septum deformity from 10º to 15º; the group III with nasal septum deformity degrees more than 15º. The degree of nasal septum deformity was diagnosed by computed tomography (CT) nasal findings as an angle made of a line from *cristae gali* to *spinae nasalis anterior inferior* and a line drawn from *cristae gali* to the point where the most striking deformity of nasal septum was. The values of deformity degree were expressed in full numbers.

Every examinee subjectively assessed their nasal breathing on the deformity side and afterwards on the nose as a whole. Their marks ranged from 0 to 10 on the visual analogue scales (VAS), with 0 marking no breathing troubles at all, whereas 10 meant nasal total nasal breathing disability. Rhinomanometry and acoustic rhinometry was performed on the side of nasal septum deformity as well as on the other sides of the nose using an Interacoustics SRE 2000 device.

Rhinomanometry is a method based on indirect resistance determination (r) in the nasal air flow. The differences in air pressure are measured directly (ΔP) at the nose entrance as well as in the nasopharynx, along with the proportion of the air flown in the time unit (V/s). On the basis of these data, nasal air flow resistance (r) is worked out by a computer using the $r = \Delta P / V/s$ formula, and it is expressed in Pas/cm³ for each side of the nose, respectively. The total nose air flow resistance is calculated according to a formula $R(t) = R(l) \times R(r) / R(l) + R(r)$. In this paper, anterior active rhinomanometry was used with nose adaptors.

Acoustic rhinometry is a method based on the time of functional nasal septum sound wave reflection analysis. It makes possible obtaining data on the size of decussated in-

tersections of various nasal septum cavity as well as air volumes in the previously examined nasal septum regions. Even the MCA has were marked and expressed in cm². The values of VOL were measured in the nose at the distance between 2 and 5 centimeters and they were expressed in cm³. For rhinometric measurements the measuring tube with the nose adaptor was used. It was shown that the deformation of the vestibulum by the anatomical nose adaptor is less than by the conical nosepiece inserted into the nostril.

For this study we provided the consensus of the Ethical Committees of Vojvodina Clinical Center and Medical Facult y in Novi Sad.

For the measured parameters, the following was calcuted and shown: arithmetic mean, median and standard deviation. To examine linking of the two traits the Pearson's correlation coefficient was used.

Results

Table 1 shows the average values of subjective nose breathing assessment on the nasal septum deformity side and the nose as a whole in all the groups, as well as standard deviations and median. In the group I there was no statistically significant difference in the subjective nose breathing assessment between the side with nasal septum deformity and the nose as a whole ($p > 0.05$), while in the groups II and III this difference was statistically significant ($p < 0.05$).

Table 2 shows average rhinomanometric values in groups as well as their standard deviations and median on the nasal septum deformity side and the nose as a whole.

Tables 3 and 4 show the average rhinometric VOL and MCA values in the groups as well as their standard deviations and median both on the nasal septum deformity side and the nose as a whole.

In the groups II and III there was a positive correlation between a subjective nose breathing assessment and rhinomanometric values both on the nasal septum deformity side and the nose as a whole, ($p < 0.05$), while in the group I there was no correlation between these traits ($p > 0.05$), (Table 5).

None of the examined groups had any correlation between a subjective nose breathing assessment and rhinometric values, both MCA and VOL values either on the nasal septum deformity side or the nose as a whole ($p > 0.05$), (Tables 6 and 7).

Table 1

The subjective assessment of nose breathing on the nasal septum deformity side and the nose as a whole

The group of patients*	The nasal septum deformity side			The nose as a whole		
	Mean	SD	Median	Mean	SD	Median
I	1.80	1.13	2.00	1.70	1.05	2.00
II	3.67	1.06	4.00	1.86	1.22	2.00
III	6.73	0.98	7.00	3.70	1.54	3.00

***see section Methods**

Table 2

Rhinomanometric values on the nasal septum deformity side and the nose as a whole

The group of patients*	The nasal septum deformity side			The nose as a whole		
	Mean	SD	Median	Mean	SD	Median
I	0.71	0.19	0.63	0.23	0.05	0.23
II	0.73	0.16	0.72	0.26	0.07	0.25
III	1.60	0.86	1.26	0.34	0.13	0.32

***see section Methods**

Table 3

Rhinometric minimal cross-sectional area (MCA) values (cm^2) on the nasal septum deformity side and the nose as a whole

The group of patients*	The nasal septum deformity side			The nose as a whole		
	Mean	SD	Median	Mean	SD	Median
I	0.39	0.05	0.39	0.98	0.15	0.96
II	0.34	0.06	0.35	0.88	0.09	0.87
III	0.26	0.10	0.25	0.77	0.10	0.76

***see section Methods**

Table 4

Rhinometric volume (VOL) values (cm^3) on the nasal septum deformity side and the nose as a whole

The group of patients*	The nasal septum deformity side			The nose as a whole		
	Mean	SD	Median	Mean	SD	Median
I	3.12	0.48	3.10	6.80	0.85	6.72
II	2.81	0.42	2.83	6.33	0.82	6.39
III	2.33	0.39	2.33	5.86	0.92	6.05

***see section Methods**

Table 5

Correlation between subjective nose breathing assessment and rhinomanometric values on the nasal septum deformity side and the nose as a whole

The group of patients*	The nasal septum deformity side		The nose as a whole	
	r	*p*	r	*p*
I	0.227	0.229	0.213	0.257
II	0.485	0.007	0.471	0.009
III	0.420	0.021	0.504	0.005

***see section Methods; r – coefficient of correlation**

Table 6

Correlation between subjective nose breathing and rhinometric minimal cross-sectional area (MCA) values (cm^2) on the nasal septum deformity side and the nose as a whole

The group of patients*	The nasal septum deformity side		The nose as a whole	
	r	*p*	r	*p*
I	0.146	0.442	0.037	0.848
II	- 0.066	0.728	- 0.062	0.745
III	- 0.340	0.066	- 0.188	0.320

***see section Methods; r – coefficient of correlation**

Table 7

Correlation between subjective nose breathing and rhinometric volume (VOL) values (cm3) on the nasal septum deformity side and the nose as a whole

The group of patients*	The nasal septum deformity side		The nose as a whole	
	r	*p*	r	*p*
I	- 0.069	0.716	0.034	0.859
II	0.033	0.862	- 0.008	0.962
III	- 0.049	0.798	0.001	0.994

***see section Methods; r – coefficient of correlation**

No correlation was found between rhinomanometric and rhinometric MCA and VOL values both on the nasal septum deformity side and the nose as a whole in any of the examined groups, ($p > 0.05$), (Tables 8 and 9).

Table 8

Correlation between rhinomanometric and rhinometric minimal cross-sectional area (MCA) values (cm^2) on the nasal septum deformity side and the nose as a whole

The group of patients*	The nasal septum deformity side		The nose as a whole	
	r	*p*	r	*p*
I	- 0.146	0.440	0.028	0.885
II	- 0.178	0.346	0.124	0.513
III	- 0.100	0.599	0.096	0.615

***see section Methods; r – coefficient of correlation**

Table 9

Correlation between rhinomanometric and rhinometric volume (VOL) values (cm^3) on the nasal septum deformity side and the nose as a whole

The group of patients*	The nasal septum deformity side		The nose as a whole	
	r	*p*	r	*p*
I	0.013	0.947	0.129	0.495
II	0.155	0.413	0.012	0.950
III	0.064	0.738	0.060	0.754

***see section Methods; r – coefficient of correlation**

Discussion

An objective assessment of nose breathing function is one of the most frequent problems in everyday ENT routine [9]. The need for its objectiveness is especially important in making a difference between mucous and mechanical causes of difficult breathing as well as establishing the proper indication for a surgical treatment of nasal septum deformity. Although anterior rhinoscopy is a routine method in diagnosing every patient complaining about impaired nose breathing, this clinical finding is often not in accordance with the degree of the subjective suffering of the patients [10]. The subjective feeling of obstruction of the nose is a complex phenomenon and depends on more than anatomical and

functional details and airflow characteristics [11]. It is known that a slight septal deviation in the nasal valve region can cause clear symptoms, whereas a much lager deviation in the back of the nasal cavity may result in far fewer symptoms [8]. Anterior rhinoscopy by means of nasal speculum risks masking abnormalities by distortion of nasal lumen in the valve area [12]. Until today, there has been no ideal clinical test of nasal patency giving the dynamic nature of the nose [13], that can translate that patient's evaluation of nasal obstruction into a specific figure, as it is the case with the audiogram for hearing, the vision test for sight, and spirometry for lung function [14].

The results of our study suggest a correspondence with a subjective nose breathing assessment and rhynoanometric findings in the examinees in the groups II and III, regardless nasal septum deformity side or the nose as a whole. Also, the examinees of these two groups experienced a significantly impaired breathing function on the side of the nasal septum deformity in relation to the nose as a whole, while the examinees of the group I did not experienced it at all. These results correspond with those obtained by Sipilä et al. [15] showing the difficulties in assessing their noses breathing in case the difference in rhinoanometric findings between the nose side is less than 60–70%. McCaffrey and Kern [16] as well as Roithman et al. [12] have also found a correspondence between these traits. On the other hand, Kim et al. [1], Tomkinson and Eccles [11] as well as Naito et al. [17], Thulesius et al. [18] do not find any correspondence between a subjective nose breathing assessment and rhinomanometric findings. Mygind [19] is of the opinion that rhinomanometry has only a scientific importance, whilst its clinical significance is very little.

We found no correspondence in any of the examined groups between a subjective nose breathing assessment and rhinometric values (either MCA or VOL) regardless the nasal septum deformity side or the nose as a whole. Similar results were reported by the majority of other authors [1, 11, 17]. Contrary to them, Roithmann et al. [12] found a correspondence between a subjective nose breathing function assessment and rhinometric MCA values.

Thulesius et al. [18] found that older age significantly lowers rhinomanometric values and are of the opinion that this is a consequence of nasal mucus atrophy and nose bones growth which lead to nasal cavity enlargement. Also, Kalmovich et al. [20] have found, endonasal volumes and minimal cross sectional areas increase in elderly people as measured with acoustic rhinometry.

There was no correspondence between rhinomanometric and rhinometric (MCA and VOL) values in any of the examined groups regardless the nasal septum deformity side or the nose as a whole. Our results coincide with the ones obtained by Warren et al. [21] and Naito et al. [17]. Nevertheless, Yaniv et al. [22] as well as Tomkinson et Eccles [11] do find correspondence between these traits.

Conclusion

Rhinomanometry which, notably in greater nasal septum deformities, significantly correlates with a subjective nose breathing function assessment, can be an objective indicator of nasal breathing function in everyday clinical practice. Acoustic rhinometry that does not correlate with a subjective nose breathing function assessment, might have a greater scientific significance than clinical application.

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Dermatoglyphic characteristics of digito-palmar complex in autistic boys in Serbia

Dermatoglifske karakteristike digitopalmarnog kompleksa kod autističnih dečaka u Srbiji

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Abstract

Introduction/Aim. Dermatoglyphics is a science that examines dermal patterns on volar side of both palms and soles. Since dermatoglyphs are unique for each person, by examining them a number of parameters can be determined. These parameters could help to diagnose and treat examined individulas. The aim of this study was to determine possible differences of the dermathoglyphic characteristics of digito-palmar complex (DPC) comparing the autistic boys with the healthy examinees. **Methods**. This study was conducted on a group of 182 boys with infantile autism, aged from 5 to 15 (average age 7.2 years) while the control group consisted of 182 healthy men from 30 to 50 years (average age 38.7 years). Within the digital scope of DPC we examined tree types of dermatoglyphic patterns on fingertips (arch, loop and whrol), as well as dermal ridge count on each finger separately (FRC – finger ridge count) and total dermal ridge count on all the ten fingers (TRC – total ridge count). Within the palmar DPC area we measured the angles between the triradius (atd, dat, adt, atb, btc, ctd), as well as dermal ridge count (RC – ridge count) between the triradius a-b, b-c and c-d. **Results.** The autistic boys had a significantely higher count of arches (9.17%) on fingertips of both hands when compared to the control group of examinees (4.34%), and the lower count of loops (28.40%) compared with the control group (32.42%). A higher count of arches was especially expressed on the fourth and fifth finger of both hands. Beside this characteristic, the autistic boys had a lower TRC and ab-RC as well as a wider atd angle. **Conclusion.** Dermatoglyphic analysis could help in diagnosing autism but only as an additional method, never as a dominant diagnostic procedure.

Key words:
dermatoglyphics; autistic disorder; child; fingers; hand; diagnostic techniques and procedures; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Dermatoglifika je nauka koja se bavi proučavanjem dermalnih šara (dermatoglifa) na volarnoj strani šaka i tabanima. Pošto su dermatoglifi specifični za svaku osobu njihovim proučavanjem mogu se utvrditi brojni parametari koji olakšavaju dijagnostikovanje i lečenje ispitivanih osoba. Cilj istraživanja bio je da se utvrde moguće razlike u dermatoglifskim karakteristikama digitopalmarnog kompleksa (DPK) kod autističnih dečaka i zdravih osoba. **Metode.** Ispitivanje je obuhvatalo 182 dečaka sa infantilnim autizmom, uzrasta 5–15 godina (prosečno 7,2 godine), i kontrolnu grupu od 182 zdrava muškarca, stara 30–50 godina (prosečno 38,7 godina). U okviru digitalnog dela DPK ispitivali smo tri vrste dermatoglifskih obrazaca na jagodicama prstiju (luk, petlju i spiralu), kao i broj dermalnih grebena na svakom prstu posebno (FRC – *finger ridge count*) i ukupan broj dermalnih grebena na svih deset prstiju (TRC – *total ridge count*). Kod palmarnog dela DPK merili smo uglove između triradijusa (atd, dat, adt, atb, btc, ctd), kao i broj dermalnih grebena (RC – *ridge count*) između triradijusa a-b, b-c i c-d. **Rezultati.** Autistični dečaci imali su znatno veći broj lukova (9,17%) na jagodicama obe šake u odnosu na ispitanike kontrolne grupe (4,34%), ali manji broj petlji (28,40%) od kontrolne grupe (32,42%). Veći broj lukova bio je posebno izražen na četvrtom i petom prstu obe šake. Pored ove karakteristike autistični dečaci imali su niži TRC i ab-RC kao i veći atd ugao. **Zaključak.** Dermatoglifska analiza može biti od pomoći za dijagnostikovanje autizma kao pomoćna metoda, ali nikako kao dominantna dijagnostička procedura.

Ključne reči:
dermatoglifika; autistički poremećaj; deca; prsti; šaka; dijagnostičke tehnike i procedure; osetljivost i specifičnost.

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Introduction

Clinical dermatoglyphics is a science that studies dermal patterns (dermatoglyphs) on the volar side of hands and soles. Dermatoglyphs are unique for each person, therefore studying them can determine a number of parameters which could be helpful in diagnosing and treatment of examined individuals. The term 'dermatoglyphs' for dermal lines, was used for the first time by American scientists Cummins and Midla in 1926. In the same year the National Congress of American Anatomist and Morphologist officially verified dermatoglyphics as a branch of medical science.[1] In Serbia, clinical dermatoglyphs appeared in XX century, during the 50-ies and the first significant study on this area was the Doctor's Dissertation of Krstić [2]. After these pioneering attempts in Serbia there were over 20 master theses and PhD dissertations related to dermatglyphs.

Today, by using clinical dermatoglyphics over 150 diseases could be identified with 80% to 99.9% of probability. Clinical dermatoglyphics is most often used in diagnosing mental retardation [3, 4], autism [5], schizophrenia [6], Alzheimer' diseases [7], or even in predicting appearances of addiction diseases such is alcoholism [8]. Besides mentioned above, dermatoglyphs can be used to determine genetic predispositions for dyslexia [9], or hyperactivity [10], and also as clinical markers for various types of trisomy.[11] Dermatoglyphic markers of autistic patients have been poorly studied in scientific literature, therefore a very few number of researchers dealt with this problem. Because of the lack of papers on this area and nonexistence of similar researches in Serbia, we decided to conduct this research in order to determine possible differences in dermatoglyphic characteristics of the digito-palmar complex (DPC) among autistic boys and healthy population.

Methods

The research included 182 boys with autism who were on rehabilitation program in the Institute for Psychophysiological Disorders and Speach Patology "Prof. Dr. C. Brajović", in Serbia and in the Cabinet for Defectology "Stošljević" in Serbia. Testing was carried out during the period from 2005 to 2010.

To identify and classify dermatoglyphs, for taking DPC prints, we decided to use the digital scaning method in accordance with the protocols of Cummins and Midlo [12] and Penrose [13]. Dermatoglyphs of the palmar area were determined using a classical scanner type "Canon" (CanoScan 9000F, 4800 × 4800 dpi Resolution) and the software for image editing "VectorMagic" (Figures 1 and 2). Dermatoglyphic fingertip patterns of the hand were determined using a specialized scanner (AET62 NFC, Advanced Card Systems, Ltd.), and the software "VeriFinger" that semiautomatically converts data from the natural into graphic shape (Figure 3).

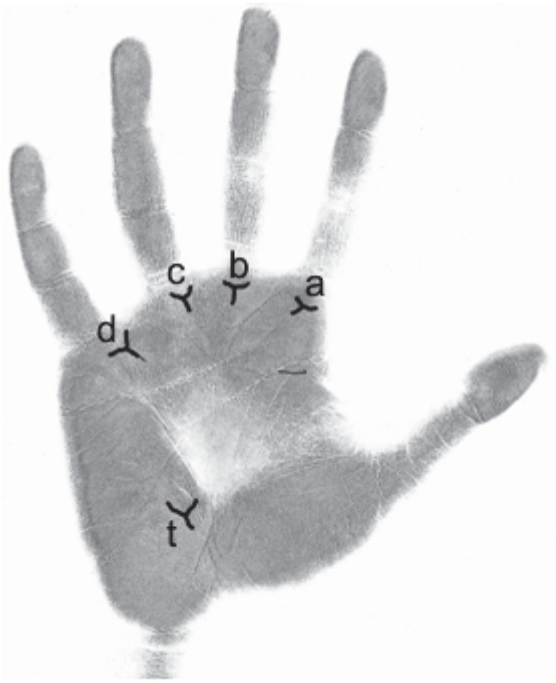

Fig. 1 – A digital hand print processed by "VectorMagic" software

Fig. 2 – An amplification of digital hand print on the level of a-b number

Natural print *Processed print*

Fig. 3 – A digital fingertip print processed by „VeriFinger" software

Qualitative-quantative analysis of the digital DPC area and quantitive analysis of the palmar DPC area were used to make a choice of variables. This implies that in the scope of digital DPC part we examined tree types of dermatoglyphic patterns on fingerprints (arch, loop and whorl) (Figure 4), as well as dermal ridge count on each finger separately (FRC – finger ridge count) and total dermal ridge count on all the ten fingers (TRC – total ridge count). At palmar DPC part we measured angles between the triradius (atd, dat, adt, atb, btc, ctd), as well as dermal ridge count (RC- ridge count) between the triradius a-b, b-c and c-d. Triradius is a spot, a point where three fields of nearly parallel lines meet. These fields form angles of 120° with each other and constrain three regions. It is important that the mutual angle of lines, of which triradius is made, must have at least 90°, so that we can talk about triradius in general. Figure 1 shows triradius a, b, c, d and t which, when connected, form above mentioned dermatoglyphic markers.

Fig. 4 – A type of dermatoglyphic patterns on the top of hand's fingertips

The results obtained by qualitative analysis are descriptively presented through absolute numbers and percentages, while the quantitative analysis results are compared using the Student *t*-test in SPSS (version 17.0.) program. The values of $p \leq 0.05$ were considered significant.

Results

The autistic examinees were from 5 to 15 years old (average age 7.2 years). Besides autism, diagnosed according to the DSM-IV classification, 32.8% examinees had profound intellectual disability (IQ below 34) combined with anxiety and incontinence, while 52.49% examinees had mild intellectual disability (IQ 35–50) followed with alalia and hyperacusis. The rest of examinees (14.64%) were in the light intellectual disability category (IQ 51–70) accompanied by echolalia and stereotypic movement disorder. Epilepsy was diagnosed in 9.56% of the examinees. The control group consisted of 182 healthy men, 30–50 years old (average age 38.7 years). Since dermatoglyphic characteristics do not change during a life time, the equalization of groups by age was not necessary.

Qualitative analysis of digital dermatoglyphic patterns implies determinating type and count of dermatoghlyphic patterns on fingertips of hands. The results of this analysis are shown in Table 1. The autistic boys, compared with the control group (4.34%), had significantly higher arch count (9.17%) on fingertips of both hands, and the lower loop count (28.40%) than the control group (32.42%).

Table 1

The results of quantitative digial dermatoglyphic pattern analysis in the autistic boys (A) and the control group (C)

Group type	Whorl		Loop		Arches	
	n	%	n	%	n	%
			The right hand			
A	595	65.38	228	25.05	87	9.56
C	604	66.37	268	29.45	38	4.17
			The left hand			
A	541	59.45	289	31.75	80	8.79
C	547	60.10	322	35.38	41	4.50
			Both hands			
A	1136	62.41	517	28.40	167	9.17
C	1151	63.24	590	32.42	79	4.34

Quantitative DPC analysis implies statistical comparison of numeric values gained from dermal ridge count and measurement of the angles between the triradius. The results of quantitave analysis of digital DPC area in the autistic boys and control group are shown in Table 2, indicating that statistical significance appeared for FRC variables of the fourth and fifth finger of both hands ($p < 0.05$), as well as for variables of dermal ridge count on five fingers of the right hand ($p < 0.001$) and the left hand ($p < 0.01$). A significant difference was also determined for TRC variable ($p < 0.001$).

The results of quantitative palmar DPC area analysis of the autistic boys and the control group are shown in Table 3 indicating that statistical significance appeared for atd angle variable ($p < 0.05$) and for ab number ($p < 0.05$) of both hands. No statistical significance was determined for other examined variables.

Table 2.

The results of quantitative digital digito-palmar complex (DPC) area analysis in the autistic boys and the control group

Localization of dermal ridges		Autistic boys group mean ± SD	Control group mean ± SD	*p*
The right hand	1st finger	18.98 ± 3.16	18.54 ± 2.84	> 0.05
	2nd finger	11.85 ± 2.35	11.35 ± 2.89	> 0.05
	3rd finger	11.87 ± 2.41	12.36 ± 2.64	> 0.05
	4th finger	14.15 ± 2.87	16.43 ± 2.93	< 0.05
	5th finger	11.27 ± 2.83	13.82 ± 2.98	< 0.05
	Total	68.12 ± 3.99	72.50 ± 4.01	< 0.001
The left hand	1st finger	19.45 ± 3.18	18.94 ± 3.76	> 0.05
	2nd finger	10.38 ± 2.96	10.80 ± 2.94	> 0.05
	3rd finger	13.02 ± 2.74	12.89 ± 3.12	> 0.05
	4th finger	12.31 ± 2.24	14.02 ± 2.83	< 0.05
	5th finger	12.32 ± 3.12	13.04 ± 2.32	< 0.05
	Total	66.38 ± 3.94	69.69 ± 4.06	< 0.01
Total count for ten fingers TRC (total dermal ridge)		134.90 ± 6.88	142.19 ± 6.03	< 0.001

Table 3

The results of quantitative digito-palmar complex (DPC) area analysis in the autistic boys and the control group

Type and localization of dermatoglyphic markers		Autistic boys group mean ± SD	Control group mean ± SD	*p*
The right hand	atd angle	46.20 ± 1.24	42.17 ± 1.25	< 0.05
	dat angle	58.79 ± 0.78	58.15 ± 0.72	> 0.05
	adt angle	82.25 ± 1.25	81.63 ± 1.23	> 0.05
	atb angle	15.97 ± 1.12	15.33 ± 0.95	> 0.05
	btc angle	12.83 ± 0.45	12.01 ± 1.13	> 0.05
	ctd angle	14.00 ± 0.66	13.28 ± 0.71	> 0.05
	a-b number	31.61 ± 0.92	34.61 ± 0.98	< 0.05
	b-c number	24.13 ± 0.84	25.75 ± 0.56	> 0.05
	c-d number	33.22 ± 0.89	34.88 ± 1.15	> 0.05
The left hand	atd angle	48.31 ± 1.65	43.06 ± 1.37	< 0.05
	dat angle	58.04 ± 0.83	58.87 ± 0.88	> 0.05
	adt angle	83.34 ± 1.15	82.21 ± 1.65	> 0.05
	atb angle	16.28 ± 1.12	15.72 ± 1.45	> 0.05
	btc angle	11.86 ± 0.85	11.27 ± 0.97	> 0.05
	ctd angle	14.18 ± 0.83	14.89 ± 1.01	> 0.05
	a-b number	32.93 ± 0.72	36.45 ± 0.88	< 0.05
	b-c number	25.88 ± 0.69	25.59 ± 0.95	> 0.05
	c-d number	34.78 ± 1.73	33.34 ± 1.28	> 0.05

Discussion

It is known that skin and brain are forming from the same ectoderm, and therefore dermatoglyphic markers could give us specific information about early brain development disorder in autistic patients. Finger dermatoglyphics and the volar side of the hand are formed at the end of the first and within the second trimester of fetal development, so it seems that during that period of time, brain disorder development can occur [14]. Namely, it is a critical period in etiology of autism and other neurodevelopment disorders. In addition to this claim, a research of Courchesne [15], on autistic patients identified agenesis of the superior olive, dysgenesis of the facial nucleus, reduced numbers of Purkinje neurons, hypoplasia of the brainstem and posterior cerebellum, and increased neuron-packing density of the medial, cortical and central nuclei of the amygdala and the medial septum. As neurogenesis occurs for these different neuron types during approximately the fifth week of gestation, the possibility is raised that this may be a 'window of vulnerability' for autism; the likely etiologic heterogeneity of autism suggests that other windows of vulnerability are also possible.

By comparing qualitative and quantitative analysis of digital DPC area it was possible to determine that autistic children had higher arch count on the fourth and fifth fingers of both hands, which is in accordance with Tarke and Barabolski [16]. A higher distribution of arches on the fourth and fifth fingers of both hands as a consequence had lower FRC on these fingers, hence lower TRC, because dermal ridges with this type of dermal patterns do not count as they do not have a Core point and delta. In his research Walker [17] got similar results. He determined that autistic population has lower dermal ridge count, not only on the fourth and fifth fingers of both hands, but for all dermal ridge counts including the palmar DPC area. Quantitative analysis of palmar DPC area showed that autistic boys had a lower a-b RC

as well as a wider atd angle on both hands, and Bujas-Petkovic got these same results [18].

The more complex researches on this area, confirming the findings of our work, dealt with the relation between dermatoglyphs and family anamnesis. That research confirmed that autistic individuals were significantly different from healthy control group, in RC on fourth and fifth fingers, in a-b RC and also in atd angles of both hands. Healthy fathers of autistic patients had different atd angles, brothers of autistic patients were different in palms variations compared with healthy control group examinees. Mothers of autistic patients as compared with healthy control group examinees, were significantly different in RC on the first, fourth and fifth fingers, in a-b and c-d RC on palms and in atd angles of both hands [5].

In addition to this research we certainly have to add the results that were obtained by Arrieta et al. [19], which also confirmed that autistic children have a lower TRC and a wider atd angle, so, it is concluded that the obtained results do not contradict the hypothesis that genetic factors might be significant in etiology of unknown origin autism.

Of course, there are researchers who completely negate the value of dermatoglyphic analysis in diagnosing autism [20], as well as researchers who show a difference in dermatoglyphic findings between autistic and healthy population, but that difference is not enough for dermatoglyphic analysis to be considered as efficient analysis [21].

Conclusion

The results of our study show that the autistic boys as compared with the healthy examinees, had higher arch count on the fourth and fifth fingers of hands, lower TRC and a-b RC as well as wider atd angle. Thus, we consider dermatoglyphic analysis helpful in diagnosing autism, but only as an additional method and never as a dominant diagnostic procedure.

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Anxiolytic and antidepressant effect of zinc on rats and its impact on general behavioural parameters

Anksiolitički i antidepresivni efekat cinka na pacove i njegov uticaj na opšte bihevioralne parametre

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Abstract

Background/Aim. Zinc is an essential element which has considerable interaction with gamma-aminobutyric acid A type receptors ($GABA_A$) and glutamate receptors in the central nervous system (CNS). It is believed that zinc acts as a potent inhibitor of glutamate N-methyl-D-aspartate (NMDA) receptors, and binding to structurally specific site on the $GABA_A$ receptor leads to inhibition of GABA-dependent Cl^- pass. The aim of our research was to test the anxiolytic and antidepressant effects of zinc after single application and its influence on general behavioural parameters after repeated administration. **Methods.** Male Wistar rats were treated with increasing doses of zinc histidine dehydrate (10, 20, 30 mg/kg, i.p.). To determine anxiolytic and antidepressant properties of zinc two models were used: elevated plus maze (EPM) and forced swim test (FST). Behavioural parameters (stillness and mobility) were, also, recorded after single and repeated administration of active substance. **Results.** Testing animals in the EPM showed a statistically significant difference as follows: dose of 20 mg/kg significantly increased the time animals spent in open arms, indicating an acute anxiolytic effect, while doses of 30 mg/kg significantly reduced the time in the open arms, indicating a potentially anxiogenic effect. Testing the animals by FST showed a statistically significant difference in immobility time of animals treated with the lowest applied (10 mg/kg) and highest applied (30 mg/kg) doses of zinc, compared to the control group. The first day of testing behavioral parameters showed the tendency to increase locomotor activity of the animals with the lowest dose of zinc (10 mg/kg), while the following day revealed a reduced activity with the highest dose applied (30 mg/kg). **Conclusion.** Zinc has important effects on the CNS: After single application, in all doses zinc showed antidepressant effects. The effects of zinc on anxiety and locomotor activity showed dose-dependent bidirectional effects.

Key words:
zinc; rats; anti-anxiety agents; antidepressive agents, second generation.

Apstrakt

Uvod/Cilj. Cink je esencijalni element, koji u centralnom nervnom sistemu (CNS) ostvaruje značajnu interakciju sa tipom A receptora za gama aminobuternu kiselinu ($GABA_A$) i glutamatskim receptorima. Smatra se da cink deluje kao snažan inhibitor glutamatskih N-metil-D-aspartat (NMDA) receptora, a vezivanjem za strukturno specifično mesto na $GABA_A$ receptoru dovodi do inhibicije GABA-zavisne Cl^- struje. Cilj našeg istraživanja bio je da ispitamo anksiolitičke i antidepresivne efekte cinka posle jednokratne primene i njegov uticaj na opšte bihevioralne parametre posle ponavljanog davanja. **Metode.** Mužjaci pacova soja Wistar tretirani su rastućim dozama cink-histidin dehidrata (10, 20, 30 mg/kg, *ip*). Za ispitivanje anksiolitičkih i antidepresivnih svojstava cinka korišćena su dva testa: uzdignuti plus lavirint (EPM) i test forsiranog plivanja (FST). Praćeni su, takođe, bihevioralni parametri (mirovanje i aktivnost životinje) tokom jednokratne i ponavljane primene aktivne supstance. **Rezultati.** Testiranjem životinja primenom EPM utvrđena je statistički značajna razlika: životinje koje su primile dozu od 20 mg/kg provodile su statistički značajno više vremena u otvorenim kracima, što ukazuje na akutni anksiolitički efekt, dok je doza od 30 mg/kg

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značajno skraćivala vreme koje životinje provode u otvorenom prostoru lavirinta. Ovo ukazuje na potencijalno anksiogene efekte cinka. Testiranjem životinja primenom FST dokazana je statistički značajna razlika u vremenu imobilnosti životinja tretiranih najmanjom primenjenom dozom (10 mg/kg) i najvećom primenjenom dozom cinka (30 mg/kg), u odnosu na kontrolnu grupu. Prvog dana ispitivanja biheviоralnih parametara pokazana je tendencija povećanja lokomotorne aktivnosti životinja sa najmanjom primenjenom dozom cinka (10 mg/kg), dok je narednog dana uočena tendencija snižavanja aktivnosti sa najvećom primenjenom dozom (30 mg/kg). **Zaključak.** Cink ispoljava značajne efekte na CNS. Jednokratna primena, cinka, u svim dozama pokazuje antidepresivne efekte. Efekti cinka na anksioznost i lokomotornu aktivnost pokazuju doznozavisne dvosmerne efekte.

Ključne reči:
cink; pacovi; anksiolitici; antidepresivi druge generacije.

Introduction

Zinc is an essential element, important for the function of over 200 enzymes. The role of zinc in humans is catalytic, structural and cofactorial, and is required for DNA replication, transcription and protein synthesis [1, 2]. In the central nervous system (CNS), the presence of zinc has been confirmed in the neocortex, amygdala and hippocampal structures. In Zn-containing neurons, zinc is stored in presynaptic vesicles and the vesicles are then released according to the depolarisation and the presence of calcium [3]. Zinc has considerable interaction with gamma-aminobutyric acid A type receptors ($GABA_A$) and glutamate receptors, as well as with voltage-dependent sodium, potassium and calcium channels [4].

The mechanism of zinc action on the CNS, to date, has not been fully determined [5]. Zinc, in the CNS, binds to glutamate N-methyl-D-aspartate (NMDA) receptors and acts as a potent modulator of glutamate neurotransmission [3, 6]. It is known that zinc binds to a structurally specific binding site on the $GABA_A$ receptor, and may lead to inhibition of GABA-dependent Cl^- ions passage [7]. It is shown that the sensitivity of different types of $GABA_A$ receptors to the effects of zinc is different and that depends on the structural subunits of $GABA_A$ receptor complex [7, 8].

Numerous studies suggest the important role of this essential element in pathogenesis of neuropsychiatric disorders, such as epilepsy [9, 10], mood disorders [5, 11, 12] and neurodegenerative diseases [13, 14]. In preclinical models, which are used for the evaluation of antidepressant activity, zinc shows effects similar to antidepressants [5, 15, 16]. It has been found that chronic use of antidepressant drugs, such as citalopram or imipramine and electroconvulsive therapy, increases the concentration of zinc in the hippocampus of rats [5, 17]. It has also been shown that chronic use of citalopram increases the concentration of zinc in serum, while imipramine and electroconvulsive therapy have not shown such an effect [17].

Clinical data show particularly low levels of zinc in the serum of patients with mood disorders, in whom there is normalisation of serum zinc levels after successful treatment with antidepressants [11, 12]. There are also some preliminary data suggesting that zinc supplementation may enhance antidepressant therapy in patients with unipolar depression [5]. Zinc supplementation significantly reduced the scores in both, Hamilton Depression Rating Scale (HDRS) and Beck Depression Inventory (BDI), measured after 6- and 12-week supplementation when compared with placebo treatment; these findings are the first demonstration of the benefits of zinc-supplementation in antidepressant therapy [12].

In general, there is much more data and studies on the effects of zinc on the immune system and peripheral tissues in the literature [18], while the central and behavioural effects are less well understood [19]. The aim of our study was to examine the behavioural effects of zinc and the effect of single and repeated application of zinc on the behavioural parameters (stillness and locomotor activity of animals, in terms of sniffing, rearing, grooming and locomotion), and antidepressant and anxiolytic effects of zinc in animals. We used zinc histidine dehydrate, as an experimental substance, for which the data in the literature suggest the optimal kinetics in terms of biological activity and bioavailability of the substance [20].

Methods

The study included male Wistar albino rats with the body mass of 180–250 g. The animals were kept in clear plastic cages and had ad libitum access to food and water. The room temperature was 22 ± 1ºC, with a relative humidity of between 40 to 70% and a 12-hour daily cycle of light and dark, with the lit beginning at 6.00 am. The experiment respected the Ethical Committee codex for work with the experimental animals of the Faculty of Medicine, University of Belgrade. The experiment was performed during the dark period of the daily cycle.

The research included altogether 84 animals, randomly divided into 3 groups of 28, and then within each group another 4 subgroups were made. The effects of zinc were followed using the active ingredients of zinc histidine dehydrate [Zn $(His)_2$]. The first subgroups of each group received the solvent (distilled water), and the three others a solution of Zn $(His)_2$ in increasing doses (10 mg/kg, 20 mg/kg, 30 mg/kg). The substances were administered intraperitoneally (i.p) in the lower right quadrant of the abdomen.

Elevated plus maze

Elevated plus maze (EPM) represents the most widely used animal model for examining anxiety [21]. The maze was elevated to the height of 1 m and consisted of 4 arms (dimensions: 50 × 10 cm). Two opposite arms were closed, and the other two open. There was a central platform (5 × 5 cm) on which the experimental animals were initially placed. The system was monitored by a digital camera, placed above the maze. Recording animal activity and processing data after the

test was conducted by a computer software Any-maze Video Tracking System – Stoelting Co., Wood Dale, IL, USA.

The basis of testing was to induce the conflict situation in experimental animals. The rats, namely, prefer dark and in closed spaces they are the safest. On the other hand, their inquisitive nature forces them to explore, so the open arms of the maze are placed in front of them, which are at the same time potentially dangerous places. It has been shown that the substances with anxiolytic action increase the number of entries into the open arms of the maze, and also prolong the time an animal spends in the open.

Testing was conducted within the first group consisting of 28 animals, 30 min after the application of the substance on each animal in the 4 subgroups. The rats were let into the maze and their spontaneous activity was monitored for 5 min.

Forced swim test

The forced swim test (FST) using the method of Porsolt et al. [22], represents the standard screening test for the evaluation of the antidepressant effects of substances. A FST (hand made) consists of a glass cylinder, 45 cm high, 20 cm in diameter. It is filled with water up to the height of 20 cm, at 21–23°C. Testing lasts for 15 min upon placing the animals into the cylinder. The first 5 min mark the habituation of the animals in the water environment. During the next 10 min the immobility time is measured. That is the time the rats spend floating in the water, so that at least 3 out of their 4 paws keep still. This condition is considered a reaction of despair and depressiveness. The substances with antidepressant potential prolong the time an animal spends in a struggle to find a way out of the cylinder, and reduce the time of immobility in relation to the control group.

The second group of 28 animals was also randomly divided into 4 subgroups of 7 animals each. Within this group the substances (the solvent and zinc histidine dehydrate in the doses of 10, 20, 30 mg/kg) were applied 30 min before placing rats in the cylinder filled with water. The animals were monitored for the next 15 min.

General behavioural parameters

Behavioural parameters were monitored after a repeated application of zinc over 4 days within the third group of 28 animals, randomly divided into 4 subgroups, each receing a competent substance (the solvent and zinc histidine dehydrate in doses of 10, 20, 30 mg/kg). Two hours after each application the behavioural parameters of each animal were individually measured over 5 min. The important parameters were stillness and the mobility of the animals, in the sense of rearing, sniffing, grooming and locomotion.

Statistical data processing

For statistical data processing we used the computer program SPSS 17.0, descriptive statistical method, *t*-test, rank sum test (Mann-Whitney), ANOVA with repeated measurements and the competent software (ANY-maze Video Tracking System – Stoelting Co., Wood Dale, IL, USA). All the numerical data presented in the figures were given as the mean ± SEM.

Results

In the forced swim test, the average immobility times of animals, in seconds, for the solvent, Zn (10 mg/kg), Zn (20 mg/kg) and Zn (30 mg/kg) were 147.5, 40.5, 98.0 and 36.5 respectively. It indicates that there is a statistically significant difference between the group with the solvent and the groups with 10 mg/kg and 30 mg/kg of the experimental substances ($p < 0.05$). There was no statistically significant difference for the group with 20 mg/kg of zinc, compared with the control group treated with the solvent (Figure 1).

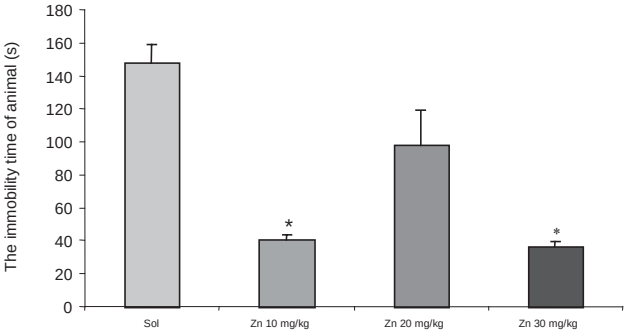

Fig. 1 – The immobility time of animals (mean ± SEM) in the forced swim test (FST), after the application of the solvent (Sol) and all the three doses of zinc

***$p < 0.05$ *vs.* Sol – statistically significant difference**

In the elevated plus maze, the number of entries into the open arms of the maze was not significantly different between the groups, while there was a statistically significant difference between the groups in the time spent in the open arms of the maze ($p < 0.05$). The animals receiving 20 mg/kg zinc spent significantly more time in the open arms of the maze, indicating an acute anxiolytic effect, while the zinc dose of 30 mg/kg significantly reduced the time the animals spent in the open arms of the maze, indicating a potentially anxiogenic effect (Figure 2).

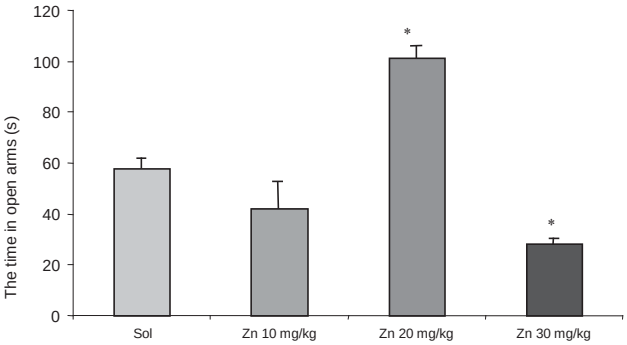

Fig. 2 – The time (mean ± SEM) that animals spent in open arms of the elevated plus maze (EPM), after the application of the solvent (Sol) and all the three doses of zinc

***$p < 0.05$ *vs.* Sol – statistically significant difference**

Analysis of the data obtained during the investigation of behavioural parameters during the 4-day experiment determined that on the first day there was a statistically significant

difference between the groups that received the solvent and the experimental substance at a dose of 10 mg/kg in terms of increased locomotor activity, $p < 0.05$; the groups that received 20 mg/kg and 30 mg/kg of experimental substances did not show a statistically significant difference as compared with the control group. However, on the second day of the experiment, there was a tendency to reduce spontaneous locomotor activity among those animals that received zinc at the dose of 30 mg/kg ($p = 0.057$). The third and fourth day of testing showed no significant differences in any tested group compared with the control group which received the solvent.

Discussion

The study animals were tested by FST 30 min after applying the appropriate substance. A statistically significant reduction in immobile time was found among the animals receiving zinc as compared with the group treated with the solvent, thus confirming the antidepressant properties of zinc. Antidepressant effects are especially significant when applying the lowest and highest doses of zinc (10 and 30 mg/kg). These antidepressant effects of zinc are consistent with the results of several previous studies [5, 23–25]. The literature gives different information about the potential mechanisms of action by which Zn exerts antidepressant effects. Antidepressant activity is mainly associated with the inhibition of glutamate NMDA and alpha-amino-3-hydroxy-5-methyl-isoxasoleprapionic acid (AMPA) receptors and an increase in brain-derived neurotrophic factor (BDNF) gene expression in the hippocampus [5, 23, 24]. Some studies suggest an interaction between the serotonergic system and Zn. Zn acts as a selective inhibitor of serotonin reuptake and enhances the pharmacological effects of standard antidepressants [26, 27].

Besides the zinc influence on the process of glutamate neurotransmission, there are more complex theories about the influence of zinc on GABA-ergic neurotransmission. Specifically, certain subtypes of $GABA_A$ receptors have specific binding sites for zinc and most studies suggest a possible inhibitory effect of zinc on GABA-ergic neurotransmission [7]. However, the data from molecular studies show that zinc has bidirectional modulatory effects on specific GABA receptors, which are mostly represented in the hippocampus. In this way, zinc is probably included in the process of GABA-ergic neuron plasticity, depending on the neurons' sensitivity to the zinc effect and also depending on the influence of glutamate neurotransmission [28]. Our study showed a dose-dependent bidirectional effect of zinc in experimental model of anxiety (EPM). The animals receiving 20 mg/kg of zinc spent significantly more time in the open arms of the maze, indicating an acute anxiolytic effect, while the doses of 30 mg/kg zinc significantly reduced the time animals spent in open space, indicating a potential anxiogenic effect.

During the 4-day tracking of the behavioural characteristics of the animals, we followed the parameters of locomotor activity among the rodents (rearing, sniffing, grooming and locomotion). Our results indicate that acute application of zinc on the first day of the test, at the dose of 10 mg/kg, significantly increased locomotor activity of the animals. The zinc dose of 20 mg/kg and 30 mg/kg acutely applied did not significantly affect the locomotor activity of the animals. However, the second day of the experiment showed a reduced spontaneous locomotor activity of animals receiving zinc at the dose 30 mg/kg, while zinc 10 mg/kg and 20 mg/kg had no significant effect on locomotor activity. These dose-dependent bidirectional effects were previously described with Zn effects on memory formation in animals [29], where lower doses of zinc show some promnesic effects, while higher doses inhibit the formation of memory. On the third and fourth day of testing there were no significant differences in any tested groups compared with the control group. The lack of influence of the third and fourth day can be explained, on the one hand, by the development of some form of tolerance to the substance, while on the other hand it is possible that after repeated applications some adaptive mechanisms start to be active. According to previous studies that negate the formation of tolerance to certain effects of zinc [16], it is most likely that repeated application of zinc increases zinc excretion through the kidneys, and its effect on locomotor activity is missing.

Conclusion

All the results of the study suggest that zinc exerts significant effects on the central nervous system. After single application, any doses of zinc showed antidepressant effects. Zinc effects on anxiety and locomotor activity showed dose-dependent bidirectional modulatory effects. The lowest applied dose (10 mg/kg) acutely increased locomotor activity, without effect on anxiety. Zinc 20 mg/kg did not significantly affect locomotor activity, but showed the anxiolytic effects. The largest applied zinc dose (30 mg/kg) showed quite a different effect, by reducing locomotor activity and showing anxiogenic potential. Thus, it can be concluded that zinc, as a fine modulator of glutamate and GABA neurotransmission, regulates specific mental functions, especially anxiety-depressive manifestations.

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Effect of pretreatment with omega-3 polyunsaturated fatty acids (PUFAs) on hematological parameters and platelets aggregation in patients during elective coronary artery bypass grafting

Efekat omega-3 polinezasićenih masnih kiselina na hematološke parametre i agregaciju trombocita kod elektivne revaskularizacije srca

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Abstract

Bacground/Aim. Using omega-3 polyunsaturated fatty acids (PUFAs) in coronary artery bypass graft surgery (CABG) could provide protection against ischemic-reperfusion damage, prevention of postoperative arrhythmia and attenuation of inflammatory response. However, omega-3 PUFAs inhibit cyclooxygenase (and thus decrease the synthesis of thromboxane A2 from arachidonic acid in platelets), which leads to decreased platelet aggregation. In cardiac surgery it is necessary to achieve a balance between inhibition and full platelets function. It is as well as important to closely follow hematological parameters, impaired by CABG itself. Therefore, the aim of the study was to establish the effects of pretreatment with omega-PUFAs on hematological parameters and plateletes aggregation in patients with elective CABG. **Methods.** This prospective, randomized, placebo-controlled, single-center trial was performed on parallel groups. The patients (n = 40) undergoing elective CABG were randomized receiving preoperative intravenous omega-3 PUFAs (Omegaven® 10%) infusion (the PUFAs group) or the same volume of 0.9% saline solution infusion (the control group). Infusion was given a day before surgery and repeated four hours before starting extracorporeal circulation (CPB) *via* the peripheral vein at single doses of 100 mL (25 mL/h). Platelet function analysis was performed using multiple electrode aggregometry (MEA, multiplate-analyzer) before starting CPB and 2 h postoperatively for the patients of both groups. **Results.** There were no clinically relevant differences in baseline characteristics between the groups. Hematological parameters were not significantly different between the groups pre-, intra- and postoperatively. During the first 24 h after surgery, the loss of blood was similar in the PUFAs and the control group (680 ± 274 mL and 608 ± 210 mL, respectively; $p = 0.356$). Postoperatively, platelet aggregation was not significantly different between the PUFAs and the control group in adenosine diphosphate (ADP) test (39 ± 11 and 42 ± 15, respectively; $p = 0.701$), arachidonic acid (ASPI) test (64 ± 24 and 70 ± 27, respectively; $p = 0.525$) and trombin receptor-activating peptide (TRAP) test (68 ± 25 and 75 ± 26, respectively; $p = 0.396$), while their aggregation in collagen (COL) test was statistically significantly lower in the PUFAs related to the control group (32 ± 15 and 47 ± 20, respectively; $p = 0.009$). **Conclusion.** Acute pretreatment with omega-3 PUFAs insignificantly affected the activity of platelets and did not influence postoperative blood loss.

Key words:
fatty acids, omega-3; coronary artery bypass; platelet agregation; hematologic tests; hemorrhage

Apstrakt

Uvod/Cilj. Primenom omega-3 polinezasićenih masnih kiselina (PUFAs – *polyunsaturated fattly acids*) kod kardiohirurških operacija može se postići zaštita od ishemijsko-reperfuzionih oštećenja, prevencija postoperativnih aritmija i smanjenje inflamatornog odgovora. Međutim, omega-3 PUFAs inhibiraju ciklooksigenazu (ovo smanjuje sintezu tromboksana A2 iz arahidonske kiseline u trombocitima), što smanjuje agregaciju trombocita. Kod kardiohirurških operacija neophodno je postići ravnotežu između inhibicije i pune funkcije trombocita. Takođe, važno je pratiti hemato-

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loške parametre koji su poremećeni samom hirurškom intervencijom. Cilj ove studije bio je da se utvrde efekte preoperativne infuzije omega-3 PUFAs na hematološke parametre i agregaciju trombocita kod bolesnika koji su podvrgnuti elektivnoj revaskularizaciji miokarda. **Metode.** Ova prospektivna, randomizovana, placebom kontrolisana studija vršena je na paralelnim grupama. Bolesnici (n = 40) planirani za elektivni hirurški zahvat revaskularizacije miokarda primali su infuziju omega-3 PUFAs (Omegaven® 10%) ili istu količinu 0,9% NaCl, po 100 mL (25 mL/h)/dan pre hirurške intervencije i četiri sata pre početka vantelesnog krvotoka. Analiza funkcije trombocita u obe grupe vršena je metodom *multiple electrode aggregometry* (MEA) pre početka vantelesnog krvotoka i dva sata nakon završetka intervencije. **Rezultati.** Nije bilo značajne razlike u vrednostima hematoloških parametara između grupa, pre-, intra- i postoperativno. Tokom 24 sata postoperativno, gubitak krvi bio je sličan u grupi koja je primala omega-3 PUFA i kontrolnoj grupi koja je primala placebo (680 ± 274 mL i 608 ± 210 mL, respektivno; p = 0,356). Postoperativo, nije postojala statistički značajna razlika u agregaciji trombocita između grupe koja je primala omega-3 PUFA i u kontrolnoj grupi koja je primala placebo u adenozin difosfat (ADP) testu (39 ± 11 i 42 ± 15, respektivno; p = 0,701), ASPI testu (64 ± 24 i 70 ± 27; respektivno; p = 0,525) i trombin receptor-aktivirajući peptid (TRAP) testu (68 ± 25 i 75 ± 26, respektivno; p = 0,396). Agregacija u kolagen (COL) testu bila je statistički značajno manja u grupi koja je primala omega-3 PUFA u odnosu na kontrolnu grupu (32 ± 14 i 47 ± 20, respektivno; p = 0,009). **Zaključak.** Preoperativna primena omega-3 PUFAs jednako utiče na agregaciju trombocita, kao i placebo u kontrolnoj grupi, osim kod COL testa čije su vrednosti statistički značajno niže u grupi tretiranoj omega-3 PUFAs u odnosu na kontrolnu grupu, ali to ne utiče na postoperativne gubitke krvi.

Ključne reči:
masne kiseline, omega-3; aortokoronarno premošćavanje; trombociti, agregacija; hematološki testovi; krvarenje.

Introduction

Bleeding is a common complication of cardiac surgery with cardiopulmonary bypass (CPB), which can require transfusion of blood products [1–3] and in 3% ± 6% of cases mediastinal re-exploration [4]. Among causes of excessive bleeding, platelet dysfunction is considered to be the most important in the early postoperative period. During coronary artery bypass graft (CABG) surgery there is the opposition between the benefit of platelet inhibition to reduce the risk of pre-operative infarction and postoperative occlusion of anastomosed coronary arteries, and the need to maintain full platelet function for optimal hemostasis in surgical incisions.

Previous studies have demonstrated beneficial effects of omega-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in platelet functions [5]. Whereas the predominant product of arachidonic acid (AA) in platelets, thromboxane A_2 (TXA_2) is a platelet agonist, the corresponding product of EPA, thromboxane A_3 (TXA_3), is virtually biologically inert. In contrast, both prostaglandin I_2 derived from AA, and prostaglandin I_3 derived from EPA, are potent vasodilators and platelet inhibitors [6]. Supplementation of omega-3 PUFAs resulted in a shift towards the production of more favorable eicosanoids that inhibit platelet aggregability [7].

Omega-3 PUFAs are commonly considered to have antithrombotic effects, based on increased bleeding times at very high doses (e.g. 15 g/day) [8]. Conversely, in human trials, omega-3 PUFA consumption has no consistent effects on platelet aggregation or coagulation factors [9, 10]. No excess clinical bleeding risk has been seen in randomized clinical trials of fish or fish oil consumption, including people undergoing surgery or percutaneous intervention and/or also taking aspirin or warfarin [8, 11].

We have recently shown that preoperative administration of omega-3 PUFAs has cardioprotective effect in patients with CPB manifested in increased oxygen extraction and lactate uptake with simultaneous decrease of serum troponin I and creatinin kinase myocardial band (MB) levels [12]. In the frame of the same study, we followed the effect of omega-3 PUFAs on their influence on postsurgical patients platelet aggregation. According to the previous findings of the other authors [11, 13] that omega-3 PUFAs do not affect bleeding in the same or similar categories of patients we assumed that they will not provoke bleeding surpassing the risk expected in the control group of patients.

The aim of the study was to establish the relationship between hematological parameters and the activity of plateletes in patients with elective CABG pretreated by 3-omega PUFAs.

Methods

This prospective, randomized, placebo-controlled study was performed on parallel groups. Study enrollment occurred between August 2010 and September 2011. The study protocol was approved by the Ethical Committee of Military Medical Academy, and all the patients gave written informed consent.

Forty patients scheduled to undergo their first on-pump CABG surgery were included in the study. For recruitment, patients needed to be older than 18 years of age, in normal sinus rhythm, and in stable hemodynamic conditions before surgery. Patients were excluded in cases of emergency CABG, redo CABG, combined CABG and any other cardiac procedure, Q-wave myocardial infarction in the last six weeks, unstable angina, or poor left ventricular function, those with abnormal coagulation tests, including a history of coagulopathy and preoperative treatment with other anticoagulants. All the participants denied the intake of any antiagregation medication during the previous five days. All the patients were treated by the same surgical and anesthesiologist team.

Eligible patients were assigned to one of the two study arms according to a computer-generated randomization list: the control (placebo) group (usual care), and the usual care plus PUFAs group.

The PUFAs infusion consisted of 100 mL of a lipid emulsion with a high content of omega-3 PUFAs (Omegaven® 10%, Fresenius Kabi, Bad Homburg, Germany). The same batch of Omegaven® was used throughout the study, and 100 mL of the lipid emulsion contained 1.25–2.82 g of EPA and 1.44–3.09 of DHA. Infusion was given a day before surgery and repeated 4 h before starting CPB *via* the peripheral vein at single doses of 100 mL (25 mL/h). The patients in the control group received an equal volume of 0.9% saline.

Preoperative sedation with 5 mg of intramuscular midazolam was administered to patients on call to the operating room. All the patients received prophylactic preoperative antibiotics (cefazolin 2 g preincision, and 2 g post-CPB; or if allergic to penicillin, vancomycin 1 g preincision and 500 mg post-CPB). The same anesthesiologist administered standardized total intravenous anesthesia using sufentanil, midazolam, propofol and pancuronium.

Immediately before CPB, 300 IU/kg heparin was administered intravenously, followed by additional doses as necessary to maintain an activating clotting time exceeding 500 sec. Protamine was administered as 1 mg /100 IU of the heparin dose after complete separation from CPB.

All the patients had CABG with the use of CPB, which was conducted with a roller pump and a membrane oxygenator primed with a solution. During CPB, pump flow was set at 2.4 times the body surface area, and mean arterial pressure maintained between 50 mmHg and 60 mmHg. The temperature was allowed to drift with active rewarming at the end of CPB. Myocardial protection was afforded with cold potassium cardioplegia. A single-clamp technique was used, and cardioplegia was given in an anterograde fashion. In all the patients, the left internal mammary artery harvested and anastomosed to the left anterior descending artery. The rest of the grafts were constructed using the great saphenous vein.

After a total release of the aortic cross-clamp, epicardial atrial or ventricular pacing wires were placed. Aortic and venous cannulas were removed after an appropriate test dose of protamine, and the surgery proceeded with closure of the pericardium and sternum.

After the surgery, the patients were followed up in the Intensive Care Unit (ICU) and were weaned off mechanical ventilation when they fulfilled the following criteria: hemodynamic stability, peripheral temperature of more than 36 °C, cooperatively, and no major bleeding.

Blood for hemoglobin (Hb) concentration, hematocrit (Hct), platelet count and coagulation profile determination including international normalised ratio (INR) and activated partial thromboplastin time (aPTT), was taken from a radial arterial catheter before start CPB and 2 h after arrival in the ICU for all the patients in the two groups. Transfusion of blood products and management of postoperative bleeding intra- and postoperatively were determined by following institutional algorithm. Platelet function analysis was performed using the multiple electrode aggregometry (multiplate-analyzer) before started CPB and 2 h after arrival in the ICU for all the patients of both groups. The method has been described in detail elsewhere [14]. Platelet aggregation was initiated using arachidonic acid (ASPI test), adenosine diphosphate (ADP test), thrombin receptor–activating peptide (TRAP test) and collagen (COL test) using commercially available test reagents. Increased impedance caused by attachment of platelets to the test cell electrodes was continuously measured over 6 min. Platelet aggregation was quantified as the area under the aggregation curve [AUC(U)]. Reference ranges for healthy subjects obtained from the manufacturer were 79–141 U for the ASPI test, 41–99 U for the ADP test, 92–151 U for the TRAP test, and 61–108 U for COL test.

The results were presented as mean values with standard deviation. The significance of differences betwen the study groups was analyzed using the *t*-test. Due to great variability of some data, the Wilcoxon matched pairs test and the Mann-Whitney *U*-test were also used. Comparison between more than two groups was done by using the Kruskal-Wallis test.

A *p*-value less than 0.05 was taken to be significant. The obtained data were processed through the Stat for Windows, R.4.5. Software package.

Results

The results of the study are presented in Tables 1 to 3 dealing with baseline and operative characteristics of the patients (Table 1), the effect of CPB procedure on hematologi-

Table 1

Baseline and operative characteristics of the patients in the control and PUFAs group

Parameter	Control group	PUFAs group	*p*
Age (years)	62.4 ± 7	65.3 ± 8	0.56
Gender (m/f)	18/2	17/3	0.36
Weight (kg)	89.8 ± 6	92.1 ± 5	0.48
Height (cm)	176.4 ± 4	178.5 ± 3	0.06
LVEF (%)	54 ± 6	53 ± 9	0.1
CPB (min)	101.4 ± 21	95.5 ± 17	0.29
Aortic cross-clamp time (min)	42.5 ± 9	38.9 ± 8	0.66
CABG (number)	2.9 ± 0.8	2.8 ± 0.7	0.65
Total heparin dose (units × 1,000)	27.7 ± 1.5	27.6 ± 2.2	0.86
Total protamin dose (mg)	279 ± 13	282 ± 19	0.638

Data presented as mean value ± standard deviation. PUFA – polyunsaturated fatty acids; LVEF – left ventricular ejection fraction; CPB – cardiopulmonary bypass; CABG – coronary artery bypass grafting.

cal parameters (Table 2), the effect of CPB procedure on the activity of platelets in the control and the PUFAs group of patients (Table 3), and peri- and postoprative complications.

and postoperative requirements for allogenic RBCs, FFP and platelet units were similar in both groups of patients, with no statistically significant difference.

Table 2

Hematological data in the patients subjected to CPB

Parameter	Control group	PUFAs group	*p*
Prior to operation			
hemoglobin (g/L)	134 ± 4.2	134 ± 3.2	0.535
hematocrit (%)	38 ± 2.7	37 ± 2.4	0.628
platelets (x10^9/L)	255 ± 42	259 ± 57	0.823
INR	1.06 ± 0.03	1.08 ± 0.03	0.139
aPTT (sec)	38.8 ± 3.9	39.6 ± 3.3	0.493
On arrival to ICU			
hemoglobin (g/L)	111 ± 8.3	109 ± 8.5	0.542
hematocrit (%)	29 ± 6.9	29.4 ± 2.3	0.832
platelets (× 10^9/L)	132 ± 34	129 ± 43	0.796
INR	1.17 ± 0.07	1.18 ± 0.14	0.703
aPTT (sec)	44.2 ± 4.5	45.8 ± 5.9	0.345
Transfusion requrements			
Intraoperative			
allogenic RBCs (units)	1.3 ± 0.7	1.4 ± 0.8	0.738
FFP (units)	0.5 ± 0.8	0.4 ± 0.8	0.946
platelets (units)	0	0	
Postoperative (0–24 h)			
allogenic RBC (units)	1.9 ± 0.7	1.9 ± 0.8	0.946
FFP (units)	1.7 ± 1	1.9 ± 0.9	0.529
platelets (units)	1.2 ± 1.3	1.35 ± 1.35	0.738
Postoperative blood loss 0–24 h (mL)	608 ± 210	680 ± 274	0.356

Data presented as mean value ± standard deviation. CPB – cardiopulmonary bypass; PUFA - polyunsaturated fatty acids; INR-international normalization ratio; aPTT – activated partial thromboplasin time; ICU – intensive care unit; RBC – red blood cells; FFP – fresh frozen plasma.

Table 3

The influence of polyunsaturated fatty acids (PUFAs) on the platelet aggregation in multiple electrode aggregometry

Parameter	Area under curve (U), $\bar{x}$ ± SD		*p*
	control group	PUFAs group	
ADP test (41–99 U)*			
preoperative	61.4 ± 20	57.8 ± 20	0.587
postoperative	42.3 ± 15	39.4 ± 11	0.701
ASPI test (79–141 U)*			
preoperative	92.6 ± 23	90.2 ± 20	0.719
postoperative	70.1 ± 27	64.9 ± 24	0.525
TRAP test (92–151 U)*			
preoperative	96.8 ± 23	95.4 ± 23	0.845
postoperative	75.1 ± 26	68.1 ± 25	0.396
COL test (61–108)*			
preoperative	68.2 ± 17	64.4 ± 16	0.465
postoperative	47.7 ± 20	32.3 ± 15	0.009

Data presented as mean value ± standard deviation. ADP – adenosine diphosphate; ASPI – arachidonic acid-induced platelet aggregation; TRAP – thrombin receptor activating peptide; COL – collagen; *reference ranges for healthy subjects.

Table 1 shows that the baseline and operative characteristics of the patients included in the study did not differ between the control and the PUFAs group in any of the observed parameters. This equally relates intraoperative CPB (101.4 min *vs* 95.5 min; $p = 0.29$), aortic cross-clamp time (42.5 min *vs* 38.9 min; $p = 0.66$), CABG number (2.9 min *vs* 2.8; $p = 0.65$) and total heparin use (27.7 min *vs* 27.6 units × 1,000; $p = 0.86$), and postoperative interventions: total protamine dose (279 mg *vs* 282 mg; $p = 0.63$).

Table 2 shows that hematological data regarding preoperative and on the arrival to ICU, as well as intraoperative

Regarding transfusion requirements and postoperative blood loss, there were no statsitically significant differences between the control and the PUFAs group in allogenic red blood cells (RBCs) (1.9 *vs* 1.9; $p = 0.94$), fresh frozen plasma (FFP) (1.9 *vs* 1.7; $p = 0.52$) and platelete (1.2 *vs* 1.35; $p = 0.73$) units, as well as in postoperative blood loss (608 ± 210 mL *vs* 680 ± 274 mL, $p = 0.356$).

Table 3 shows that the level of platelet aggregation reached the reference values in both groups of patients and in all four performed tests, indicating thus their normal values. In all instances, the observed values were above, but closer

to the lower levels of aggregators reference potencies given in the brackets.

The second part of the results concerns the intergroup differences in the platelet aggregation pre-, and postoperatively. In that respect, almost all the tests showed equal activity of plateletes before the surgical intervention in the PUFAs group in relation to the control group of patients. Postoperatively, platelet aggregation was not significantly different between the PUFAs and the control group in the ADP test (39 ± 11 *vs* 42 ± 15; $p = 0.701$), ASPI test (64 ± 24 *vs* 70 ± 27; $p = 0.525$) and TRAP test (68 ± 25 *vs* 75 ± 26; $p = 0.396$), while their aggregation in COL test was statistically significantly lower in the PUFAs related to the control group (32 ± 15 *vs* 47 ± 20; $p = 0.009$).

Postoperative complications were similar in both groups of patients. In the control group, one patient died of cardiac failure on the second postoperative day, two patients had perioperative infarction, three patients needed inotropic support. In the PUFAs group, one patient underwent reexploration for bleeding, one had a respiratory failure and two patients needed inotropic support. Due to the low number of the observed complications, no statistical comparison was performed.

Discussion

We studied the relatoinship between omega-3 PUFAs, which may influence the activity of platelets, and the hematological parameters liable to imapirment in patients with CPB, and found that in spite of the marked decrease in the postoperative activity of platelets, more pronounced in the PUFAs group of patients in relation to placebo in the COL test, they did not affect any of the observed intra- and postoperative hematological parameters (blood loss, RBC, FFP and platelet requirements).

Due to the separate study types, discussion is given in two parts: the effect of CPB on hematological parameters, and the influence of omega-3 PUFAs in CPB procedure on the activity of platelets.

The effect of CPB on hematological parameters

Statistically significant differences were postoperatively found in both groups of the studied patients in some hematological parameters, like Hb and Htc levels and platelet counts, but not in the others as INR and aPTT. This is very important, since bleeding after cardiac surgery may ensue either from surgical (anastomoses, sternum, cannulation sites) or nonsurgical sites. If bleeding becomes excessive or causes hemodynamic disorders, reexploraton of mediastinal wound is necessary. In our study, one patient in the PUFAs group underwent reexploration for bleeding. Studies from the other authors have shown that reexploration can be associated with multiple negative outcomes such as renal failure, sepsis, atrial fibrilation, prolonged mechanical ventilation and hospital stay and, most notably, increased mortality and costs [1–4].

There have also been studies in which patients with coronary bypass grafting [15–17], endarterectomy [18, 19], and femoral artery catheterization [20–22] were given omega-3 PUFAs. In these studies, identically with our results, the risk of clinically significant bleeding was virtually nonexistant. However, in this respect, one has to keep in mind the review of Bays [23] in which he concluded that although there is little evidence for increased risk of clinically significant bleeding with omega-3 PUFAs supplementation, clinicians should be aware of this as a theoretical possibility.

The influence of omega-3 PUFAs in CPB procedure on the activity of platelets

The results of our study show that preoperative activity of platelets in both groups of patients were in the range of reference values for all of the four used tests. Conversely, after the surgical intervention, their activity was statistically significantly reduced, with easily noticable lower values in PUFAs group in relation to placebo, particularly in COL test.

This finding is very relevant, because platelets play an important role in maintaining normal hemostatic function. Their disfunction is a major cause of excessive bleeding in the early postoperative period after CPB procedures [24, 25], not found in our patients. Transient impairment of platelet function is mediated by platelet activation during passage through the synthetic, nonendothelial surface of the extracorporeal circuit, used also in our patients with twofold decrease in their count, and involves the secondary release and partial depletion of α-granules. Platelet dysfunction may also be related to other factors [24]. Hypothermia related CPB influences platelet function and coagulation [26], the effect which can persist into the ICU [27], and be more pronounced as the time on CPB increases [28, 29].

Multiple electrode platelet aggregometry (MEA) used in our study, allows the assessment of platelet function without centrifugation steps [30], and has proven sensitive for platelet inhibition induced by aspirin and clopidogrel, as well as for the effects of CPB and of hypothermia on platelet aggregation [31–34]. The test has also been found to be able to detect impaired hemostasis after CPB surgery [35–37] and to identify patients before and after cardiac surgery with enhanced risk of bleeding and of blood transfusion [38, 39].

The mechanism of favorable antithrombotic effects of omega-3 PUFAs found in our study are complex. It has been shown that alteration of fatty acid composition by omega-3 PUFAs incorporation into platelet membranes can alter not only membrane permeability, but also modulate function and activity of membrane receptors and transporters [40, 41]. The COL test reagent, being the most sensitive in our study, contains collagen, which activates the platelets by the collagen receptor. Following its binding to the receptors, AA is released, which is the substrate of platelet enzyme cyclooxygenase (COX). COX transforms AA into TXA_2, a potent platelet activator. With a blockade of COX, the formation of TXA_2 is inhibited and therefore inhibited platelet activation is usually detected, as happened to be in our patients.

In most studies either no effect on platelet aggregation was found with omega-3 fatty acids or no difference in effect was seen between the treatments and the control. Kwon et al. [42] noted that with 2 mg of collagen, a significant decrease in

platelet aggregation was found at three weeks on canola oil diet, which reverted to baseline by eight weeks. Freese et al. [43] reported that the decrease in collagen-induced aggregation in the fish oil supplement arm did not return to baseline during a 12 week follow-up period.

Overall, although there is heterogeneity among the studies, there is a trend toward a net reduction of coronary artery restenosis with fish oil supplementation, estimated by the meta-analysis to lower such a risk for 14% [21, 44]. The optimal degree of platelet inhibition is unclear and must be confirmed in trials evaluating cardiovascular outcomes and sould be balanced with the excessive risk of bleeding [45]. In any case, the results of our study show that the postoperative inhibition of platelet aggregation by PUFAs, particularly pronounced in the COL test, did not affect the intra- and postoperative hematological parameters, with the risk of bleeding, as the most dangerous, being equal to the placebo group. This finding undoubtedly speaks in favour of omega-3 PUFAs use as cardioprotectors in patients with open heart surgery, found in our recent study [12].

Conclusion

The results of our study show that acute omega-3 PUFAs pretreatment of patients subjected to CBP grafting did not affect INR, aPTT and bleeding volume, while the postoperative platelet count dropped twofold, equally in the PUFAs and placebo treated groups. The activity of platelets was statistically significantly lower after surgical intervention in both groups of patients, particularly markedly pronounced in the COL test in the PUFAs group, but with no negative effect on bleeding.

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Declaration of Conflicting Interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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GENERAL REVIEWS

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Programmed cell death in sepsis in Balkan nephropathy

Programirana ćelijska smrt kod sepse u balkanskoj nefropatiji

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Key words:
sepsis; balkan nephropathy; renal insufficiency, chronic; inflammation mediators; biological markers; cells; apoptosis; mitochondria.

Ključne reči:
sepsa; nefropatija, balkanska; bubreg, hronična insuficijencija; zapaljenje, medijatori; biološki pokazatelji; ćelije; apoptoza; mitohondrije.

Introduction

Sepsis is defined as a suspected or proven infection in the systemic inflammatory response syndrome (SIRS) [1]. From the beginning of a systemic infection and over sepsis peaks of immune-mediators characteristic of SIRS and for the compensatory anti-inflammatory response syndrome (CARS) may be seen in sequence or in parallel [2] that enlighten the reason why broad investigation of inflammatory biomarkers during the last decade, including members of cytokine network versus sepsis outcome or patient survival did not satisfactorily pass the validation tests.

Inflammation biomarkers

Inflammation biomarkers were not more efficient than standard clinical parameters in the intensive care patients. Pierrakos and Vincent [3] displayed the results of a total of 3,370 studies that assessed 178 different biomarkers in sepsis, among them apoptotic related biomarkers and sepsis outcome. A relation between inflammation control and programmed cell death (PCD) – apoptosis type I both of immunocytes and parenchymal cells in sepsis development and regulation has been recognized. There are numerous examples of the dualism in activity of a stimulus in cell fate. Several proinflammatory cytokines (TNF, IL-6, IL-18) may trigger apoptosis through several caspases activation rather than inflammation stimulation. Conversely, caspases, as classical mediators of cell death may trigger apoptosis pathway or upregulate some (proinflammatory) cytokines that in turn induce cell survival and proliferation. Oligomerization of cell surface death receptor Fas, a member of TNF receptor family, by their cognate ligands results in formation of death-inducing signaling complex (DISC), additionally including adapter protein Fas associated death domain receptor (FADD) and caspase-8. The death domain (DD) in FADD interacts with DD in the cytoplasmic tail of the Fas, while the death effector domain (DED) in FADD binds to a DED within the prodomain of caspase-8. This promotes the autocatalytic activation of caspase-8, which then cleaves downstream effector caspases that eventually will induce TUNEL+ DNA fragmentation in apoptosis [4]. Caspases may signalize the pathway of antigen activation of immunocytes, when another adapter molecule named FLICE inhibitory protein (FLIP), which is the Fas inhibitory protein, be incorporated into the DISC. In that case caspase-8 may promote lymphocyte activation and proliferation [5, 6]. Cells have given alternative splicing of the *flip* gene with the possibility for the FLIPs short protein and the FLIPl long protein production. The FLIPl contains two DED domains and caspase-8 like p20 and p10 domains without enzymatic activity, so that the accumulation of FLIPl in DISC prevents recruitment of caspase-8 [7]. Newton and Strasser [4] proposed that FLIPl may act as a scaffold protein; and gathering of high amount of FLIPl and FLIPs to Fas may inhibit apoptosis, low level of FLIPl facilitates apoptosis, enabling FADD to assist in caspase-8 activation. Caspase-1 may activate caspase-3 and triggers cascade activation of enzymes that will lead to DNA fragmentation. Caspase-1 may purposely induce synthesis of IL-1 beta and IL-18 cytokines in activated monocytes in sepsis. IL-18 is a factor of potent IFN gamma induction in Th1 lymphocytes, regarding it stimulates them together with IL-12 to clonal expansion, promoting inflammation. TNF not only induces apoptosis by activating caspase-8 and -10, but can also inhibit apoptosis signaling through NF-kappa B stimulation, which induces the expression of IAP, an inhibitor of caspases-3, -7 and -9. In patients in septic shock serum caspase-1 is significantly increased [8] that may be the biomarker of dramatically

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amplified apoptosis, regarding the finding that an early apoptotic marker anexin V binding was also importantly higher than in control animals [9]. It would seem that widespread TUNEL+ apoptosis of immunocytes may be deleterious during sepsis.

Mitochondrial function

Adrie et al. [10] have shown failing mitochondrial function in circulating monocytes from 18 patients with severe sepsis. Opening of permeability transition pores in the mitochondrial inner membrane is followed by the change in mitochondrial transmembranes potential. The subsequent release of mitochondrial intermembrane proteins (cytochrome c, apoptosis-inducing factor -AIF) into the cytosol may activate caspases [11]. However, mitochondrial membrane alterations may also lead to ATP synthesis arrest with subsequent cell necrosis [12]. T lymphocyte mitochondrial alterations have also been described in septic mice [13]. Fas dead receptors may transmit proapoptotic signal into the cell, after oligomerization with soluble Fas ligands (sFasL), while soluble Fas (sFas) inhibits it with sFasL binding outside the cell. Doughty et al. [14] have shown that severe pediatric sepsis with poor survival is coincided with the rise of sFas blood levels in correlation with IL-6 and IL-10. sFasL does not increase. Pursuant to these results, the link of apoptosis prevention with sFas and systemic inflammation in severe sepsis with multiple organ failure syndrome (MOFS) has been proposed. The same has been noted in adult patients, as well [15]. Nevertheless, other investigators have shown that the increased sFas, which correlates with nitric oxide and circulating nitrates, does not suggest reduced apoptosis of blood mononuclear cells (MNC). On the contrary, a completely different expression of Fas and FasL on blood MNC has been noted suggesting high apoptosis rate of MNC in severe sepsis. Thus, correlations of raised blood IL-6 and TNF alpha with sFas level in these patients may be a reliable prognostic marker of poorer survival, but it does not imply infrequent lymphocyte and monocyte apoptosis. Instead, apoptosis is increased in sepsis [16].

Immune response

Non-survivors have shown increased number of peripheral monocytes with depolarized mitochondria prone to apoptosis. During the first days of sepsis anti-apoptotic Bcl-2 monocyte expression decreases *ex vivo* [10]. Apoptosis of monocytes manages complex immunomodulation in sepsis, and this may compromise host defense against microbes. Namely, stimulation of Th1 or Th2 rules out each other's response. Lymphocytes may exchange their roles and acquire or renew characteristics of either Th1 or Th2 cells, after antigen (re)stimulation and depending on the cytokine and co-stimulatory molecules from monocytes and dendritic cells, as professional antigen-presenting cells (APC), or under the influence of surrounding accessible cytokines. Mature dendritic cell may provide signals positive for the production of Th1 and other signals negative for the production of Th2 cells, following TLR activation on dendritic cell [17, 18]. Signals from APC influence whether the tolarogen or an active immune response would occur in lymphocytes to a particular antigen. Dendritic cell uptake of apoptotic cells in the absence of maturation signals induces tolerance [19]. Namely, type of lymphocytes death triggered by the pathogen is one of the leading mechanisms to define the immune response for inflammation or immune suppression in the development of sepsis. Macrophages and dendritic cells that phagocyte necrotic cells start inflammation by stimulation of mainly Th1 cell response, while macrophages and dendritic cells after phagocyting apoptotic cells stimulate preferentially the Th2 response. One has to bear in mind that necrotic debris may stimulate TLR and innate immunity, while apoptotic cells avoid TLR signaling and do not initiate innate immune response, which is in turn essential for adaptive immune response to microbes, consequently both would be silenced. Under certain conditions, proinflammatory cytokines may induce apoptosis of immunocytes, as it has been already explained for IL-18 [20]. Apoptosis may not potentiate synergistic stimulation between innate and adaptive pro-inflammatory response. This will support Th2 cell prevalence. Th2 anti-inflammatory cytokines may suppress and extinguish further function of antigen-presenting cells or induce their apoptosis. All these events lead to the so-called 'inflammatory immune suppression', and finally to anergy that happens in (lethal) sepsis [20]. The net result is a severely compromised innate and adaptive immune system with poorly functional "exhausted" CD8 and anergic CD4 T cells. *Post mortem* immunohistologic findings in septic patients reveal vast apoptosis of immune system cells, particularly B and CD4+ lymphocytes, as well as follicular dendritic cells [21, 22]. The finding of "waste spleen" is conspicuous, while natural killer (NK) cells and CD8+ lymphocytes are spared. This also implies systemic immune suppression when the immune cells die, instead of expected clonal expansion. Lymphocytopenia is evidenced. Also, massive apoptosis of intestinal epithelial cells and vascular endothelial cells is noted. It is present also in the kidneys, heart and liver. Apoptosis of non-immune cells also may induce hyporeactivity of monocytes or other APC following uptake of apoptotic bodies. Prevention of lymphocyte apoptosis in an experimental model of sepsis improves animal survival [22–25]. Intervention to suppress apoptosis with rIL-7 treatment may have influence on better severe sepsis outcome; however it is still an experimental effort [26].

Sepsis in patients with kidney disease

The site of prime infection, such as urosepsis seems to be also important for specific immune system modulations including apoptosis rate of monocytes, as APC, and a biomarker behavior in the progression to severe sepsis. In the field of acute pyelonephritis the expression of HLA-DR on monocytes, the rate of apoptosis of monocytes and the rate of apoptosis of NK cells decreased first 24 h of severe urosepsis/septic chock calculating in 42 patients, 9.3% with chronic renal failure (CRF), quite different from abdominal sepsis with decreasing CD8 count and apoptosis score [27]. In patients with kidney disease at least two additional factors potentially influence the sepsis course and outcome. These are the nature of underlying kidney dieses and the chronic renal fail-

ure. All these factors should be calculated to decide what a biomarker does say to us about the sepsis state and sepsis severity. In patients with CRF sepsis may be prolonged with predominant immunosupression reaction from the beginning of the sepsis. CRF is a state of chronic inflammation with remarkably deregulated monocyte function. The co-stimulation impairment for T and B lymphocytes acts together with monocyte aberrant cytokine secretion. Monocytes release more proinflammatory cytokines, and blood levels of TNF alpha, IL-1 beta, IL-6, IL-12 and IL-18 are progressively increased [28–30]. However, some lymphokines secreted by activated T cells, e.g. IFN gamma, are missing hypothetically due to poor lymphocyte function. Quite the opposite, when exposed to signals from normal APC (monocyte), isolated T and B cells from the blood of CRF or uremic patients are directed to function normally [31]. Blood cells of these patients stimulated by *Staphylococcus epidermidis* in culture realize significantly lower IFN-gamma synthesis than cells of healthy subjects [32]. It has been concluded that the link between innate and adaptive immunity is impaired in patients with CRF, resembling endotoxin tolerance.

Increased rate of monocyte and Th1 lymphocyte apoptosis in CRF is another important disorder affecting the immune response dysfunction in sepsis in these patients. Plasma of CRF patients has increased the pro-apoptotic potential to U937 monocytes in culture, correlating with TNF plasma levels and independently of IL-1, IL-2 or IL-10 [33, 34]. In CRF patients, inflammatory cytokine IL-18 may also participate in increased apoptosis rate of Th1, monocytes or parenchymal cells, via Fas system [35].

Sepsis in patients with Balkan endemic nepharopathy and associated upper-urothelial carcinoma

Especially intriguing is the occurrence of post-operation sepsis in the patients with Balkan endemic nephropathy (BEN) and associated upper urothelial carcinoma (UEM), which is highly prevalent malignancy in endemic areas [36, 37]. BEN is slowly progressive tubulointerstitial disease, now regarded as toxic (possibly aristolochic acid) nephropathy. Low pro-inflammatory immune response may explain almost acellular foci of interstitial fibrosis that surround progressive tubule atrophy. Savin et al. [38] discovered considerable tubule cell apoptosis in BEN that is greatly important in disease development and one may describe pathogenesis of BEN as a human apoptotic model of kidney injury [39]. In addition, half of BEN patients may develop CRF, which additionally manages apoptosis increase in sepsis. Petkovic [40] originally displayed endemic appearance of upper-UEM in Serbia, and noticed an extraordinary favorable outcome of these patients after nephroureterectomy, for even 20 years, and a 5-year survival rate was 72% for the conservative kidney operation in the Urology Clinic, Clinical Centre of Serbia, Belgrade. The same survival trend has been shown by a more detailed epidemiological investigation of the endemic village Petka in Serbia by Radovanović et al. [36]. Later on, Petronić et al. [37] suggested slow growth of these tumors in BEN patients on hemodialysis; a new or recurring urothelial carcinoma has been evidenced in 20% of patients for 5–12 years, and that indirectly imply a long survival of patients from BEN regions.

Pyelonephritis is common in patients with BEN and urothelial carcinoma, practically the same as in the patients with upper-UEM outside endemic regions. It is rational expecting greater incidence of postsurgical (uro)sepsis in BEN patients with upper-UEM and worse outcome of BEN patients in sepsis in the setting of chronic exposure to environmental toxin attacks that induce apoptotic injury of the kidney, as well. Surprisingly, by our pilot study sepsis following surgical removal of the kidney similarly occurred in patients with BEN from affected households, as in upper-UEM patients without BEN outside endemic regions (27.3% and 30%, respectively), regardless more advanced azotemia detected in BEN patients in sepsis ($p = 0.008$). Furthermore, analysis of the patient survival *vs.* sepsis after total nephrouterectomy due to upper-UEM (n = 37) denied an influence of added deleterious factors – BEN or chronic renal insufficiency in poor outcome, excepting unfavorable long-lasting effect on chronic hemodialysis, and great apoptosis in tumor before sepsis in BEN patients [41]. A possible explanation is that TUNEL+ apoptosis (PCD type I) is not the only apoptotic form in BEN, as concomitant autophagy (PCD type II) may play a protective role against toxic (kidney) injury in these patients, at least on glomerular cells.

It would be of interest to analyze the influence of those "chronic" apoptosis attacks of renal tubular cells, such as in BEN, and sepsis outcome initiated from different localization of primary infection, outside the urinary tract that may open a new approach to patients with particular tumor origin and sepsis [42].

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Early rehabilitation in patients operated for breast carcinoma

Rana rehabilitacija bolesnica operisanih zbog karcinoma dojke

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Key words:
breast neoplasms; rehabilitation; rehabilitation centers; postoperative complications.

Ključne reči:
dojka, neoplazme; rehabilitacija; rehabilitacioni centri; postoperativne komplikacije.

Introduction

The most often complication of breast surgery with dissection of axilla is decrease in the range of shoulder joint of the ipsilateral arm motion, the feeling of heavy arm, secondary lymphedema of the arm, and very rarely pain and weakness of the arm's muscles. Persistence of these symptoms leads to permanent dysfunction of the arm [1–4].

Decrease in the range of motion is a consequence of surgery and scarring of the healed wound, which decreases the amount of movement at each joint on the operated side [4, 5]. A reduced range of shoulder joint motion is diagnosed in 2%–51% patients who underwent surgery for breast carcinoma [2, 4].

Secondary lymphedema of the arm is a consequence of mechanical insufficiency of the lymphatic system caused by the surgery and later, by post-irradiation fibrotic changes, and is manifested by abnormal accumulation of interstitial fluid, rich in proteins [6]. In the majority of studies, secondary lymphedema of the arm occurs in 10%–30% of patients following the breast carcinoma therapy [4].

For postoperative complications reduction, numerous rehabilitation programs and instructions were developed with the aim of damage prevention, maximizing the occurred damage (range of motion, muscle power) and minimizing the risk for development of secondary lymphedema of the arm [1–4, 7–12]. In breast carcinoma patients, rehabilitation has become more significant due to quality of life awareness of the oncological patients [8].

It arises dilemma when to start with the rehabilitation program: most of the authors agree in that the program should start in the first several days after the surgery [1–4, 7–12], while the other authors consider that early beginning of rehabilitation in patients with axilla dissection is associated with an increased risk from postoperative complications: longer drainage period, seroma formation, postoperative infection and consequential longer hospitalization [1, 2, 8]. In a controlled, randomized study, a hypothesis that exercises do not increase the risk of occurrence of secondary lymphedema of the arm has been confirmed [13].

Exercises are efficient, safe and preferred interventions in a postoperative period [4]. Early rehabilitation and later home-based exercises program, education [14, 15], as well as a continuous follow-up of patients [7, 9] were identified as interventions for the improvement of life in women with breast carcinoma in all 4 dimensions: physical, emotional, social and cognitive [15]. Type, duration, frequency and intensity of exercises vary in the studies [16]. Education and follow-up of patients with breast carcinoma enable prevention, detection of early and late occurrences of postoperative damages [8].

A lack of rehabilitation interventions in patients operated for breast cancer is a consequence of no standardized exercises program avalable, so it is necessary to homogenize a reproducible regime [9].

Early rehabilitation in breast carcinoma patients who underwent surgery at the Oncology Institute of Vojvodina

The Rehabilitation Department was founded in 1996 as an organizational unit of the Oncology Institute of Vojvodina. Its activities are designed for preventive oncological rehabilitation in breast carcinoma patients, and, to some less extent, for other segments of oncological rehabilitation – restitute, supportive and palliative oncological rehabilitation [17]. Cooperation with other medical personnel, based on

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the horizontal correlation from the moment of diagnosis, during the therapy and the post-therapeutic period, enabled a continuous follow-up of all breast cancer patients by the physiatrist at the Oncology Institute of Vojvodina (Figure 1).

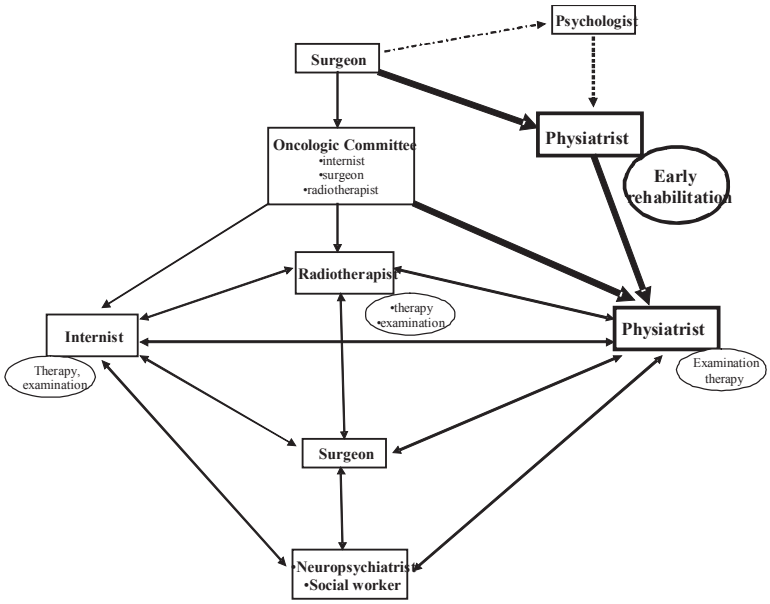

Fig. 1 – Significance and role of rehabilitation in the patients surgically treated for breast cancer at the Oncology Institute of Vojvodina

Based on the data from the literature, clinical experience, presented results [18–21] and current possibilities, early rehabilitation algorithm in patients with breast carcinoma diagnosis was defined in the Rehabilitation Department (Figure 2).

Evaluation

Basic data – preoperative measuring includes measuring shoulder joint range of motion for both arms (flexion, abduction, external and internal rotation and extension); the borderline value of the motion reduction range is ≥ 10°; measuring the volume of both arms at 5 symmetrical, clearly defined levels; the borderline value of the volume is ≥ 2 cm; psychological evaluation.

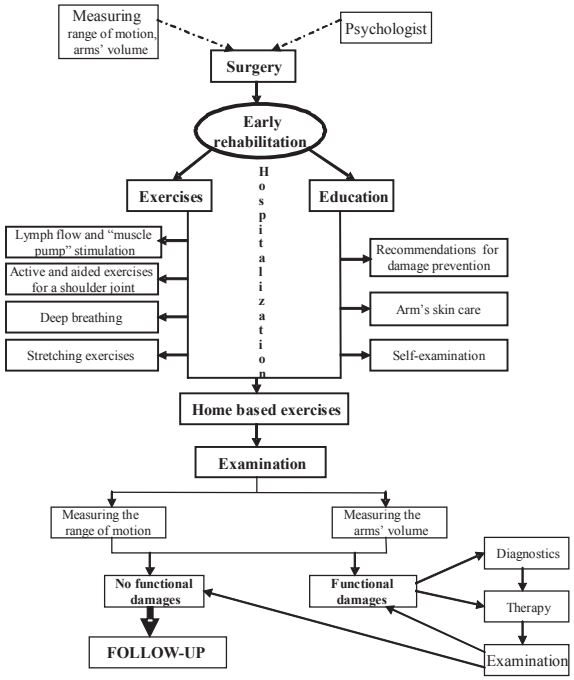

Fig. 2 – Early rehabilitation and follow-up in the breast cancer operated patients at the Oncology Institute of Vojvodina

According to the defined algorithm preoperative measurements are not mandatory because the first contact the physiatrist – the physiotherapist – the patient is most often immediately after the surgery. If there are any functional damages, a preoperative evaluation of the functional status is performed in cooperation with the surgeon.

Also, psychological evaluation is not obligatory since it is impossible to have a permanenthy engaged professional (no legal obligation to employ a psychologist or a defectologist) (Figure 1).

Early rehabilitation program during hospitalization

Kinesitherapy program starts on the 2nd postoperative day. It includes active exercises for the hand, radiocarpal joint and elbow to stimulate lymph flow and strengthening of the "muscle pump"; exercises with active and actively-aided movements in the shoulder joint (shoulder circling, wall climbing, elbow pushing); deep breathing; stretching exercises (neck movements, arm lifts).

The exercises are designed to maintain or increase the range of motion, to provide and increase lymph flow, to prevent fibrous adhesions and maintain the muscle power. The exercises are performed each day during hospitalization.

In patients with signs of wound infection or febrile state, kinesitherapy program is postponed until thay become is stabile.

Educating a patient includes recommendation on later complications prevention (secondary lymphedema of the arm, brachial plexus damages), i.e. how to behave and what to avoid (risk factors) [17]; on skin care of the ipsilateral arm; and education on haw to notice any changes in the skin of the arm, postoperative cut, drain (self-examination).

Home-based rehabilitation exercises include practising these exercises at home 3 times a day, 5–10 repetitions; up to the pain limit. If infection occurs in the area of the postoperative cut, the residue of the breast tissue or ipsilateral arm, the patient should stop the exercises (seroma formation is not a contraindication) and be referred to the surgeon for examination.

Follow-up

First examination in the Rehabilitation Department follows the Oncological Committee (4–6 weeks after the surgery) in accordance with the "horizontal correlation" system (Figure 1), for examination by the physiatrist including measuring the range of motion and registration of the obtained parameters; measuring the volume of extremities and registration of the obtained parameters.

The next examination is perfermed after 3 months, while the following ones comply with the therapeutic procedures, or, if any of post-therapeutic complications appears, it is necessary to make the diagnosis according to indications (magnetic resonance imaging, ultrasound diagnostics, electromioneurography, etc.). The principles of restitute, supportive or palliative oncological rehabilitation are also applied.

This procedure in the Rehabilitation Department at the Oncology Institute of Vojvodina, horizontal correlation of all medical segments that participate in the breast carcinoma treatment, implementation of principles of preventive oncological rehabilitation, continuous follow-up and early detection of complications, significantly reduce the number and severity of post-therapeutic complications.

Out of 360 randomly selected patients, surgically treated at the Oncology Institute of Vojvodina, in the period 2000–2009, reduction of the range of motion in the shoulder joint (≥ 10°) was registered in 96 patients (26.67%) (Table 1). The most usual range reduction were in two movements (flexion and abduction). In more than half of the patients, the reduction was up to 30% for abduction and flexion movements and up to 20% for movements of internal rotation, external rotation and extension (Table 2).

Table 1

Incidence of movement reduction in the shoulder joint in the patients surgically treated for breast carcinoma at the Oncology Institute of Vojvodina

Number of reduced movements	Patients n (%)
0	264 (73.33)
1	25 (6.94)
2	27 (7.5)
3	19 (5.28)
4	18 (5.0)
5	7 (1.95)

Low incidence of secondary lymphedema of the arm in comparison to data from the literature [4] and high presence of mild clinical forms are presented in Table 3 and Table 4 respectively.

Damages of the brachial plexus were actually individual cases, mostly of less severe degree.

Table 2

Degree of the reduction in the shoulder joint motion range in the patients surgically treated for breast carcinoma at the Oncology Institute of Vojvodina regarding the type of motion

Type of motion	Mild reduction (%)	Modest or severe reduction (%)
Abduction	45.63*	54.37†
Flexion	63.49*	36.51†
Interval rotation	63.46•	36.54+
Exsternal rotation	64.59•	35.41+
Extension	92.68	7.32+

***reduction range < 30º; • reduction < 20º; † reduction ≥ 30º; + reduction ≥ 20º**

Table 3

Secondary lymphedema of the arm (SLEA) in the patients surgically treated at the Oncology Institute of Vojvodina, 2003–2007

Year	Number of surgically treated patients	Patients with SLEA n (%)
2003	409	40 (9.78)
2004	362	30 (8.3)
2005	362	38 (10.5)
2006	318	28 (8.81)
2007	384	35 (9.11)

Table 4

Clinical forms of secondary lymphedema of the arm in the patients surgically treated for breast carcinoma at the Oncology Institute of Vojvodina, 2003–2007

Clinical forms	Patients n (%)
Mild[1]	109 (63.74)
Moderate[2]	43 (25.15)
Severe[3]	19 (11.11)

[1] arm volume difference of 2–2.9 cm at at least 1 level; [2] volume difference of 3–4.9 cm; [3] volume difference of ≥ 5 cm

The results of postoperative breast cancer treatment in the Rehabilitation Department including the designed algorithm were recognized by the National Committee for preparation of the National Guide of Clinical Practice for Breast Carcinoma (one author of this paper is a member of the team). This is the first time rehabilitation in breast carcinoma is placed within the legal framework.

In conclusion, our answer to the question "Is physiotherapy useful for the breast cancer patients?" [21] is: Yes, indeed!

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CASE REPORTS

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Percutaneous transluminal renal angioplasty application effect on renal function in patients with renal artery stenosis – a case report on 4 patients

Uticaj primene perkutane transluminalne renalne angioplastike na funkciju bubrega kod bolesnika sa stenozom bubrežne arterije

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Abstract

Introduction. Renal artery stenosis (RAS) is narrowing of one or both renal arteries or their branches. Clinically significant stenosis involves narrowing of the lumen, which is approximately 80%. The two most common causes of its occurrence are atherosclerosis and fibromuscular dysplasia. Percutaneous transluminal renal angioplasty (PTRA) with stent implantation is an effective treatment modality that leads to lower blood pressure and improvement of kidney function. **Case report.** We presented 4 patients with significant stenosis of one or both renal arteries followed by the development of arterial hypertension and renal insufficiency. The causes of RAS were atherosclerosis in two patients and fibromuscular dysplasia in one patient. One of the patients had renal artery stenosis of transplanted kidney that developed 9 month after transplantation. In all the patients, in addition to clinical signs, doppler screening suspected the existence of significant renal artery stenosis. The definitive diagnosis was made by applying computed tomographic angiography (CTA) of renal arteries in 3 of the patients and in 1 patient by percutaneus selective angiography. All the patients were treated by application of PTRA with stent implantation followed by improvement/normalization of blood pressure and kidney function. **Conclusion.** Application of PTRA with stent implantation is an effective treatment of significant stenosis of one or both renal arteries followed by renal insufficiency.

Key words:
renal artery obstruction; kidney function tests; diagnostic techniques and procedures; angioplasty, balloon.

Apstrakt

Uvod. Renalna arterijska stenoza (RAS) predstavlja suženje jedne ili obe renalne arterije ili njihovih grana. Klinički značajna stenoza podrazumeva suženje lumena koje iznosi približno 80%, a dva najčešća uzroka njenog nastanka su ateroskleroza i fibromuskularna displazija. Perkutana transluminalna renalna angioplastika (PTRA) sa implantacijom stenta, predstavlja efikasan modalitet lečenja koji dovodi do sniženja krvnog pritiska i poboljšanja bubrežne funkcije. **Prikaz bolesnika.** Prikazali smo četiri bolesnika sa značajnom stenozom jedne ili obe renalne arterije, praćene razvojem arterijske hipertenzije i bubrežne insuficijencije. Uzrok nastanka RAS bili su ateroskleroza kod dva i fibromuskularna displazija kod jednog bolesnika. Kod jednog bolesnika devet meseci nakon transplantacije bubrega došlo je do razvoja stenoze na mestu anastomoze renalne arterije donorskog bubrega i hipogastrične arterije recipijenta, najverovatnije uzrokovane aterosklerozom. Kod sva četiri bolesnika pored kliničkih pokazatelja, doplersonografskim skriningom postavljena je sumnja na postojanje značajne stenoze renalne arterije. Definitivna dijagnoza postavljena je primenom multislajsne skenerske angiografije renalne arterije kod tri bolesnika, a kod jednog bolesnika selektivnom angiografijom. Sva četiri bolesnika lečena su primenom PTRA sa implantacijom stenta, nakon čega je došlo do poboljšanja/normalizacije krvnog pritiska i bubrežne funkcije. **Zaključak.** Primena PTRA sa implantacijom stenta predstavlja efikasan modalitet lečenja značajne stenoze jedne ili obe bubrežne arterije, praćene bubrežnom insuficijencijom.

Ključne reči:
a. renalis, opstrukcija; bubreg, funkcijski testovi; dijagnostičke tehnike i procedure; angioplastika, balonska.

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Introduction

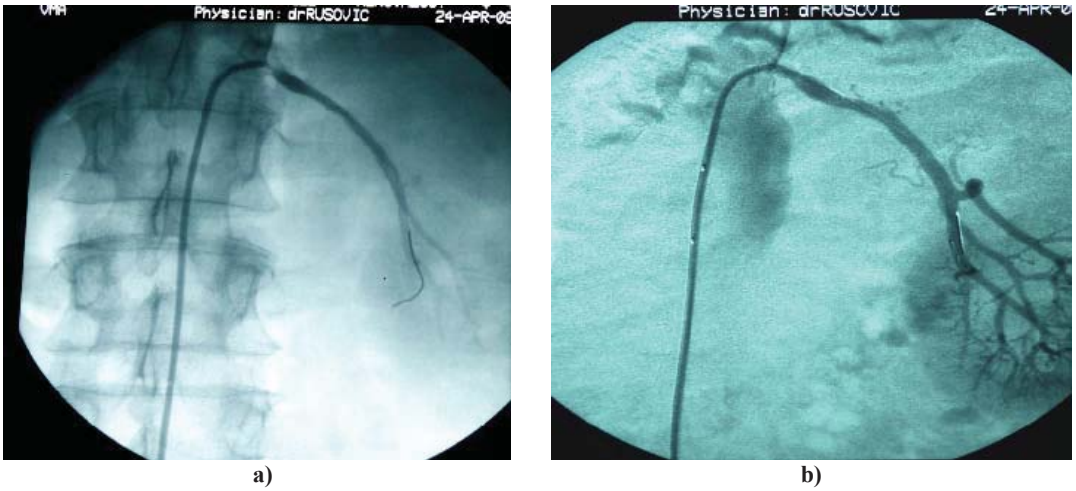
Renal artery stenosis (RAS) is narrowing of one or both renal arteries or their branches [1]. Clinically significant stenosis involves narrowing of the lumen, which is approximately 80%, resulting in renal hypoperfusion, activation of the renin-angiotensin system, increase in systemic blood pressure and reduction of the glomerular filtration rate [2, 3]. The two most common causes of RAS are atherosclerosis and fibromuscular dysplasia (FMD). Atherosclerotic renal artery stenosis (ARAS) is common in adults older than 65 years with hypertension, generalized atherosclerosis disease and renal impairment, as the main clinical signs [4]. The reported prevalence of clinically manifested ARAS in the general population is 0.5% overall and 5.5% among patients with chronic kidney disease. Since patients are often asymptomatic, the actual frequency is higher and ranges up to 7% [5, 6]. Unlike ARAS, FMD is a nonatherosclerotic and noninflammatory vascular disease that most commonly affects the renal and internal carotid arteries but has been described in almost every arterial bed in the body, occuring frequently in young women [7]. Diagnosis of RAS can be set using various functional and morphological tests [8]. Duplex ultrasonography is noninvasive tool which provides a functional assesment of the severity of stenosis because higher velocity correlates with a greater pressure differential across the stenosis [9]. Renal angiography using computed tomography (CTA) or magnetic resonance (MRA) are noninvasive and sophisticated diagnostic techniques. Sensitivity/specificity of these methods is an average of 64/92% for CTA and 62/84% for MRA [10]. In patients with chronic kidney disease, the use of CTA and MRA are limited by toxicity of the contrast medium or risk from development of nephrogenic systemic fibrosis, associated with gadolinium [11]. The gold standard to diagnose RAS still remains digital-subtraction angiography but it is invasive diagnostic procedure with risks from different vascular complications.

While the application of percutaneous dilatation is an effective modality of treatment FMD [12], optimal therapy of ARAS is still controversial [13].

The aim of this case reports was to show the effect of applying PTRA with stent implantation on renal function in four patients with significant renal artery stenosis. Under significant stenosis we assumed renal artery lumen narrowing greater than 70%. Two patients had atherosclerotic RAS, one patient FMD and one patient had renal artery stenosis of transplanted kidney. In all the patients endovascular intervention led to the normalization or improvement of renal function.

Case 1

A 57-year-old former smoker, with the history of arterial hypertension, abdominal aneurism and renal insufficiency was admitted with following signs and symptoms: shortness of breath, edema and oliguria. Laboratory findings showed an increase in serum creatinine from 230 to 881 μmol/L, with normal results of urine. Hemodialysis treatment was initiated through a central venous catheter. A total of four procedures was carried out, by means of which we achieved a good control of blood pressure and decreased values of serum creatinine to 365 mmol/L. Ultrasound examination revealed the right kidney of reduced size (8.2 cm), with thin parenchyma (0.7 cm) and without doppler signal. Left kidney diameter was 11.5 cm, with thin parenchyma in the upper half of the kidney -0.9 cm, without doppler signal. In the lower half of the left kidney parenchyma was a 1.6 cm and intrarenal doppler examination showed that the resistive index (RI) was 0,45. Based on clinical features and the finding of a low RI, the patient was suspected to having significant stenosis of the left renal artery. Multislice computed tomography (MSCT) arteriography was performed and diagnosed the existence of the sclerotic right kidney with an occluded artery, while the left kidney had two arteries. Artery in the upper half was occluded and the artery of the lower half had ostial and subtotal stenosis/narrowing of more than 95% lumen (Figure 1a). By the interventional radiologist the patient underwent percutaneous transluminal renal angioplasty (PTRA) with dilation and implantation of a stent size 3 × 18 mm in the artery to the lower half of his left kidney (Figure 1b). The achieved response was well and clinically manifested in the



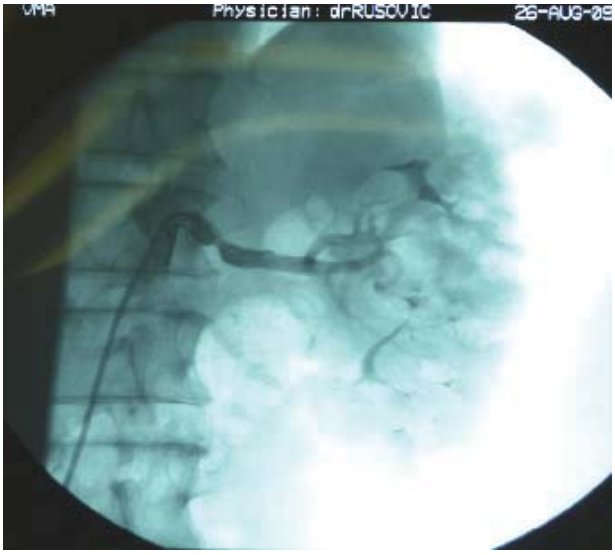
a) **b)**

Fig. 1 – The case 1: (a) subtotal ostial stenosis of the artery in the lower half of the left kidney before percutaneous transluminal renal angioplasty (PTRA) and (b) after PTRA

decline of serum creatinine value of 222 μmol/L at discharge and normalization of blood pressure values, which with application of antihypertensive medications was 130/70 mmHg. Interventional procedures and time to discharge passed without complications.

Case 2

A 61-year-old smoker was admitted due to worsening of arterial hypertension with a max. value of blood pressure up to 180/110 mmHg. Laboratory analysis showed elevated serum creatinine, which amounted to 121 μmol/L. Ultrasound finding in the kidneys was normal. Intrarenal doppler obtained the normal value of RI, which in the right kidney was 0.65, and 0.64 in the left kidney. MSCT angiography was done. We diagnosed infrarenal localized abdominal aortic aneurysm and significant stenosis of the left renal artery to 1.5 cm from the ostium (Figure 2a). The right kidney was vascularized with 2 arteries. The artery for the upper half of the right kidney had significant stenosis at 1 cm from the ostium (Figure 2b). We applied PTRA to the left renal artery with dilation and implantation of two stents of the dimension of 6 × 18 mm and obtained an excellent angiographic response (Figure 2c). A month following the previous, we performed PTRA to the significantly narrowed one of the existing two right renal arteries, with dilation and implantation of a stent size 3.5 × 18 mm (Figure 2d). Both interventions were performed without complications. After the treatment, the patient had normal values of blood pressure and creatinine, which was 93 μmol/L at discharge.



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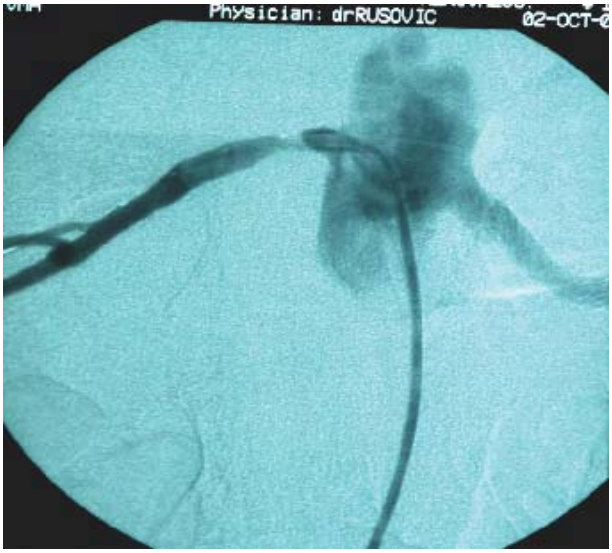

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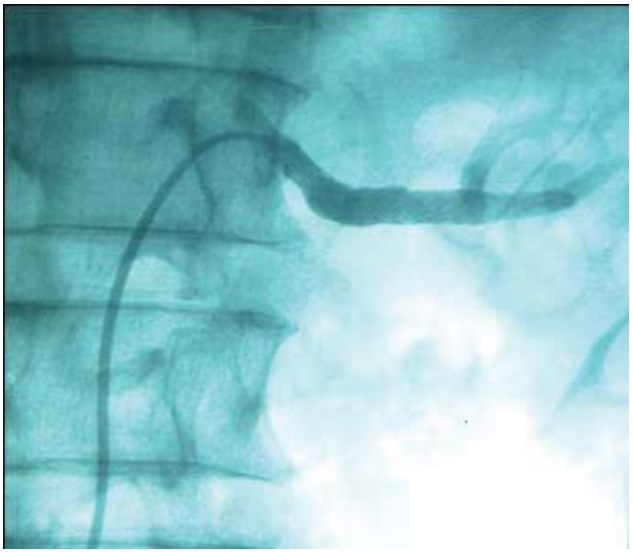
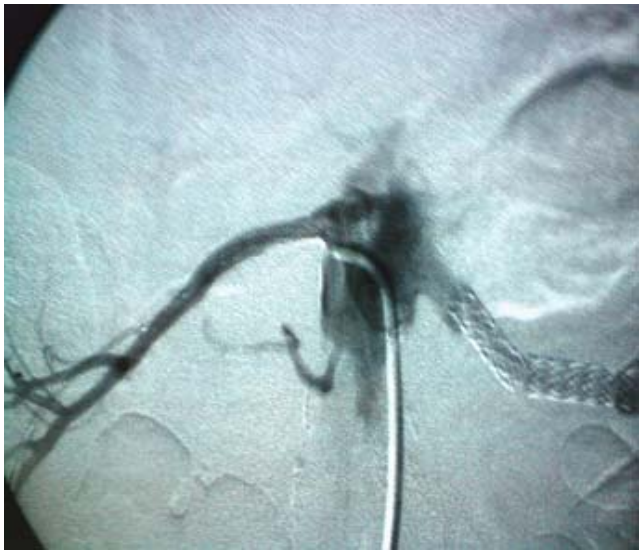


c)



d)

Fig. 2 – The case 2: (a) significant stenosis of the left renal artery before percutaneous transluminal renal angioplasty (PTRA); (b) significant stenosis of the artery for the upper half of the right kidney before PTRA; (c) the left renal artery after PTRA; (d) the artery for the upper half of the right kidney after PTRA

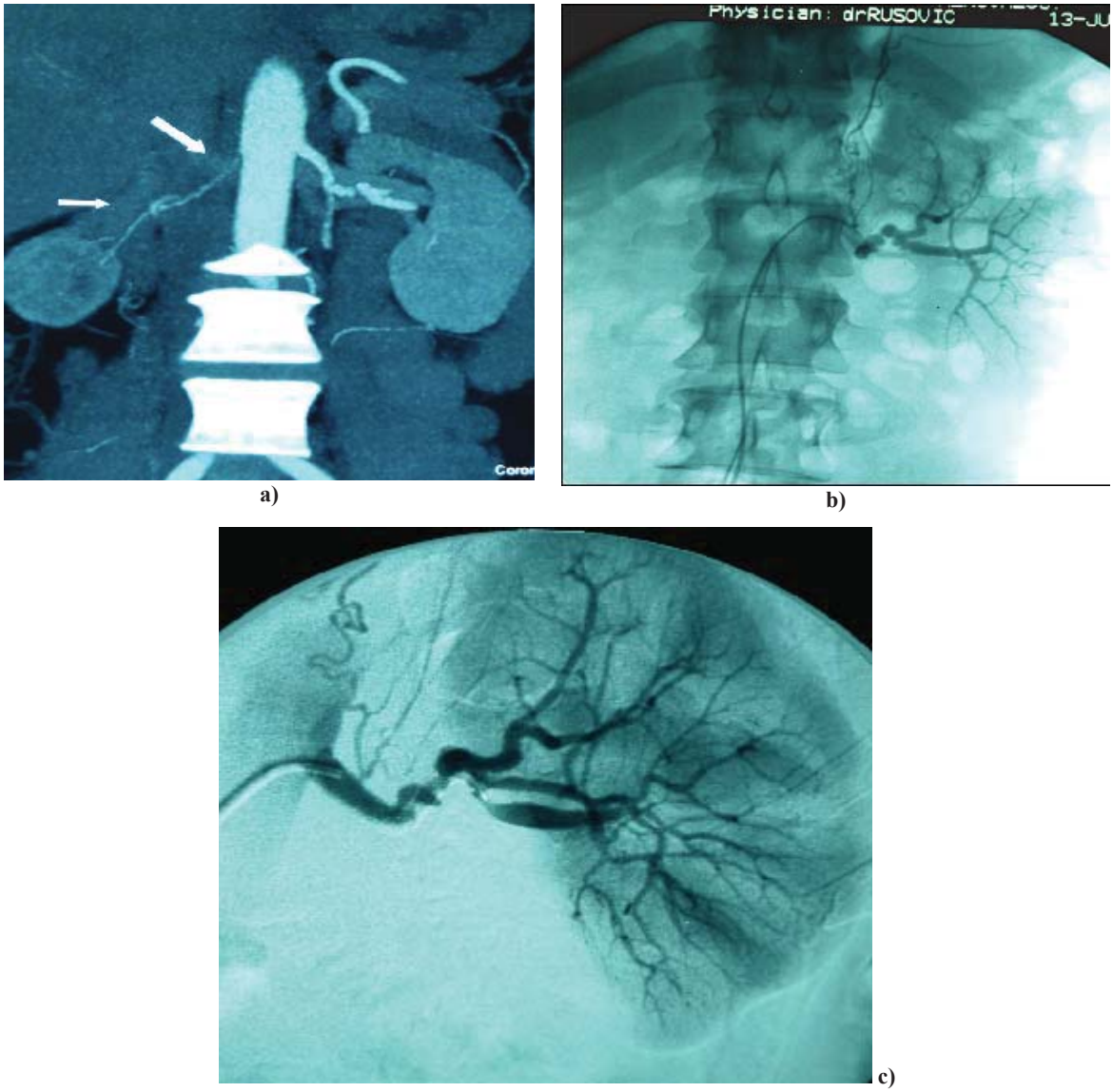
Case 3

A 43-year-old patient was admitted due to worsening of arterial hypertension, with a maximum value of blood pressure 240/130 mmHg and the development of renal insufficiency with serum creatinine value of 148 μmol/L. The personal history showed that the patient had subarachnoid bleeding in February 2008. Angiographic examination diagnosed the presence of an aneurysm of *a. communicans cerebri anterior.* Embolization was done.

Ultrasound examination revealed the right kidney of reduced-size (7 cm), with thin parenchyma (0.7 cm) and without doppler signal. The left kidney diameter was 11.2 cm, normal widh of parenchyma (1.6 cm) and intrarenal doppler examination showed that the RI was 0.41. Based on clinical features and the finding of low RI, the patient was suspected to having significant stenosis of the left renal artery. MSCT arteriography was performed and diagnosed the existence of the sclerotic right kidney with the graceful and ocluded right renal artery (Figure 3a). The left renal artery had numerous stenosis, with classic "string of beads" appearance (Figure 3b). By the intervention radiologist, the patient underwent PTRA with three dilations, but there was no satisfactory angiographic response, so a stent size 3 × 30 mm was placed (Figure 3c). Interventional procedure was performed without complications. At discharge the patient had normal value of serum creatinine which was 102 μmol/L and normal blood pressure of 120/80 mmHg, by applying an antihypertensive medication.

Case 4

A 37-year-old patient, with living-related kidney transplantation 9 months before, was admitted because of deterioration of the function of renal graft. Laboratory findings showed an increase in serum creatinine from 130 to 286 μmol/L, which coincided with the introduction of antihypertensive drugs from the group of ACE inhibitors. The extra renal doppler examination showed the peak sistolic velocity of 280 cm/s at the site of anastomosis of the renal and hypogastric artery. We performed percutaneous renal angiography



a) b) c)

Fig. 3 – The case 3: (a) ocluded right renal artery; (b) the left renal artery with the classic "string of beads" appearance before percutaneous transluminal renal angioplasty (PTRA); (c) the left renal artery after PTRA

and diagnosed the significant (narrowing of 85% lumen of artery) annular stenosis at the site of anastomosis. Interventional radiologist performed dilation with implantation of a stent size 5 × 20 mm. After the procedure there was a decline in serum creatinine, the value of which at discharge was 150 μmol/L. The patient was regularly controlled and graft function was stable. In June 2009, due to worsening of blood pressure, restenosis was suspected. MSCT angiography showed stent patency, with no signs of stenosis (Figure 4).

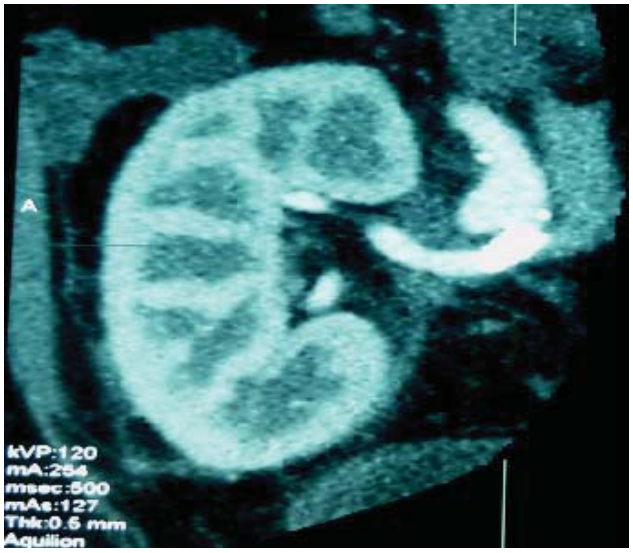


Fig. 4 – The Case 4: the renal artery of transplanted kidney without stenosis

Discussion

The optimal therapeutic treatment of ARAS is still unclear. Until now randomized clinical studies comparing the effects of combined application of balloon angioplasty with stent implantation and drug therapy as opposed to the application of drug therapy alone, have shown no significant higher survival rate of patients in the first group [14–16]. There is an ongoing largest, multicenter, randomized, controlled clinical trial on 1080 patients, CORAL (*Cardiovascular Outcome in Renal Atherosclerotic Lesions*) study, with better defined criteria for treatment of renal artery stenting, the aim of which is to avoid shortcomings of previous studies. The results of this study are expected by the end of 2011. Until completion of the results the CORAL study, endovascular intervention should be implemented only in patients with tight RAS of the single functioning kidney or with bilateral stenosis in patients with recurrent pulmonary oedema or when arterial hypertension is refractory to medication with rapid reduction of renal function. The presence of a small, sclerotic kidney is an obvious contraindication for endovascular intervention [17].

Two of the patients with atherosclerotic RAS were older, with signs of generalized atherosclerotic disease, including the existence of abdominal aortic aneurysm. In both patients there were clear indications for implementation of PTRA and stent implantation. In the first patient there was significant ostial stenosis of the single functioning kidney, followed by rapid deterioration of renal function and the development of hypervolemia with the signs of heart failure. Kane et al. [18] demonstrated that renal artery revascularization resulted in improved heart failure control and reduction in the number of hospitalizations. In the patient number 2 there was significant bilateral renal artery stenosis accompanied by refractory arterial hypertension and worsening of renal function. Ischemic damage led to no reduction in kidneys size. Upon completion of the treatment in both patients there was an improvement or normalization of renal function, so good control of blood pressure was achieved.

In contrast to ARAS, FMD is more common in younger female persons and changes typically occur in the middle or distal arterial segments [19]. The cause is less than 10% of cases of renovascular hypertension. In addition to renal artery, FMD can occur in the cerebral and visceral arteries, and may clinically manifest as arterial hypertension, stroke, abdominal angina and claudications. The incidence of unruptured intracranial aneurysms in patients with FMD vary widely, from 7% to more than 50% [20]. Percutaneous balloon angioplasty is a therapeutic modality of treatment in patients with poorly regulated arterial hypertension and renal failure. Stent implantation is applied in the case of obtaining suboptimal response or dissection of renal artery [21].

In our patient number 3 the diagnosis of renal artery FMD was preceded by subarachnoid hemorrhage caused by rupture of aneurysm *a. communicans cerebri anterior*. Renal artery stenosis is clinically manifested by resistant arterial hypertension and the development of acute renal failure. Although the loss of renal mass occures in up to 63% of patients with renal-artery FMD, renal failure is rare in these patients [22]. Occurrence of acute renal failure in our patients may be partly explained by the existence of a hypoplastic right kidney. After applying PTRA we obtained a suboptimal angiographic response, which was the reason for stent implantation. After the treatment the patient achieved normalization of blood pressure and kidney function.

Renal transplant artery stenosis is often an unrecognized vascular complication that can occur several months and years after kidney transplantation. The published incidence ranges from 1% to 23% depending on the criteria used for diagnosis [23]. It occurs more frequently at the anastomotic site compared with the distal part of donors artery [24]. Stenosis is usually manifested as difficult-to-treat hypertension, with deterioration of renal function, in the absence of rejection, recurrence of primary disease, calcineurins toxicity, infections and ureteral obstruction [25–28]. Duplex-Doppler examination is the ideal test for screening and follow-up of stenosis. Balloon-angioplasty is a therapeutic method of choice in comparison with surgical revascularization and drug therapy [29, 30].

In our patient number 4 the graft renal artery stenosis was diagnosed 9 months after the renal transplantation. Transplantation was complicated with endarterectomy of hypogastric artery of recipient and thrombosis at the anastomosis. It was the reason to do thrombectomy and reanastomosis. The above surgical complication probably represented the predisposing factor for the development of stenosis. Graft renal artery stenosis manifested with deterioration of its

function, which coincided with the application of drugs from the group of ACE inhibitors. Findings obtained by doppler examination indicated the presence of stenosis at anastomosis. We applied percutaneous selective angiography and confirmed the existence of significant annular stenosis of the renal artery at anastomosis. At that time our institution had no MSCT. After application of PTRA with stent implantation graft function improved. At outpatient control, the patient maintained stable graft function 7 years after the transplantation. The value of creatinine was 124 μmol/L at the last ambulatory control in June 2011.

Conclusion

In patients with acute or worsening chronic renal insufficiency, the existence of significant stenosis of one or both renal arteries should be considered. Timely application of PTRA in these patients leads to preservation of renal function.

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CASE REPORT

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Ambulatory phlebectomy under tumescent local anesthesia in a kidney-transplant patient

Ambulantna flebektomija u uslovima tumescentne lokalne anestezije kod bolesnika sa transplantiranim bubregom

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Abstract

Introduction. Tumescent local anesthesia (TLA) is widely used for ambulatory surgery. Patients with transplanted organs are on immunosuppressive therapy and with risk for organ rejection or severe infection. **Case report.** Saphenectomy with phlebectomy on the left leg under TLA was performed in a patient with kidney transplantation performed four years ago. A combination of 35 mg of 1% prilocaine-hydrochloride, 5 mL of 8.4% sodium bicarbonate and 500 μg of epinephrine in 460 mL of normal saline was used for TLA. Overall 750 mL of the solution was used. The patient had satisfactory postoperative analgesia and was discharged home on the same day. Blood levels of urea, creatinine, estimated glomerular filtration rate (eGFR) and tacrolimus concentration, measured preoperatively and on the second postoperative day, were in a regular range. Prilocaine blood concentrations determined on the 4th, 10th and 16th postoperative hours, were below toxic levels. **Conclusion.** TLA in a kidney-transplanted patient performed for saphenectomy with phlebectomy proved to be a safe and reliable anesthesia method.

Key words:
varicose veins; vascular surgical procedures; anesthesia, local; ambulatory care; treatment outcome; kidney transplantation.

Apstrakt

Uvod. Tumescentna lokalna anestezija (TLA) koristi se za ambulantne procedure. Nakon transplantacije organa bolesnici su pod rizikom od odbacivanja organa i teške infekcije. **Prikaz bolesnika.** Safenektomija sa flebektomijom na levoj nozi izvedena je u uslovima TLA kod bolesnika kome je četiri godine pre toga izvršena transplantaciju bubrega. Na osnovu kliničkog nalaza, kolor dopler ultrazvučnog prikaza i *Clinical Etiological Anatomical and Pathophysiological* (CEAP) klasifikacije doneta je odluka o neophodnosti hirurškog zahvata. Kombinacija 35 mg 1% prilokain-hidrohlorida, 5 mL 8,4% natrijum bikarbonata i 500 μg adrenalina u 460 mL fiziološkog rastvora korišćena je za TLA. Ukupno je primenjeno 750 mL rastvora. Analgezija je bila zadovoljavajuća, bez potrebe za dodatnim analgeticima. Nivo uree, kreatinina, stepen glomerularne infiltracije (eGFR) i koncentracija takrolimusa u krvi, mereni preoperativno i drugog postoperativnog dana, bili su u referentnim granicama. Koncentracija prilokaina u serumu određivana četvrtog, desetog i šesnaestog postoperativnog časa bili su ispod toksičnih nivoa. **Zaključak.** Tumescentna lokalna anestezija za ambulantno izvođenje safenektomije sa flebektomijom kod bolesnika sa funkcionalnim transplantiranim bubregom obezbeđuje adekvatnu perioperativnu i postoperativnu analgeziju bez komplikacija.

Ključne reči:
vene, varikozne; hirurgija, vaskularna, procedure; anestezija, lokalna; lečenje, ambulantno; lečenje, ishod; transplantacija bubrega.

Introduction

Tumescent local anesthesia (TLA) assumes subcutaneous infiltration of a large volume of diluted local anesthetics that provides extensive regional anesthesia of skin and subcutaneous tissue. The targeted tissue becomes swollen and firm, or tumescent. TLA is widely used in ambulatory surgery since it is a safe and reliable method with low complication rate [1–4].

TLA hypothetically can reduce the incidence of surgery and anesthesia complications in the patient on immunosuppressive therapy. Complications of general anesthesia

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are mostly associated with tracheal intubation [5]. Laryngeal mask airway has become a popular alternative to the endotracheal tube, but its use is not complication-free. The most serious complication is regurgitation of gastric content and possible aspiration [6]. Neuroaxial or regional anesthesia provides analgesia and reduce pulmonary complications. However, patients under immunosuppressive therapy after solid organ transplantation are rarely considered as candidates for neuraxial techniques as the risk of central nervous system infection is increased [7]. Risk of hemorrhagic or neurologic complications is higher in patients with altered immune status compared with healthy patients [7]. Immunodeficient-state patients are at increased risk for infectious complications [8].

Pharmacological characteristics of many drugs used for general anesthesia and during postoperative period can be modified by immunosuppressive medications [9]. Also, it was noted that perioperative massive fluid infusion can cause a significant tacrolimus blood level decrease [9].

TLA is a method with low percentage of complications. It provides analgesia in a postoperative period for up to 24 h [10–12].

We presented a case with performed phlebectomy under TLA in the patient with the history of kidney transplantation who suffered from verified varicose syndrome class III in accordance with clinical, ethiology, anatomic, pathophysiology (CEAP) classification of chronic venous insufficiency [13].

Case report

A 40-year-old patient (body weight 90 kg) was presented for surgical repair of varicose veins in the left leg. Four years earlier the patient underwent a successful living kidney transplantation. The patient was on triple immunosuppressive therapy including tacrolimus, mycofenolate mofetil and prednisolone.

Indications for phlebectomy as a surgical treatment were based on a clinical exam, color doppler sonography and CEAP class III classification. Blood concentration of urea, creatinin and tacrolimus level were assessed preoperatively and on the 2nd postoperative day. The estimated glomerular filtration rate (eGFR) was calculated using Modification of Diet in Renal Disease (MDRD) formula pre- and postoperatively ($eGFR=186 \times (S_{Cr})^{-1.154} \times (age)^{-0.203} \times (0.742$ for women) $\times$ (1.212 for black)) [14]. Normal values of eGFR in healthy male patients aged 20–40 years are from 90 to 138 mL min^{-1}.

A TLA solution, prepared before surgery by the surgeon, was consisted of 35 mg of 1% prilocaine-hydrochloride (Xylonest®, Astra Zeneca), 5 mL of 8.4% sodium bicarbonate and 500 μg epinephrine (0.5 mL) in 460 mL of normal saline. For infiltration 750 mL of the solution was used, a total amount of 525 mg prilocaine hydrochloride, or 5.83 mg kg^{-1}. Cefazolin 1.0 g intravenously (IV) was used preoperatively, and low molecular weight heparin, nadroparine 0.6 mL, postoperatively. Prilocaine-hydrochloride concentration in blood was tested 4, 10 and 16 hours after the surgery.

Surgical procedure took 65 minutes and went uneventfully. Postoperatively, there was no need for additional analgesia. Plasma prilocaine concentration was below toxic levels measured 4 hours (0.13 μg mL^{-1}), 10 hours (0.27 μg mL^{-1}) and 16 hours (0.06 μg mL^{-1}) after operation.

The preoperative and postoperative values of blood urea and creatinine levels, tacrolimus concentration (Po) and eGFR are shown in Table 1. After elastic bandage removing local findings were normal. After several hours of observation the patient was discharged home.

Table 1

Blood urea and creatinin levels, tacrolimus concentration and estimated glomerular filtration rate (eGFR) measured preoperatively and on the 2nd postoperative day

Parameters	Average values	
	preoperative	postoperative (the 2nd day)
Urea ($mmolL^{-1}$)	6.9	7.3
Creatinine ($mmolL^{-1}$)	91	93
Tacrolimus concentration ($ngmL^{-1}$)	5	5.1
eGFR ($mLmin^{-1}$)	85	83

Discussion

After organ transplantation surgery patients are on immunosuppressive therapy and every surgical procedure is considered to be a risk for transplanted organ rejection and serious infections. Preoperative risk assessment, optimal surgical treatment, and anesthesiology approach are carefully analyzed [15]. The presented patient with the history of kidney transplantation had varicose veins on his left leg CEAP class III with inappropriate response to noninvasive treatment. TLA seemed to be anesthesia of choice for this patient based on data from the literature becouse of lowest incidence of complications during and after the surgery compared with general anesthesia and neuroaxial blocks [16–18].

Prilocaine is amide-type and long-acting local anesthetic medium with high potency. Prilocaine has three times faster clearance compared to mepivacaine and 1.5 hour shorter half-life compared to other anesthetics. Prilocaine is mostly metabolized in lungs by amidase and to a lesser extent in the liver and kidneys [19, 20]. For phlebectomy the presented patient received a total dose of 525 mg prilocaine. That dose was sufficient to provide an analgesic effect, and at the same time, due to low concentrations in the tissue, absorption was slow, allowing sufficient time for plasma prilocaine metabolism [19]. Measured prilocaine plasma concentrations were far below the toxic threshold (5 mg mL^{-1}). With low concentrations of prilocaine, renal graft function was not compromised.

Moreover, blood concentration of immunosuppressive agents remained stable in postoperative period. Kidney-transplanted patients usually have depressed values of eGFR and frequently they have (second or third stage of graft failure). Based on preoperative value of eGFR (85 mL min^{-1}) the presented patient had second degree renal insufficiency, which stayed unchanged after the surgery.

Epinephrine added to the solution reduced intraoperative blood loss and obviously prolonged analgesia. In addition, the possibility of postoperative hematoma and wound infection was minimized. TLA took more than 24 hours, and there was no need for additional analgesia.

Conclusion

TLA with prilocaine in the presented kidney-transplanted patient proved to be a safe and reliable anesthesia method considering unchanged values of eGFR, stable tacrolimus blood concentration and low prilocaine blood levels.

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CASE REPORT

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Oxime and atropine failure to prevent intermediate syndrome development in acute organophosphate poisoning

Neuspeh sprečavanja razvoja intermedijernog sindroma kod akutnog trovanja organofosfornim insekticidima primenom oksima i atropina

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Abstract

Introduction. Intermediate syndrome (IMS) was described a few decades ago, however, there is still a controversy regarding its exact etiology, risk factors, diagnostic parameters and required therapy. Considering that acute poisonings are treated in different types of medical institutions this serious complication of organophosphate insecticide (OPI) poisoning is frequently overlooked. The aim of this paper was to present a case of IMS in organophosphate poisoning, which, we believe, provides additional data on the use of oxime or atropine. **Case report.** After a well-resolved cholinergic crisis, the patient developed clinical presentation of IMS within the first 72 h from deliberate malathion ingestion. The signs of IMS were weakness of proximal limb muscles and muscles innervated by motor cranial nerves, followed by the weakness of respiratory muscles and serious respiratory insufficiency. Malathion and its active metabolite were confirmed by analytical procedure (liquid chromatography-mass spectrometry). Pralidoxime methylsulphate, adiministered as a continuous infusion until day 8 (total dose 38.4 g), and atropine until the day 10 (total dose 922 mg) did not prevent the development of IMS, hence the mechanical ventilation that was stopped after 27 h had to be continued until the day 10. **Conclusion.** Continuous pralidoxime methylsulphate infusion with atropine did not prevent the development of IMS, most likely due to the delayed treatment and insufficient oxime dose but also because of chemical structure and lipophilicity of ingested OPI. A prolonged intensive care monitoring and respiratory care are the key management for the intermediate syndrome.

Key words:
poisoning; phosphoric acid esters; neurotoxicyty syndromes; atropine; oksimes; respiration, artificial; treatment outcome.

Apstrakt

Uvod. Intermedijerni sindrom (IMS) opisan je pre nekoliko decenija, međutim i dalje postoje kontroverze u vezi sa njegovom etiologijom, faktorima rizika, dijagnostičkim parametrima i potrebnom terapijom. S obzirom na to da se akutna trovanja leče u medicinskim ustanovama različitog tipa, ova teška komplikacija akutnih trovanja organofosfornim insekticidima (OFI) često se ne prepoznaje. Cilj rada bio je da se prikaže slučaj akutnog trovanja organofosfornim insekticidom koji će dati dodatne podatke o upotrebi oksima i atropina. **Prikaz bolesnika.** Nakon kupirane holinergičke krize kod bolesnika, 72 h od namerne ingestije malationa, došlo je do razvoja kliničke slike IMS. Znaci IMS su uključivali slabost mišića gornjih ekstremiteta i mišića inervisanih motornim kranijalnim nervima, što je bilo praćeno slabošću respiratorne muskulature i teškom respiratornom insuficijencijom. Malation i njegov aktivni metabolit potvrđeni su analitičkom procedurom (tečna hromatografija-masena spektrometrija). Kontinuiranom infuzijom pralidoksim metilsulfata do osmog dana (ukupno 38,4 g) i atropina do desetog dana (ukupna doza 922 mg), nije sprečen razvoj IMS, te je mehanička ventilacija, koja je prekinuta nakon 27 h, morala biti nastavljena do desetog dana. **Zaključak.** Kontinuiranom infuzijom pralidoksim-metilsulfata i atropina nije sprečen razvoj IMS, najverovatnije zbog odloženog početka lečenja i nedovoljne doze primenjenog oksima, ali i hemijske strukture i lipofilnosti ingestiranog OFI. Istaknut je značaj produžene opservacije u jedinici intenzivne nege i respiratorne podrške u lečenju intermedijernog sindroma.

Ključne reči:
trovanje; estri fosforne kiseline; neurotoksičnost, sindromi; atropin; oksimi; disanje, veštačko; lečenje, ishod.

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Introduction

The intermediate syndrome (IMS) is a delayed onset of muscular weakness and paralysis that occurs 1–4 days after the resolution of acute cholinergic syndrome in acute organophosphate (OP) poisoning [1]. It was first reported by Wadia et al. [2] in 1974 as the "type II paralysis after organophosphate poisoning". In 1987 Senanayake and Karalliede in 1987 termed this pattern of weakness as "intermediate syndrome" as the symptoms and signs occurre before OP induce delayed polyneuropathy. Clinically, IMS is characterized by acute paralysis and weakness in the territories of several cranial motor nerves, neck flexors, facial, extraocular, palatal, nuchal, proximal limb, and respiratory muscles. Although this syndrome has been described for decades, due to sometimes diverse clinical picture, it often remains undiagnosed, at least until the occurrence of significant respiratory weakness. The controversy exists regarding not only the question of whether IMS is a clearly defined entity, but also its exact etiology, risk factors, diagnostic parameters and required therapy [1–3].

Case report

A farmer, at the age of 54, was brought to the Emergency Department of a regional medical center in a coma, with miosis, muscle fasciculations, hypersalivation and rales on auscultation. His wife stated that he drank malathion 2 h earlier and explained that during the last month he had been depressed and refused to eat. After intubation, gastric lavage was done. Atropine 3 mg *iv* and infusions were administered. At admission to the National Poison Control Center (NPCC), 5 h after deliberate ingestion of malathion, the patients was in a coma, unresponsive to noxious stimuli. His vital signs were as follows: blood pressure 120/80 mmHg, pulse 40/min, and respiratory rate 26/min. Physical examination showed copious bronchial secretion and pinpoint pupils. Soon after admission, myoclonic leg jerks and respiratory insufficiency developed. During the first 30 min the patient received 12 mg of atropine and activated charcoal was administered. The patient was transffered to Intensive Care Unit (ICU) in NPCC where intermittent positive pressure ventilation (IPPV) was started and atropine continued as intravenous infusion. Pralidoxime methylsulphate (200 mg/h as a continuous infusion) and supportive treatment were administered. Routine laboratory tests were within normal limits, except for white blood cell count 18.9×10^9/L (reference range, $4.00–10.80 \times 10^9$/L). Blood gases showed acidosis (pH 7.212, pO2 123 mmHg, Sat O2 97.2%, pCO2 35.5 mmHg, ABE – 12.6 mmol/L, and lactate 1.1 mmol/L).

First measurement of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) showed activity of 1,806 U/L and 1,586 U/L. A significant reduction of activity of AChE 1198 U/L and BChE to 277 U/L occurred after 6 hours. Organophosphate and metabolites were confirmed in serum and urine by liquid chromatography-mass spectrometry, based on solid-phase extraction procedure, a chromatographic separation using an ACQUITY UPLC® HSST3 column and mass spectrometric detection in the positive ion mode. A mobile phase consisted of Solvent A (5 mM ammonium formate pH 3.0) and Solvent B (0.1% acetic formate in methanol), in a linear gradient (constant flow-rate 0.3 mL/min). Malathion was confirmed in blood in concentration of 1.21 mg/L on the day 1, and 0.22 mg/L on the day 2. In urine it was confirmed in concentration of 0.54 mg/L the day 1, and 0.34 mg/L the day 2, and malaoxon was detected in concentration of 0.12 mg/L and 0.13 mg/L respectively. After the first 27 h the patient was recovering and MV was no longer needed.

Two cholinesterase assays, monitored daily, showed that AChE did not correlate well with the severity of poisoning, whereas BChE was more sensitive index of OP poisoning (AChE 1806–1198–1273 U/L–1384 U/L–1295 U/L–1447 and BChE 1586–277–391 U/L–476 U/L–585 U/L–670 U/L).

After a well-resolved cholinergic crisis during the first 27 h, the generalized flaccid weakness of upper extremities and neck muscles, worsening of ophtalmoplegia, with progressive respiratory insufficiency was registered on the day 3, which was explained as intermediate syndrome. The repetitive nerve stimulation test (hypothenar – *n. ulnaris* system at 3 Hz), at admission showed insignificant compound muscle action amplitude (CMAP) decrement, that progressed to 30% of CMAP amplitude decrement on the day 5, related to post-synaptic failure of neuromuscular transmission. Pralidoxime methylsulphate therapy was administered until the day 8 with the total dose of 38.4 g, and atropine until the day 10 (total dose 922 mg). The mechanical ventilation was continued from the day 3 until the day 10. After that the of the patient condition improved steadily and on the day 21 he was disharged from the hospital.

Discussion

The pathophysiology of IMS, despite a high incidence (10%–68%), remains unclear [1]. Some clinicians suggest that IMS may result from inadequate oxime therapy (subdosage, shorter duration of therapy, modality of administration), albeit there are others who feel that oximes are not necessary or even deny the existence of IMS explaining delayed deterioration among OP-poisoned patients by hypo-atropinisation [3–5].

The case presented in this paper refer to the patients with severe malathion poisonings, admitted to hospital more than 5 h after ingestion, implying possible risk for poor response to the therapy. Gastric lavage was made in the local hospital, 2 h after the ingestion. The American Academy of Clinical Toxicology and the European Association of Poison Centers and Clinical Toxicologists position paper suggests that "gastric lavage should not be performed routinely, but it should be considered only if a patient has ingested a potentially life-threatening amount of a poison, and the procedure can be undertaken within 60 minutes of ingestion" [6]. Administration of activated charcoal in conventional doses, is generally recommended for reducing further absorption of OP pesticides, and all of our patients received it in single doses. During the last few years much has changed in toxicology

and some new data from a randomized controlled trial (RCT), aimed to assess efficacy of routine treatment with multiple-dose activated charcoal, showed no benefit compared to a treatment with the single use of charcoal [7].

Pralidoxime methylsulphate was applied as a continuous infusion (200 mg/h), but it could not prevent the development of IMS. The explanation for that might be a delayed treatment and insufficient pralidoxime dose (200 mg/h) but also the chemical structure and lipophilicity of ingested OP. The clinical usefulness of oximes has been challenged for decades by toxicologists throughout the world [8–9]. The paucity of data from RCT, disparate results with oxime treatment ranging from benefit to harm, could be explained by substantial delay to the treatment, type and dose of OP, and different therapeutic protocols that included pralidoxime in doses from 1g every 6 h to 1g per h. Only one RCT so far compared the World Health Organization (WHO) recommended doses (30 mg/kg/h followed by 8 mg/kg/h continuously) with placebo. This trial showed no clinical benefits and a trend towards harm, despite a clear evidence that reactivation of acetylcholinesterase was achieved [9].

There are other proposed mechanisms of IMS which include different susceptibility of various cholinergic receptors, muscle necrosis, downregulation or desensitization of postsynaptic acetylcholine receptors, failure of postsynaptic acetylcholine release, and oxidative stress-related myopathy [10–14]. In our patient, who had nicotinic sings of OP poisoning, the level of creatine phosphokinase was normal as well as the level of aspartate aminotransferase normal, so this could not have been the cause of IMS.

The presented patient developed the clinical picture of IMS within the first 72 h after the ingestion of OP formulation in a suicide attempt. Though other signs of IMS poisoning were present, it was the respiratory insufficiency that drew medical attention to the onset of the syndrome. At that time, no significant decrease of AChE and BChE compared to the initial values was registered.

Repetitive stimulation test in the patient with malathion poisoning showed post-synaptic failure of neuromuscular transmission, implying the development of IMS, in accordance with the study of Jayawardane et al [15]. In a prospective study of 70 patients with OP poisoning, the authors identified a series of stereotipic electrophysiological changes associated with IMS.

The risk of death in IMS is as high as it is in the cholinergic crisis. A prolonged clinical medical supervision after recovery from the cholinergic crisis is necessary because of the risk of IMS appearance. Based on this presentation, that the cornerstone of IMS management is supportive therapy, essentially directed towards the treatment of rapidly developing respiratory distress and respiratory failure. Any delay in instituting mechanical ventilation will result in death [1–3, 15]. However, whilst it is not expected that atropine therapy would merit in the IMS, as the symptoms and signs are clearly not muscarinic, oxime given during the cholinergic crisis might be effective if it reactivates AChE. However, the optimal oxime dose is yet to be determined [16–17].

Conclusion

IMS is a major contributing factor of morbidity and mortality related to organophosphate poisoning. In our patient, continuous pralidoxime methylsulphate infusion did not prevent the development of IMS, most likely due to the delayed treatment and insufficient oxime dose, but also the chemical structure and lipophilicity of ingested OP. Although the efficiency of atropine and oxime in IMS is limited, the administration of these drugs, after early aggressive gastrointestinal decontamination, is recommended to be continued for a longer period. However, prolonged clinical medical supervision and respiratory care are specifically emphasized as the key of management for the intermediate syndrome.

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Writing biomedical research papers in English - a challenge for non-Anglophone authors

Pisanje biomedicinskih istraživačkih radova na engleskom jeziku - izazov za autore kojima engleski jezik nije maternji

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Ključne reči:
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Introduction

English has emerged as the main language for publication of scientific and medical research and is often used in international gatherings of specialists in biomedicine. This trend facilitates smoother communication between scientists and, consequently, more rapid progress in science. Because English is recognized as the primary medium of international specialized publication, non-English speaking scientists like rather to publish in English than in their native language. However, rules for scientific writing are not always easy to follow for authors writing papers in a foreign language.

Clear writing is essential for effective convenience of information in written form, but one of the major problems in scientific communication in English is the correct use of this language by authors for whom English is not a mother tongue. There are at least two reasons for this [1]: the first, to be sure that you yourself know what you mean and the second, to be sure that you get your message across to your reader. Many books and papers have been written on how to improve style and publish scientific papers in English. The aim of this paper was to provide guidelines on how to achieve clarity in biomedical scientific writing in English for non-English speaking (non-Anglophone) authors. These guidelines refer to: writing style (choice of simple, precise and, whenever possible, short words; proper use of tense and voice (active or passive); mechanics (avoidance of unnecessary words and phrases, abstract terms, jargon, and excessively long compound terms, so-called "freight-train phrases"; and, avoidance of non-English expressions and grammatical errors.

Medical research papers – style and structure

The term style has at least two meanings [3]. In its literary sense, style is a manner of language expression, such as a "prose style", or "writing style" can be used. However, style is also used to denote in more specific terms the custom followed in punctuation, abbreviation, capitalization, reference citation, format and content. This is known as "publication style" [2], or "house style" [3].

Writing style helps achieve brevity and clarity [4]. Many publications recommend how to accomplish that goal [1–4]. Some suggestions are intended for non-Anglophone authors and refer to the proper use of English. More precisely, they indicate the need not only for grammatically correct English, but also for simple and clear expressions that are readily understood by readers, regardless of their native language.

Scientific language does not need complicated or convoluted expressions, or words transliterated or derived from other languages where English words suffice [5]. For example, it is preferable to say "now" instead of "at the present moment" or "at this point of time"; "because of" instead of "as a consequence of"; "most" instead of "a majority of"; "believed" instead of "was of the opinion that", etc [6]. There are many more examples of how inexperienced authors mistakenly think that pretentious and abstract words will improve the scientific content of their papers; they forget the importance of simple and short words for the necessary clarity of scientific communication [6].

Authors should select words that accurately, precisely and correctly convey the intended meaning (Table 1) [2]. Certain words can be confused or misused for various reasons: some sound the same although they are spelled differently and have different

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meanings (homophones). Mistakes can occur if the author is uncertain of the spelling and misuses one word for its homophone pair. The most common errors occur when authors fail to make precise distinctions among words of similar meaning. Table 1 gives some examples of real or apparent synonyms, together with their correct usage. "Which" and "that" are often misused, and their incorrect usage can change the meaning of the sentence substantially. "That" begins an essential adjectival clause (fundamental to the meaning of the sentence), and "which" begins a non-essential adjectival clause (one that merely adds interest to the sentence and could be omitted) [4]. Table 1 gives examples: the first sentence where "which" indicates that all oral bacteria are sensitive to penicillin compared to the second sentence where "that" indicates that only some oral bacteria are sensitive to penicillin, and that they cause dental infection.

Table 1

Similar words and their precise use*

Words	Meaning / Explanation	Examples
among	relationship involving more than two units of the same kind	Among oral *penicillins*, amoxicillin is the best choice
between	relationship involving two units of the same kind	They chose an appropriate antibiotic between *penicillin* and *cephalosporin*
as	has temporal sense	As we were completing the paper, new evidence came to light
because	shows cause	Because clinical experience in patients with severe liver disease is limited, caution should be taken when administering the drug.
since	preferably shows temporal relation	He has done nothing since he recovered.
compared to	compared, to emphasize contrast	Compared to us, they have achieved much better results
compared with	looking for similarities or differences	*Lidocaine* was compared with *procaine*
majority	a number of items greater than half of total	The majority of the patients had received prior chemotherapy.
most	preferred when quantitative expression is not needed	Most operations are successful. The most he can hope for is a symptom-free interval.
presently	currently, soon, shortly	The MR machine is presently out of order.
at present	now	No effective drug is available on the market at present.
varying	changing	Because of the varying prices medical material has become very expensive.
differing	to have unlike characteristics	The two methods, although differing greatly in their technology, are equally used in practice.
different	to have unlike characteristics	Different therapies are used for cancer treatment.
which	used in non-restrictive sense	Oral bacteria, which are sensitive to *penicillin*, also cause dental infections
that	introduces an essential clause	Oral bacteria that are sensitive to *penicillin* also cause dental infections
while	indicates a period of time under consideration	While there is life there is hope.
although	should be used for a conditional state	Although breast cancer maps provide visuals, they don't tell the whole story.

*** Adapted from: "The CBE Manual for Authors, Editors, and Publishers" [2].**

Short sentences are the crux of good scientific writing [4]. Sentences with fewer words not only convey their meaning clearly at first reading, but also provide fewer opportunities for non-English constructions and grammatical errors. Short sentences provide text clarity and make it easier for authors to follow basic linguistic rules.

Objectivity

Information and facts are more important than personal opinions. It is the task of the writer to address the topic in an objective manner. An objective style puts a certain distance between the writer and the arguments proposed.

Clarity

Apart from accurate reporting the results of the study, clarity is the most important element in medical scientific writing. The reader should be told why the study was performed and what the research is about (introduction), what was done (material and method), what was found (results) and what the results mean (discussion). This presentation style is known as the Introduction, Material and Method, Results and Discussion (IMRaD) structure. A paper with the IMRaD structure, is generally preceded by an abstract.

Abstract – An abstract provides a shortened version of the full paper. Since abstracts may be reprinted without the full paper, they must be self-explanatory. Abstracts describe the purpose of the research, how the research was conducted, what the main findings were, any limitations of the applied method, what the findings mean and what can be recommended for further research. Abstracts do not include information not in the paper itself, tables or diagrams, or citations of other work.

There are two kinds of abstract. A descriptive abstract tells what is in the paper; what the author will attempt to prove, rather than a synopsis of the results. It is appropriate for longer papers, such as review articles and can be written before the paper itself is drafted. An informative abstract not only describes what is in the paper, but also summarizes factual information, including methods, results and conclusions. This type of abstract is suited to reports about original

research and is usually written after the paper is finished. A structured abstract, similar to the informative one, follows the IMRaD formula but uses specific content headings instead of a single paragraph format.

Introduction – The introduction to a research paper presents the topic in general and expresses the central research question or hypothesis to be proved through evidence and examples. It should tell readers why the study was done and why it is important. Only those references that are essential to justify proposed study should be cited.

Material and Method(s) – This section of a research paper describes all of the specific method used. Every detail is important and must be completely documented so that other researchers can repeat the studies and verify the results. The failure to list relevant variables will call into question the reported results and conclusions. A writer should consider three basic questions: Where? (location of the work, if relevant); What? (equipment and other materials used); How? (procedures and methods used in the research).

How could the research be done differently to verify the findings?

Conclusion – This is an optional part of the research paper. It can summarize the main points and the obtained results.

The Proper Use of Tenses – the problem of tense is not merely a grammatical point. It relates to style of particular sections of the research paper. The convention commonly in use requires that the present tense be used to quote previously published work as a sign of respect for established knowledge. When referring to one's own work, the past tense should be used, as this work is not presumed to be established knowledge until after it has been published [7]. Generally, the Abstract is written in the past tense because it refers to the author's present results. Likewise, the Material and Method and Results sections should use the past tense. On the other hand, much of the Introduction and Discussion should be in the present tense (Table 2).

Table 2

Tenses in scientific writing*

Section	Correct use of tense
Abstract	The antimicrobial activity of three root canal sealers on five standard bacteria strains was tested
Introduction	The root canal sealers have antimicrobial activity against some bacteria
Material and Method	The antimicrobial activity of three root canal sealers was tested against five standard bacteria strains
Results	The tones of inhibition were greatest with Endomethasone against all of the tested bacteria
Discussion	Antimicrobial activity of Endomethasone against oral bacteria is doubtful

*** Adapted from "Todorović G, Matejašev S, Todorović Lj. How to Make Writing in English Easier for Non-Anglophone Authors. *Balk J Stom*, 2003; 7:66-70"[7]**

Results – This section presents the data and findings from the research. Data may be effectively presented in charts, tables, graphs, diagrams, or figures, which should be accompanied by explanatory text. Descriptions within this section may refer to trends or preference. Some of the useful vocabulary items for describing tables and graphs include: "to increase, to rise, to grow, to improve, to go up"; or "to decrease, to fall (off), to drop, to decline, to go down, to slip"; "to remain stable, to stay at the same level, to remain constant, to stagnate, to stabilize". The degree and speed of change may be described by some of the following adjectives and adverbs: "dramatic/dramatically", "considerable/considerably", "slow/slowly", "significant/significantly", "quick/quickly", "slight/slightly", substantial/substantially", "sudden/suddenly", "rapid/ rapidly", "moderate/moderately", "steady/steadily", "gradual/ gradually".

Discussion – The discussion section may restate the hypothesis or intent and follow with the interpretation of findings and an evaluation of the research. It determines whether the work supported the hypothesis or failed to do so. This section may also discuss the limitations of various research methods and how the studies might be done differently. It considers the following questions: 1) Did the research support the hypothesis? 2) What interpretations can be made from the results? 3) Were the research methods adequate? 4)

Grammar Matters

For some, grammar is a mystery or a collection of incomprehensible rules; for others, it is about knowing why something reads badly and how to fix it [6]. Although most native-English speakers simply "know" when a sentence reads well, non-English authors must learn certain rules of grammar to help them write efectively [4]. Apart from learning the basic rules of grammar, the best way to avoid making mistakes in English is to analyse the troublesome sentences and errors after correction by reviewers.

Authors who do not distinguish between singular and plural forms of nouns often fail to match subjects and predicates correctly [7]. Such errors are most frequently made with words taken from other languages, especially Latin-derived nouns. Plural endings of these words differ from the English, although there are some anglicised forms, such as index-indices/indexes, fungus-fungi/funguses (Table 3, section a).

The agreement in number between subject and predicate is also a problem in sentences containing numerals [6]. For example, verbs should be in the plural for all values greater than one, even if these are less than two (Table 3, section b). Noun-verb agreement also pertains to fractions.

Gerund (the -ing form with features of both noun and verb) can sometimes be substituted by an infinitive. How-

ever, the gerund, and not the infinitive, should be used in these instances [8]: a) after words followed by preposition; b) after verbs such as avoid, risk, or stop; c) after some adjectives (busy, worth); and d) after certain phrases - look forward to, or it's no use (Table 4).

The frequent use of the passive voice in medical writing is impersonal and objective and creates a certain distance between the writer and the arguments proposed.

Unneeded Words and Phrases, Abstract Nouns, and Jargon

Lengthy sentences are tiresome to read [6]. The reader has to search for the main message while trying to remember and place all of the subtopics and asides [3]. Table 5 gives examples for using unneeded and wordy phrases.

Table 3

Agreement in number between subject and predicate*

	Incorrect	Correct
a	Words are media of expression.	Words are a medium for expression.
	Patients with following criteria i**s** not eligible for randomisation.	Patients with following criteria are not eligible for randomisation.
	Drug resistance phenomena was recognised very early in the history of cancer chemotherapy.	Drug resistance phenomena were recognised very early in the history of cancer chemotherapy.
b	Twenty percent of time are spent on administration.	Twenty percent of time is spent on administration.
	Four-fifths of the area are contaminated.	Four-fifths of the area is contaminated.
	A number of respondents was verbose in their answers.	A number of respondents were verbose in their answers.
	The number of respondents were surprising.	The number of respondents was surprising.

*** Adapted from: Todorović G, Matejašev S, Todorović Lj. How to Make Writing in English Easier for Non-Anglophone Authors. *Balk J Stom*, 2003; 7:66-70[7]**

Table 4

Misuse of the infinitive*

	Incorrect	Correct
a	He is a man capable to judge art.	He is a man capable of judging art.
	We insist to check all records.	We insist on checking all records.
	You should not risk to get your life in danger.	You should not risk getting your life in danger.
	He can't stop to talk about his illness.	He can't stop talking about his illness.
b	He was busy to get ready for his journey.	He was busy getting ready for his journey.
	His books are not worth to read.	His books are not worth reading.
c	I always look forward to hear from you.	I always look forward to hearing from you.
	It's no use to ask her for an advice.	It's no use asking her for an advice.

*** Adapted from: Todorović G, Matejašev S, Todorović Lj. How to Make Writing in English Easier for Non-Anglophone Authors. *Balk J Stom*, 2003; 7:66-70 [7]**

Table 5

Unneeded words and phrases

Verbose	Concise
it is reported by Smith that	Smith reported
are of the same opinion	agree
as a consequence of	because
as far as our own observations are concerned, they show	we observed
despite the fact that	although
was of the opinion	believed

Modal verbs are also frequently used for hedging, or expression of tentativeness and possibility. This allows the author to present statements with appropriate accuracy and caution, by expressing possibility rather than certainty and prudence rather than overconfidence [9–11]. Hedging plays a major role in medical discourses [12, 13] where the accreditation of knowledge depends on the consensus of the research community. Where evidence must be evaluated, and there is a need to comment on its reliability, hedging helps to avoid potentially hostile responses, and it may facilitate acceptance of research claims. Research writing is necessarily a balance of fact and evaluation as the writer tries to present information as fully, accurately, and objectively as possible. Alternatively, a writer may wish to anticipate the possible negative consequences of being proven wrong and a claim disputed [10, 13, 14].

Abstract nouns formed from verbs (by adding "ion" at the end of the word) increase sentence length unnecessarily because of the need to add prepositions and verbs [6]. Examples include "interpretation" from "interpret" or "production" from "produce", etc. Replacing abstract nouns with their equivalent verbs makes the sentence more vivid [3] (Table 6).

Jargon [3] is often characterized by slang or obscure meaning. It is always preferable to use simple English words instead of foreign words, phrases or jargon [6]. For example, it is better to say "the patient could walk" than "the patient was mobile", or "arms and legs" than "upper and lower extremities". Furthermore, the use of informal idiom in a scientific paper can be quite unintelligible to many readers, especially the non-native English speaker.

Table 6

Replacement of abstract nouns*

Sentences with abstract nouns	Sentences without abstract nouns
A direct correlation between serum antibiotic concentration and resolution of infection was seen	The resolution of infection correlated directly with the serum antibiotic concentration
Following termination of the treatment, there was a substantial decrease of pain, resolution of bone infiltrates, and partial improvement of function	After the treatment, pain greatly decreased, bone infiltrates resolved, and function partially improved

*** Adapted from: Todorović G, Matejašev S, Todorović Lj. How to Make Writing in English Easier for Non-Anglophone Authors. *Balk J Stom*, 2003; 7:66-70 [7].**

"Verbosity" – the use of long instead of one-syllable words [7], should also be avoided. Words and phrases often used in medical conversation, such as "blood sugar" (glucose concentration in blood), should be avoided in scientific writing, as well as terms like "diabetics", "psychotics" and similar labelling of participants in the study. It is better to write "patients with diabetes" than "diabetic patients" even though the first expression is longer. The word "participant" is frequently used in clinical studies. The terms "subjects" and "individuals" are acceptable, but the term "participants" is more correct because it reflects the role of people in the research process [13]. Throughout papers on clinical studies, authors should refer to their patients rather than cases, and they should be careful not to dehumanise their participants (patients) by using the wrong pronoun. For example, it is correct to write "participants who" and dehumanising to write "participants that".

Un-English Expressions

When writing in English, non-Anglophone authors should always consider the need to transmit content, i.e. meaning and essence of the sentence rather than its form [6]. Errors often occur when authors translate expressions from their native language directly into English, following structure rather than meaning.

To sum up instead of a conclusion

Medical writing is a particular skill set in scientific communication. To accommodate an international readership, it needs to be clear and concise, and written in plain English, with the reader in mind. Research that furthers the progress of science deserves to be presented in the best possible way. The simple guidelines for the grammar and language of written biomedical communication described in this paper are intended to help authors improve the style and structure of their medical research papers. Acknowledging many difficulties of writing in a foreign language, before submitting a paper to an English-language journal, a non-English author is advised to seek review by a reader who knows the English idiom well [3]. This final step will ensure that the contents of papers are clear and enjoyable to read for a wide professional audience.

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I N M E M O R I A M

prof. dr sc. med. MIODRAG ODAVIĆ pukovnik u penziji (1930–2012)

U Beogradu je 30. 11. 2012. godine preminuo pukovnik u penziji, profesor dr sc. med. Miodrag Odavić, višegodišnji član kolektiva Vojnomedicinske akademije (VMA) u Beogradu.

Rođen je 12. 02. 1930. godine u službeničkoj porodici u Vršcu, gde je završio osnovnu školu i realnu gimnaziju. Otac mu je bio profesor književnosti i latinskog jezika u toj istoj gimnaziji. Kao vojni stipendista, profesor Odavić je 1956. godine diplomirao na Medicinskom fakultetu u Beogradu. Nakon poslediplomskog staža u VMA 1957. godine odlazi u Sanitetsku oficirsku školu, posle čega biva upućivan u trupnu službu u Garnizon Raška, a zatim u Sanitetski centar u Kragujevcu. Od 1963. do 1967. godine bio je na specijalizaciji iz interne medicine u VMA. Nakon toga odlazi u Vojnu bolnicu, Skoplje.

Godine 1969. dolazi u Odeljenje za nuklearnu medicinu Klinike za unutrašnje bolesti VMA (današnji Institut za nuklearnu medicinu VMA). Dalje se obrazuje u oblasti nuklearne medicine u INN Vinča, kao i u centrima nuklearne medicine u Zagrebu, Ljubljani, Sarajevu, Skoplju i Beču. Godine 1970. formirao je radioimunološku laboratoriju u okviru Odeljenja i uveo brojne RIA analize za određivanje koncentracije mnogobrojnih hormona i drugih biološki aktivnih supstanci. Kasnije se posebno bavio dijagnostikom karcinoma štitaste žlezde, kao i imunoscintigrafijom.

Od 1972. godine do penzionisanja prof. Odavić učestvuje u svim vidovima nastave u VMA, u Centru za permanentno obrazovanje Institut za nuklearne nauke „Vinča“, kao i na raznim seminarima i kursevima za zdravstvene radnike. Na tom polju istakao se kao vrstan nastavnik, s obzirom na njegovu elokvenciju i sposobnost da jasno izlaže.

Polovinom 1970-ih godina uključuje se u naučnoistraživački rad u Institutu rukovodeći i učestvujući u više naučnoistraživačkih zadataka. U tom periodu nastala je i njegova doktorska disertacija koju je odbranio u martu 1978. godine. Njegova posvećenost nauci ogledala se u činjenici da je neke eksperimente stresa hipoksije, koja je bila predmet njegove disertacije, izvodio na sebi.

Profesor Odavić sve vreme svog rada učestvuje na sastancima posvećenim nuklearnoj medicini u Jugoslaviji. U periodu od 1973. do 1991. godine učestvuje sa oko 30 radova na evropskim i svetskim kongresima nuklearne medicine. Neki od tih radova su posebno isticani kao značajan doprinos nuklearnomedicinskoj nauci. Objavio je preko 190 stručnih i naučnih radova u zemlji i inostranstvu, učestvovao u pisanju 14 priručnika i monografija za specijalizante nuklearne i interne medicine.

Bio je član Srpskog lekarskog društva od 1956. godine, a od 1969. bio je na raznim dužnostima u Sekciji za nuklearnu medicinu čiji je i bio predsednik u dva mandata. Bio je član predsedništva Udruženja za nuklearnu medicinu Jugoslavije, kao i član Evropskog udruženja nuklearne medicine.

Dr Odavić izabran je za docenta za predmet Interna medicina 1981. godine, za vanrednog profesora 1987, a za redovnog profesora 1990. godine.

Za načelnika Instituta za nuklearnu medicinu postavljen je jula 1984. godine i tu dužnost obavljao je sve do penzionisanja 1994. godine.

Pored mnogobrojnih stručnih i društvenih priznanja, prof. Odavić šest puta dobio je vojna odlikovanja, a 1991. godine i najvišu vojnu nagradu za dostignuća u naučnoistraživačkom radu – Nagradu 22. decembar.

Profesor Odavić potekao je iz poznate hercegovačke porodice u kojoj su mu bliži i dalji rođaci bili književnici, pesnici, kulturni radnici i lingvisti. S obzirom na zanimanje oca u rodnoj kući, posebna pažnja bila je posvećena književnosti, pisanoj reči i govoru sa insistiranjem na čistoti srpskog jezika. Profesor Odavić 1972. godine počinje ozbiljno da se bavi lingvistikom uopšte, a posebno medicinskom terminologijom. U toku toga rada počinje intenzivnu saradnju sa prof. dr Aleksandrom Kostićem, koji mu je bio uzor i napismeno ostavio u amanet da „brani i odbrani našu medicinsku terminologiju“ od najezde tuđica. Kao rezultat toga rada jula 2001. godine profesor Odavić, kao svoje životno delo, objavljuje Enciklopedijski latinsko-srpski medicinski rečnik.

Često se, nažalost, dešava da ne shvatimo vrednost čoveka dok je među nama. Nestankom prof. Odavića njegove vrednosti kao da izbijaju na videlo. Dok je prof. Odavić prikupljao građu za rečnik, nismo bili svesni značaja tog dela. Evo nekoliko citata iz recenzija koja ga, možda, najbolje odslikavaju:

„Ne čekajući da sličan medicinski rečnik urade timovi stručnjaka ili određene institucije, profesor Odavić je, zahvaljujući svojoj fanatičnoj odanosti, a posebno i istinskoj zaljubljenosti u lepotu srpskog jezika, sam završio ovo monumentalno delo.” (Dejan Medaković)

„Ubeđen sam da Medicinski rečnik profesora Miodraga Odavića po svom obimu, sveobuhvatnosti, detaljnim i jasnim opisima pojmova prevazilazi mnoga slična leksikografska izdanja u svetu.” (Prof Zlatimir Kecmanović)

„Prof. dr Miodrag Odavić je autor silne intelektualne snage, velikog znanja i plemenitog entuzijazma, čije će kapitalno delo biti uvršćeno u najveću dragocenost ne samo naše medicinske nauke, već i našeg jezika i naše duhovnosti uopšte.” (Prof dr Vladimir Ilić)

„Sažeto rečeno, ovakva dela služe na čast ne samo autoru, nego i naciji i jeziku na kome su nastala.” (Dr sc. Drago Ćupić)

Profesor Odavić sa suprugom Mirom, koja je preminula tri meseca pre njega, živeo je u srećnom dugogodišnjem braku u kom su dobili sina Darka, danas poznatog oftalmologa (sa suprugom i ćerkom živi i radi u Beogradu).

Mi, njegovi saradnici, zbog svega rečenog o njemu, kao izuzetnom stručnjaku i dobrom čoveku, zadržaćemo ga u trajnoj i prijatnoj uspomeni sa osećanjem dubokog poštovanja prema njegovim naporima, njegovom delu i zaslugama za uspešni rad Instituta za nuklearnu medicinu VMA.

Neka mu je večna slava i hvala!

prof. dr sc. med. Boris Ajdinović,
načelnik grupe Instituta za dijagnostiku i terapiju VMA

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Na drugoj stranici nalazi se strukturisani apstrakt sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **uvod i cilj** rada, osnovne procedure - **metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi - **rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt (**250** reči) ima podnaslove: *uvod/cilj, metode, rezultati* i *zaključak*. Za apstrakte na engleskom dozvoljeno je i do **450** reči. Strukturisani apstrakt je obavezan za metaanalize (istog obima kao i za originalne članke) i kazuistiku (do 150 reči, sa podnaslovima *uvod, prikaz slučaja i zaključak*). Ispod apstrakta, pod podnaslovom „Ključne reči“ predložiti 3–10 ključnih reči ili kratkih izraza koji oslikavaju sadržinu članka.

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

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Primeri referenci:

Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

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

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Examples of references:

Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinska-Mladenovska O, Arsov T, Strezova A, et al. Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl 2003; 60(6): 657–612.

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

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Časopis „Vojnosanitetski pregled“ izlazi godišnje u 12 brojeva.

Godišnja pretplata za 2013. godinu iznosi: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za strane državljane i ustanove. Pretplate: žiro račun br. 840-314849-70 MO – Sredstva objedinjene naplate – VMA (za Vojnosanitetski pregled), poziv na broj 12274231295521415. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om). Za zaposlene u MO i Vojsci Srbije moguća je i pretplata u 12 mesečnih rata putem trajnog naloga, tj. „odbijanjem od plate“. Popunjen obrazac poslati na adresu VSP-a.

PRIJAVA ZA PRETPLATU NA ČASOPIS „VOJNOSANITETSKI PREGLED“

Ime i prezime ili naziv ustanove	
Jedinstveni matični broj građana	
Poreski identifikacioni broj (PIB) za ustanove	
Mesto	
Ulica i broj	
Telefon / telefaks	

Pretplata na časopis „Vojnosanitetski pregled“ (zaokružiti):

1. Lično. Dokaz o pretplati dostavljam uz ovu prijavu.
2. Za pripadnike MO i Vojske Srbije: Dajem saglasnost da se prilikom isplate plata u Računovodstvenom centru MO iz mojih prinadležnosti obustavlja iznos mesečne rate (pretplate).
3. Virmanom po prijemu profakture.

Datum _____	Potpis _____