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Alzheimer's disease (AD), a chronic neurodegenerative disease, accounts for most cases of dementia. It usually starts slowly with difficulties in remembering recent events, as the most common early symptom, and, as the disease advances, gets worse including disorientation, problems with language, mood swings, loss of motivation and behavioral issues. The cause of AD is yet poorly understood and there are no treatments that can stop or reverse its progression, although may temporarily improve some symptoms. The disease most often begins in those over 65 years of age. Since the human life extended, AD is becoming increasingly present. In order to draw public attention to its growing presence, since 1994, 21 September, and since 2012, the entire month of September have been dedicated to this disease and celebrated as the World Alzheimer's Day, and World Alzheimer's Month, respectively (see Editorial, p. 763–4).

Alchajmerova bolest (AB), hronično neurodegenerativno oboljenje, obuhvata najveći broj demencija. Ima spor početak, sa teškoćama u pamćenju skorijih događaja, kao najčešćim ranim simptomom, a kako bolest napreduje, javljaju se i drugi simptomi poput dezorijentacije, problema sa govorom, promenama raspoloženja, gubitkom motivacije i bihevioralnim problemima. Uzrok AB još uvek nije dovoljno razjašnjen i za sada ne postoji način da se bolest zaustavi ili da dođe do njene reverzije. Bolest se najčešće javlja kod osoba starijih od 65 godina. Kako se ljudski vek produžio, AB postala je sve prisutnija. Da bi se skrenula pažnja javnosti na njenu sve veću zastupljenost, od 1994. godine, 21. septembar, a od 2012. godine i čitav mesec septembar posvećeni su ovoj bolesti i obeležavaju se kao Svetski dan Alchajmerove bolesti, odnosno Svetski mesec Alchajmerove bolesti (vidi Uvodnik, str. 763–4).

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

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Alzheimer dementia – a problem of individuals, families, medicine and society

Alchajmerova demencija – problem pojedinca, porodice, medicine i društva

Smiljana Kostić

Neurology Clinic, Military Medical Academy, Belgrade, Serbia

Since ancient times the term dementia, derived from the Latin word demeans ("no mind"), has marked a specific, acquired state of impaired intellectual and mental abilities of an adult which interfere with his her social and working abilities, making him her dependent on others.

At the beginning of the 2nd century Roman poet Junius Juvenal described how the weakness of the mind is worse than any physical illness, "*Sed omni membrorum damno maior dementia, quae nec nomina servorum nec vultum agnoscit amici cum quo praeterita cenavit nocte, nec illos quos genuit, quos eduxit*". ("But worse than any loss in body is the failing mind which forgets the names of slaves, and cannot recognize the face of the old friend who dined with him last night, nor those of the children whom he has begotten and brought up").

According to the assessment of the group of experts commissioned by the Alzheimer Disease International in 2005 about 24 million people suffered from dementia at that time, with 4.6 million new cases arising every year [1]. The lifespan of the world's population has extended and there is a trend of increase of the elderly population. It is predicted that in 20 years the number of people affected by dementia will be nearly doubled. A frightening prediction says that by the year 2050 115 million people will be suffering from this disease and that 1 out of 85 persons will be demented.

Alzheimer dementia (AD) is the most common and accounts for about 70% of all dementias. The prevalence rates increase with age and double every 5 years after age 65.

Based on its age of onset, AD is classified into early onset AD, before the age of 65, accounting for 1–5% of all cases, and late-onset AD, after the age of 65, which occurs in 95% of patients.

Although there are no significant differences in the clinical symptoms of the two types of the disease, it is considered that the early onset AD is connected with faster progression and a different inheritance patterns. Mutations in three genes (APP, PSEN1, PSEN2) that participate in the encoding of proteins involved in the pathophysiological processes of the synthesis of the amyloid precursor protein and amyloid-beta (Aβ) are inherited autosomal dominant with high penetrance. In late-onset sporadic form there is an increased tendency towards manifestations of the disease in relatives of the patients, and the risk is doubled if the disease exists in the first instance relatives.

AD is result of a progressive neurodegenerative process whose pathological characteristics represent diffuse extracellular senile plaques made up of Aβ and intracellular neurofibrillary tangles consisting of tau protein aggregates, associated with reactive microgliosis, dystrophic neurites and neuronal and synaptic loss. In its clinical course this multifactorial neurodegenerative disease progresses from the prodromal phase to the late stages and histopathological substrate often cannot fully explain the clinical features of the disease.

Today we know that the pathological process begins decades before the first manifestation of the clinical symptoms and it is still assumed that the disorder of Aβ protein metabolism, the amyloid cascade, in the central nervous system is the initial and significant factor causing the disease. Hence, the main therapeutic strategies aim at modifying the abnormal production, accumulation and depositing of Aβ. Application of drugs that could potentially change the course of the disease would find its place in the earliest stages, asymptomatic- molecular, prodromal and early onset AD.

Until recently, the diagnosis of the disease, according to the valid criteria of Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association (NINCDSADRDA) from 1984, relied mainly on clinical characteristics of the disease reaching the reliability level of probable, but definite diagnosis requested an autopsy, histopathologic confirmation.

In the light of new knowledge, with a reliable and early recognition of the disease being the aim, diagnostic criteria

Correspondence to: Smiljana Kostić, Neurology Clinic, Military Medical Academy, Belgrade, Serbia;
E-mail: popovicsmiljana@gmail.com

for AD have been revised with a more precise definition of the clinical phenotype, including neuroimaging biomarkers: structural (MRI), metabolic (FDG PET), molecular (Aβ ligand PET), as well as biomarkers of cerebrospinal fluid (Aβ 1-42, total tau and p-tau).

Since AD is a disease of a still unknown etiology with a modest impact of so far available therapies, knowledge of risk factors is significant. An increased risk of illness showed a correlation with age, the presence of mild cognitive impairment, family manifestation of the disease, the presence of the APOE ε4 allele, vascular risk factor and traumatic brain injury. Identification of populations at risk leaves space for the preventive measures and neuroprotective strategies.

Among protective factors the concept of "cognitive reserve" is emphasized. Individuals with intellectually enriched life-styles, such as those with high educational and occupational attainment, have a reduced risk of expressing AD pathology clinically [2, 3]. Mediterranean diet, as well as regular physical activity also have a protective effect and are associated with a reduced risk of a progression from mild cognitive impairment to AD [4].

In terms of treatment of AD in the past decades a large number of drugs with potential disease modifying effect were examined but they have not given significant results. The encouraging results of experimental therapies with anti-amyloid beta monoclonal antibodies in the early stages of AD have been published recently, and we are expecting the results of other clinical studies in search for a cure that would stop or slow down the pathological processes.

The current treatment of AD is still based on the use of symptomatic drugs from the group of acetylcholinesterase enzyme inhibitors and NMDA receptor antagonists that have a modulating effect on the neurotransmitter activity and lead to a certain improvement in cognitive status, functionality, and behavioral symptoms.

Dementia is a growing issue throughout the world and is getting the status of the priority problem in the health care system worldwide. This disease leads to significant physical, psychological and social disability of patients and represents an enormous worry and burden for the family that takes care of the patient, as well as a great burden for the society. Financial expenses of the treatment of dementia in the United States range from 159 to 215 billion $ *per* year, which exceeds the cost of treating cardiovascular and malignant diseases. The largest economic cost of the treatment is set aside for the provision of institutional and long-term home care, followed by medical services.

According to the last official census in 2011 Serbia has a population of 7.2 million people, of which 17.4% are over 65 years old. Epidemiological studies have not been conducted yet, but it is estimated that approximately 80,000 people live with dementia. In 2004 the first pilot population survey was conducted in Belgrade and the estimated prevalence of unspecified dementia F03 was 6.7% which indicated the need for more reliable diagnosis by conducting broader population studies, as well as the education of professionals and the public [5]. In Serbia there is only one specialized clinic for patients with dementia, a few centres and outpatient clinics, mainly in larger cities. There is the lack of specialized institutions for long-term care and the majority of patients are placed in residential care institutions of the elderly. There is also the lack of daily care centers for dementia patients and the support services for patients and their families, which would contribute to the prolongation of stay of those patients in local communities. Most of the costs and almost the entire burden of care for the patients are born by the families of patients themselves. In the past decade a lot of efforts were made to raise awareness about the disease in the general population, to train health professionals and improve communication between general practitioners, families and institutions of the tertiary level. There remains the problem of delayed and misdiagnosis, as well as failure to recognize the seriousness and severity of the problems that these patients and their caregivers face every day. That leaves us to think, as individuals and as a society, about the future that could become our cruel present.

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ORIGINAL ARTICLE

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A clinical study on the influence of suturing material on oral wound healing

Klinička studija o uticaju materijala za šivenje na zarastanje oralne rane

Dragan Gazivoda*†, Dejan Pelemiš*, Goran Vujašković‡

***Clinic of Oral Surgery, Military Medical Academy, Belgrade, Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia; ‡Faculty of Dentistry, University of Belgrade, Belgrade, Serbia**

Abstract

Background/Aim. Suture materials play an important role in healing, enabling reconstruction and reassembly of tissue separated by the surgical procedure or trauma, and at the same time facilitating and promoting healing and hemostasis. Suture materials are used daily in oral surgery, and are considered to be substances most commonly implanted in human body. The aim of this clinical study was to examine the speed of wound healing and complications incidence, after the use of three different absorbable synthetic suture materials in oral surgery (catgut, Dexon and Vicryl rapide), and to ascertain which one is the most suitable for oral surgery. **Methods.** The study was conducted on 96 patients undergoing root resection or surgical extraction of third molars. Each of the suture materials (catgut, Dexon and Vicryl rapide) was used for 8 root resections and 8 surgical third molar extractions in the maxilla, as well as in the mandible (a total of 32 surgical interventions for each suture material). **Results.** The faster wound healing was obtained with Vicryl rapide compared to other two suturing material tested. There was no significant difference regarding the presence of local reaction in all the three groups of patients on the 21st postoperative day. **Conclusion.** The results of our clinical study point out that Vycril-rapid contributes more than catgut or Dexon to faster healing of human wounds, with fewer incidences of wound dehiscence and milder local reactions.

Key words:
oral surgical procedures; sutures; treatment outcome.

Apstrakt

Uvod/Cilj. Materijali za šivenje igraju važnu ulogu u zarastanju rane, omogućavajući rekonstrukciju i vraćanje razdvojenog tkiva tokom hirurške procedure ili traume, i u isto vreme omogućavaju i pospešuju zarastanje i hemostazu. Materijali za šavove svakodnevno se koriste u oralnoj hirurgiji i smatraju se supstancama koje se najčešće implantiraju u ljudsko telo. Cilj ove kliničke studije je da se ispita brzina zarastanja rana, učestalosti komplikacija nakon primene tri različita resorptivna sintetička materijala za šivenje u oralnoj hirurgiji (catgut, Dexon i Vicryl rapide), i da se utvrdi koji je od njih najpogodniji za upotrebu u oralnoj hirurgiji. **Metode.** Studija je sprovedena na uzorku od 96 pacijenata kod kojih je urađena resekcija korena i hirurška ekstrakcija trećih molara. Svaki od materijala za šavove (catgut, Dexon i Vicryl rapide) upotrebljen je prilikom osam resekcija korena i osam hirurških ekstrakcija trećih molara u maksili i mandibuli (ukupno 32 intervencije za svaki materijal za šavove). **Rezultati.** Ustanovljeno je brže zarastanje rana uz primenu Vicryl rapide materijala za šavove u odnosu na druga dva testirana materijala. Nije ustanovljena značajna razlika u prisustvu lokalne reakcije kod sve tri grupe bolesnika 21. postoperativnog dana. **Zaključak.** Rezultati kliničke studije ukazuju na veći doprinos bržem zarastanju rane uz primenu Vicryl rapide materijala u odnosu na catgut i Dexon, sa nekoliko slučajeva dehiscencije rane i ispoljavanjem blaže lokalne reakcije.

Ključne reči:
hirurgija, oralna, procedure; šavovi; lečenje, ishod.

Introduction

Suture materials play an important role in healing of wounds, enabling reconstruction and reassembly of tissue separated by a surgical procedure or a trauma, and at the same time facilitating and promoting healing and hemostasis [1]. Suture materials are used daily in oral surgery, and are considered to be substances most commonly implanted in human body.

Usage of suture materials dates back to ancient Egypt, but significant development occurred in the 1960s and the early 1970s, when greatest innovations of synthetic sutures made of polyvinyl alcohol were introduced [2, 3].

Correspondence to: Dragan Gazivoda, Clinic of Oral Surgery, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia. Phone: +381 63 847 3735. E-mail: gazivoda55@gmail.com

Suture materials are classified on the basis of several criteria, and these are usually origin, structure, and biological properties [4]. By origin, sutures can be natural and synthetic; by structure they can be monofilament and multifilament, while by biological properties they can be absorbable and non-absorbable.

Development of synthetic suture materials introduced a few suture materials of different characteristics, good quality and acceptable price [3]. However, ideal suture material has not been manufactured yet.

Important features of absorbable suture materials are their way of absorption and loss of tensile strength over time. Both are very important for ascertaining whether the used absorbable suture material will stay intact long enough and ensure enough strength to facilitate and promote wound healing. Superficial wound tissue usually takes five to ten days to heal, but some surgical procedures require sutures to persist 14 to 28 days [5]. However, as absorbable suture materials are dissolved under the influence of proteolytic enzymes or hydrolysis, it would be preferable not to have them remain in tissue longer than necessary.

Ideal suture material should have certain physical characteristics and properties, such as resistance to traction, dimension stability, absence of memory, knot safety and flexibility sufficient to avoid damaging oral mucosa. Furthermore, ideal suture material needs to provide limited bacterial adhesion and wound contamination. Given that no ideal suture material has been manufactured yet, sutures themselves can become the source of inflammation, which may reduce or compromise the potential for reparation and regeneration. Only a few references compare or discuss properties and quality of absorbable suture materials [6–9].

Sutures used in oral and maxillofacial surgery behave differently from those used for other parts of the body due to differences in the quality of tissue involved, constant presence of saliva, high level of vascularisation and the presence of functions of speech, chewing and swallowing.

Oral surgery interventions are customarily finished by suturing surgical wound [10], and the choice of the most appropriate suture material depends on the site and depth of tissue to stitch. Given that this type of intervention includes suturing overlying tissue, non-absorbable materials (usually silk sutures) are routinely used, which are ther removed 5–7 days postoperatively.

Sometimes, however, when it is not possible to remove the placed sutures, absorbable materials are used: in case of persons with disability, not able to cooperate, given that removing sutures would imply induction of general anaesthesia; children and soldiers positioned in remote units, without adequate conditions for revisiting and removing sutures, especially in case of emergency and in wartime [11].

There is lack of information on behaviour of these materials in oral cavity. Oral environment is characterized by numerous specificities, primarily presence of saliva [10–13], which is of variable amount, pH and contents of immunoglobulins. Besides the presence of saliva, oral cavity is characterised by: local immunogenic response, presence of saprophyte bacteria that can manifest pathogenic effects in some circumstances, accumulation of plaque on the surface of suture material (especially on knots), capillary effect and subsequent absorption of fluid by material [14, 15].

The aim of this clinical study was to examine the speed of wound healing and complications incidence, after the use of three different absorbable synthetic suture materials in oral surgery [catgut, polyglycolic acid (Dexon) and polyglactin 910 (Vicryl rapide)], and to ascertain which one is the most suitable for oral surgery.

Methods

The study was conducted on 96 patients undergoing root resection or surgical extraction of third molars. The patients were chosen randomly, paying attention to equal distribution of interventions in both upper and lower jaw (medical indication was the only criterion for choice, not sex, age or general health condition). Therefore, each of the suture materials (catgut, Dexon and Vicryl rapide) was used for 8 root resections and 8 surgical third molar extractions in the maxilla, as well as in the mandible (total of 32 surgical interventions for each suture material).

With the aim of unification of healing conditions, the same incisions were used (Rëin-Müler and Novak-Peter for root resection and standard edge cut with relaxation for surgical extractions of impacted third molars). In the postoperative period, patients were followed-up on the days 1, 3, 7, 14 and 21. The following indicators of wound healing efficiency were monitored: a) incidence of wound dehiscence (postoperative days 1, 3 and 7); b) occurrence of local tissue reaction (postoperative days 1, 7, 14 and 21).

In the postoperative period, all the patients were monitored, in specified intervals, for the presence (absence) and incidence of the following side effects: edema, hematoma, infection, as well as administration of analgesic or antibiotics.

Changes of protruding for more than 1 cm relative to the opposite side were registered as edema, and changes of mucosa color to purple were considered as hematoma. Particular attention was paid to the presence of dehiscence and local reaction (redness of the mucosa, edema, inflammation) for all the three types of suture materials.

The results are presented in tables and figures, and χ^2 test was used for determining differences in the incidence of particular change. All indicators had frequency distribution identified, with frequency differences determined with chi-square test. Differences were accepted as statistically significant if *p*-value was 0.05 or less.

Results

Our study was finalized by summing up the obtained results regarding the registered manifestations: dehiscence and local reactions in the postoperative period, as well as an incidence of postoperative complications, such as edema, hematoma and infections.

On the 1st postoperative day, dehiscence was registered in two patients (6.2%) with catgut applied, and not registered in the patients with Dexon or Vicryl rapide applied. By comparing all the 3 groups on the 1st postoperative day, it is ap-

parent that this difference was not significant (Table 1). On the 3rd postoperative day, dehiscence was registered more frequently, and it was statistically significant when comparing catgut and Vicryl rapide (Table 1). On the 7th postoperative day, Dexon was associated with dehiscence in 10 patients, catgut in 16 patients, and Vicryl-rapid in 6 patients, which was statistically significant (Table 1).

On the postoperative day 1, the presence of some mild local reaction (mostly just redness of the mucosa and slight edema) was registered in more than 50% of the patients. Comparison of all the 3 groups on the postoperative day 1 revealed no significant difference (Table 2). On the postoperative day 7, the presence of local reaction was still registered in approximately 50% of the patients. However, comparison of the results showed a significant difference in favor of Vicryl rapide when compared to the other two materials (Table 2). On the postoperative day 14, the presence of local reaction was registered in only 3 (9.3%) of the patients with applied catgut and Dexon. The patients treated with Vicryl rapide had no local reaction. There was no significant difference when comparing all the 3 groups on the day 14. There was no significant difference regarding the presence of local reaction on the postoperative day 21 also (Table 2).

Fluctuation of local reactions during the postoperative period is presented in Figure 1, and mean values of local reactions for the entire postoperative period are presented in Figure 2.

Table 1

Number of patients with dehiscence on the days 1, 3 and 7 postoperatively

Suture materials	Postoperative day		
	1	3	7
Dexon	0	6	13
Catgut	1	9	8
Vicryl rapide	0	3	5

Table 2

Number of patients with the presence of local reaction on the postoperative days 1, 7, 14 and 21

Suture materials	Postoperative day			
	1	7	14	21
Dexon	17	16	3	0
Catgut	20	17	3	1
Vicryl rapide	17	4	0	0

Discussion

There are not many references comparing or discussing properties and quality of absorbable suture materials [6–9, 16]. Many authors compare non-absorbable and absorbable sutures; however, just a few of them define properties of suture materials used in oral cavity. Given that there is no ideal suture material, it is necessary to conduct large number of studies that would help clinicians to consider all characteristics of suture materials, with the emphasis on biological properties and their applicability in oral cavity. Oral cavity is a specific environment relative to other human tissues, due to the presence of saliva and specific microorganisms, strong vascularisation, as well as associated functions of speech, chewing and swallowing.

On the basis of their comparative study, Filho et al. [17] concluded that polyglactin 910 is one that is preferred in dentistry. It has proved to be clinically excellent because it does not allow adherence of plaque and is well suited for handling. In addition, it shows no intensive local reaction. It is excellent clinically because it does not allow plaque adherence, being coated with Ca-stearate, which enables easy and efficient passing through tissue with minimum resistance [16, 17]. Our experiences with Vicryl are positive and agreeable with these. A shortcoming of Vicryl rapide could be a possibility of knots loosening (because it is coated). However, this deficiency may be overcome by tying more knots [3–5].

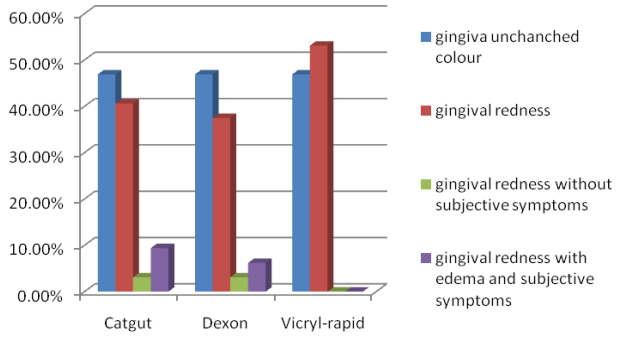

Fig. 1 – Local tissue reactions after suturing with the tested suture materials.

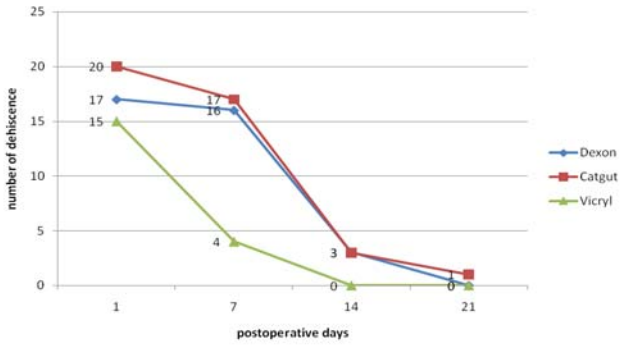

Fig. 2 – Local reaction in the postoperative period after the use of the tested suture materials.

Duprez et al. [18] conducted a clinical research including surgical procedures on children's hips. Breaking of suture material was registered after 12 to 16 days. Absorption mechanism includes hydrolysis. Inflammatory reaction was obvious, with abundant macrophages containing suture material fragments. It is also possible that inflammation cells release lytic enzymes, which increase spontaneous lysis and lead to fragmentation of suture material. These authors found Vicryl-rapid to be perfect, tolerant and breaking after 12 to 16 days, with moderately present reaction of macrophages.

The objective of our study was to examine clinical characteristics of the 3 most commonly used absorbable suture materials (catgut, Dexon and Vicryl rapide). References suggested that inflammatory tissue reaction is strongest with catgut. Our research results showed the highest level of inflammatory response in the observation period – on the postoperative days 3, 7, 14 and 21 – with Dexon, then with catgut, and the lowest with Vicryl rapide.

Shaw et al. [19] conducted a prospective study in which patients with oral surgery reported by mail the moment when absorbable stitches disappeared from their mouth. A study of this type is interesting, but not very significant, given that patients gave personal subjective perception of the moment of suture absorption, which did not necessarily need to be authentic.

Search for ideal suture material does not imply only biological compatibility, but also good clinical behaviour – resistance to traction, dimensional stability, low memory effect, good knot security and good flexibility with mild capillary effect [20].

Wallace et al. [21] compared polyglycolic acid, silk, chromic and flat catgut used on 52 respondents. Tissue reaction was studied and classified clinically and histologically on the days 3, 5, 7 and 14 after oral surgical intervention. Polyglycolic acid stitch (Dexon) caused the mildest tissue reaction, milder than silk, chromic and flat catgut. Besides suggesting easier stitching with Dexon than with silk, they said that Dexon stayed present in tissue after implanting for 16–20 days; catgut was absorbed in 3–5 days, and chromic in 7–10 days. According to these authors, Dexon seems to have properties closer to ideal suture material than any other tested.

Besides being conditioned by the degree of local reaction, time of absorption of suture material is also conditioned by the quantity of present electrolytes, as well as by basal metabolism. The only logical explanation for misbalance in absorption time of Dexon lies in the fact that Wallace et al. [21] equals disappearing of knot from oral cavity with absorption, which does not necessarily imply the moment of complete absorption of suture material, since it can be registered only with histopathological examination, which was not indicated as the method used in that study.

Our study confirms that Vicryl rapide provokes mildest local reaction, while most severe local reaction is not caused by catgut, but by Dexon, which differs from most other study results and manufacturers' information. It is also confirmed that Vicryl rapide contributes to faster healing of wounds in humans, with the lower incidence of dehiscence and milder local reactions than with applied catgut or Dexon. Besides these manifestations, it is important that intensity of local reaction stays as mild as possible (low antigenic potential), with the least possible wound dehiscence. Statistically significant difference regarding the incidence of dehiscence on the postoperative day 3 was visible when catgut and Vicryl rapide were compared, being more rare with application of Vicryl rapide. Occurrence of local reaction on the postoperative day 7 presented statistically significant difference when comparing catgut and Vicryl-rapid and Dexon and Vicryl rapide.

In oral surgery, sutures are usually removed 7–10 days upon implanting, when differences between materials do not seem to be very significant. However, some patients who do not cooperate and do not have sutures removed may benefit from using absorbable sutures with best characteristics. Moreover, histopathological examination, a part of our experimental study already performed [11], confirmed that Vicryl rapide has the best results, again approving the results of this clinical study.

Conclusion

It seems that Vicryl rapide has the best properties of the available absorbable suture materials for application in oral surgery.

The results of our clinical study point out that Vicryl rapide contributes more than catgut or Dexon to faster healing of human wounds, with fewer incidences of wound dehiscence and milder local reactions.

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Emotional reactions in patients after frontal lobe stroke

Emocionalno reagovanje kod bolesnika nakon cerebrovaskularnog inzulta u čeonom režnju

Zlatan Stojanović*, Sanja Vukadinović Stojanović†

***Department for Anatomy, Faculty of Medicine, University of Banja Luka, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; †Clinic for Psychiatry, Clinical Centre, Banja Luka, Republic of Srpska, Bosnia and Herzegovina**

Abstract

Background/Aim. Emotional reactions have been documented after tumor lesions and the other damages of the brain. The aim of this paper was to examine the correlation between frontal lobe lesions and emotional reactions in patients with stroke. **Methods.** The research included 118 patients after stroke. Lesion localization was defined on computed axial tomography records, whereas the area and perimeter of lesion were measured by AutoCAD 2004 software. Examinations by means of the Hamilton Rating Scale for Anxiety and Depression (HRSA and HRSD) were carried out 11–40 days after stroke. Statistic data were processed by simple linear/nonlinear regression, Cox's and the generalized linear model. **Results.** A higher frequency of emotional reactions, i.e. anxiety, was determined in women after stroke ($p = 0.024$). A negative correlation between the lesion size and the intensity of anxiety manifestations was determined (Spearman's $r = -0.297$; $p = 0.001$). Anxiety was more frequent in patients with frontal lobe lesions in the dominant hemisphere (interaction: frontal lesion * hand dominant hemisphere, $p = 0.017$). Also, HRSD score values showed the tendency for lesser decline in case of greater frontal lobe lesions in relation to lesions of other regions of prosencephalon (interaction: frontal lesion * lesion area, $p = 0.001$). **Conclusion.** The results of this study indicate the correlation between evolutionary younger structures of the central nervous system and emotional reactions of man. Therefore, it is necessary to undertake proper early psychopharmacotherapy in the vulnerable group of patients.

Key words:
anxiety; depression; frontal lobe; stroke.

Apstrakt

Uvod/Cilj. Uočena je pojava emocionalnog reagovanja nakon lezija moždanih struktura kod tumora mozga, povreda glave i sl. Cilj našeg rada bio je ispitivanje povezanosti između lezija čeonog lobusa i emocionalnih reakcija kod bolesnika sa moždanim insultom. **Metode.** Istraživanjem je obuhvaćeno 118 osoba nakon cerebrovaskularnog inzulta. Lokalizacija lezije određivana je na aksijalnim nekontrasnim CT snimcima, a površina i obim lezije primenom AutoCAD 2004 digitalne planimetrije. Psihometrijsko ispitivanje pomoću Hamiltonove skale za anksioznost (HRSA) i depresiju (HRSD) izvođeno je 11–40 dana nakon insulta. Statistička analiza podataka vršena je prostom linearnom /nelinearnom regresijom, Cox-ovim hazardnim i generalizovanim linearnim modelom. **Rezultati**. Utvrđena je češća pojava emocionalnih reakcija, tj. anksioznosti, nakon cerebrovaskularnog insulta kod žena ($p = 0.024$). Utvrđena je negativna korelacija između veličine lezije i intenziteta anksioznog ispoljavanja (Spearman-ov $r = -0.297$; $p = 0.001$). Anksioznost je bila češća kod bolesnika sa lezijama čeonog lobusa dominantne hemisfere (interakcija: čeona lezija * motorno-dominantna hemisfera, $p = 0.017$). Takođe, vrednosti HRSD skorova pokazale su tendenciju manjeg opadanja u slučaju većih lezija čeonog lobusa u odnosu na lezije drugih regiona prosencefalona (interakcija: čeone lezije * površina lezije, $p = 0.001$). **Zaključak.** Rezultati našeg istraživanja ukazuju na povezanost između evoluciono mlađih struktura centralnog nervnog sistema i emotivnih reakcija čoveka. U tom smislu potrebno je preduzimanje odgovarajuće rane psihofarmakoterapije kod rizične grupe bolesnika.

Ključne reči:
anksioznost; depresija; mozak, čeoni režanj; mozak, infarkt.

Introduction

Emotional reactions have been documented after tumor lesions and the other damages of the brain [1]. The aim of this paper was to examine the significance of frontal lobe lesions for the control of emotional behaviour in patients with stroke. In our study we started from the hypothesis that frontal lobe lesions (contrary to the lesions of other regions of the

Correspondence to: Zlatan Stojanović, Bulevar Stepe Stepanovića 175, 78 000 Banja Luka, Republic of Srpska, Bosnia and Herzegovina. Phone: +387 65 71 70 29. E-mail: szlatan@blic.net

forebrain) would cause statistically significant changes of emotional behaviour in patients with stroke.

Methods

Inclusion criteria of participants

The research included a total of 118 persons suffering from cerebrovascular stroke (of ischemic and hemorrhagic origin) who had no previously diagnosed psychiatric disorders: 59 male persons and 59 female persons at the age span 44–87 years. The patients were inquired at the Neurological Department of the Institute for Physical Medicine, Rehabilitation and Orthopaedic Surgery "Dr. Miroslav Zotović" Banja Luka. The study had two phases. In the first phase we assessed inclusion criteria, and in the second one we carried out psychological testing. The study included patients with first stroke and macroscopic lesions of prosencephalon on computed axial tomography (CAT) records. CAT records were done in the period of 72 h after stroke and psychometric examination 11–40 days after stroke. The exact day of psychometric testing for each patient was defined by means of the method of random selection. The patients were assessed once in the observed period.

Due to a significant mixture of influences, patients in heavier, comorbid states (heart decompensation, unstable angina, *infarctus myocardii* in the previous year and the year of examination, infective diseases, malignant and chronic immunological diseases) were excluded. Also, the study included only patients with baseline National Institute of Health Stroke Scale (NIHSS) score at the moment of psychological testing $2 \leq X \leq 10$. A total score on NIHSS scale ranges between 0–42, where higher values reflect greater weight of cerebral infarction. NIHSS score of less than 10 includes patients with mild and adequately severe neurological deficit [2]. Among the patients with mild neurological deficit, those were included with whom "drift test" was positive on the same sided extremities (NIHSS = 1 + 1) or NIHSS score had the value of minimum 2 on one of the extremities. Exclusion criteria were also moderate and severe dysphasia since they complicate to a great extent carrying out of verbal neuropsychological tests which were used in our study. The study was approved by the Faculty of Medicine in Banja Luka Ethic Committee and the participants gave informed consent prior to their inclusion in the study.

Research instruments

Thes morphometric research included superacute (up to 24 h) and acute ischemic/hemorrhagic lesions (24 h up to 3 days). Sensitivity of CAT scanner in detection of early ischemic lesions is limited, and only one half of all strokes are visualized within 48 h after the stroke [3, 4]. Brain edema and the mass effect reach their maximum values usually 3 to 5 days after the stroke [4]. Given that in this case the pathological process spreads more and more into the healthy tissue, in our study morphological research was limited to lesions that appeared up to 72 h after the stroke. The measurement of the area of hemorrhagic lesions (in 13 patients) included the zone of cytotoxic edema too.

Localization of lesions with clearly stated damages of specific morphoanatomic structures [5, 6] was defined on non-contrast CAT records (5 mm layer thickness) on the surface of the biggest lesion cross section. Cerebral lesions were classified into the following categories: frontal lobe/other forebrain segments damages; striate body damages (yes/no); limbic lobe, i.e. medial and basolateral limbic cortex, adjacent white matter, limbic nuclei damages (yes/no), and interbrain damages (thalamus and/or hypothalamus) (yes/no). The aforementioned lobe categories have included both cortical and subcortical lesions. To define deep frontal lesions, the border of the frontal lobe at the level of insular cortex and parainsular structure sections was the orthogonal line drawn through the front end of sulcus *circularis insulae* on the axis of *neuraxis* (mediosagittal plane), thus comprising precaudate structures. Mixed lesions that caught the adjacent lobes were included into frontal lesions (25 of total 35 frontal lesions were mixed). The area and perimeter of lesions were measured by AutoCAD digital planimetry (Figure 1) with previous transformation of CAT records into the digital format by means of a digital camera with resolution 8 Mpx. AutoCAD 2004 for PC Windows (developed by Autodesk, Inc. San Rafael, California, USA; see *http://usa.autodesk.com/autocad/*) belongs to programme package groups meant for drawing, projecting and other forms of computer application in engineering practice. This programme package can be used for measuring surfaces which have irregular geometric forms, such as the structures of central nervous system [7].

Fig. 1 – AutoCAD digital morphometry. Frontoparietal cerebrovascular lesion affecting the right *gyrus frontalis superior*, right *gyrus precentralis* and deep anterior groove segment of *gyrus postcentralis*. Area: 966,29 mm², perimeter: 16,577 cm.

Psychometric tests

The following psychometric tests were used to test disorders in psychic functions: the Hamilton Rating Scale for Anxiety (HRSA), 14 items [8]; values 0–13 are in the normal range

(without anxiety), 14–17 indicate mild anxiety and 18–24 moderate anxiety, whereas values ≥ 25 indicate severe anxiety; the Hamilton Rating Scale for Depression (HRSD), 17 scored items [9]; values 0–7 are normal, 8–13 indicate mild depression, 14–18 moderate depression, 19–22 severe depression, whereas values ≥ 23 indicate very severe depression; a questionnaire for qualitative evaluation of object relations in etiopathogenesis of post-stroke behavioural and emotional disturbances [10].

For the purpose of an orientation insight into childhood quality of object relations (up to the age of 18) of patients affected by cerebrovascular stroke, the following parameters were tested: patient's primary family profile compared to its integrity: divorces, death of a parent; continuous separation of the patient from his/her mother: death of mother during childbirth, custody of a child given to father after divorce, the adopted child, woman immature for the role of a mother gives her child to someone else; discontinuous separation of mother from the patient: prolonged hospitalization of mother due to mental illness, prolonged hospitalisation of mother due to somatic illness, parental substitutes – "weekend" mother, which was justified with housing and economic reasons. The patients who presented one or more positive answers were classified into category: detachment from parents = yes, which was used for further statistical analysis. We used the Handedness Questionnaire to evaluate the dominance of brain hemisphere in sensory-motor functions [11, 12].

Statistic analyses

The size of focal lesion was brought in connection with the intensity of emotional manifestations by applying following mathematical/statistical models: Pearson's coefficient of linear correlation (basic assumptions of the linear model: normality, homoscedasticity were tested) [13], simple nonlinear regression as well as Spearman's rank correlation. Besides classical parameters such as odds ratio (OR) and relative risk (RR) we also used Kaplan-Meier's and Cox's hazard model, and Generalized Linear Model. To reduce variability, one examiner, i.e. the first author, carried out all HRSA and HRSD psychometric testing. Statistic analyses were performed using SPSS version 20.0 for Windows. Statistic conclusions were derived on the basis of 2-tailed *p* values and the level of significance $p < 0.05$.

Results

The frontal lobe was affected in 35 (29.7%) of the cases, corpus striatum in 33, limbic lobe in 19, and interbrain (thalamus and/or hypothalamus) in 15 of the cases. The mean value of HRSA score on the examined sample of patients was 7.39 (SD = 4.741, n = 118). Anxiety on the same sample (HRSA positive, i.e. score > 13) was found in 17.8% of the patients and anxiety comorbid with depression (HRSD score > 7) in 11.0% of the cases. The statistical parameter values (Figure 2, Table 1) were obtained by using regression analysis of HRSA score values of all patients (anxious and not anxious) and the area of the biggest cross-section of cerebrovascular lesions.

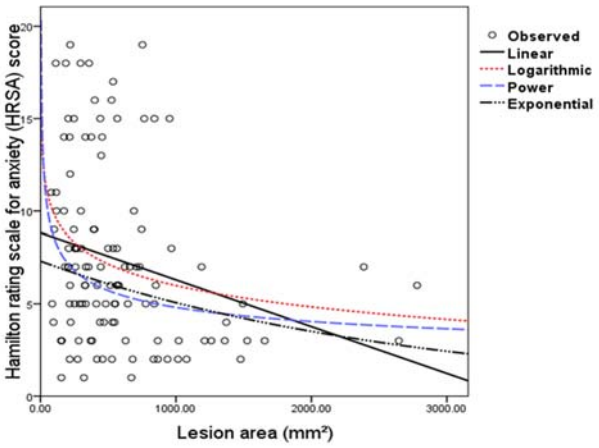

Fig. 2 – Regression analysis between the largest cross-section area of cerebrovascular lesion and the level intensity of anxiety in patients with stroke. X-axis shows values of the area of cerebrovascular lesions (in mm²) measured through the largest cross-section, while Y-axis shows the observed Hamilton Rating Scale for Anxiety (HRSA) score values of patients included in the study. Using the method of least squares the line and curves (logarithmic, power, and exponential) which best fit the observed data are plotted.

A statistically significant linear correlation between the area of the biggest cross-section of cerebrovascular lesions and the intensity of manifestations of anxiety (HRSA scores) ($p = 0.005$) was established by means of regression analysis and

Table 1

Coefficient of determination (R^2) of the largest cross-section area of cerebrovascular lesions and the intensity level of anxiety manifestations [Hamilton Rating Scale for Anxiety (HRSA) score values]

	R^2	F	df1	df2	*p*	Regression constant	Regression coefficient b1
Linear	0.066	8.168	1	116	0.005	8.831	-0.003
Logarithmic	0.068	8.411	1	116	0.004	17.521	-1.669
Power	0.070	8.727	1	116	0.004	27.150	-0.251
Exponential	0.063	7.850	1	116	0.006	7.308	-0.0004

Dependent variable: HRSA score.
The independent variable is lesion area (mm²).
Linear -0.00253 * x + 8.8314.
Power $27.149 * x^{-0.2506}$.

the coefficient of determination (R^2). Due to violation of the basic assumptions of the linear model: normality (Shapiro-Wilk, $p < 0.001$) and homoscedasticity, we examined the Spearman's rank correlation coefficient. A negative correlation between the size of cerebrovascular lesions and the intensity of manifestations of anxiety in the patients with stroke was obtained (Spearman's r = -0.297, $p = 0.001$). Exclusion of high leverage values and values with a high Cook's distance from the regression model did not change the direction of Pearson's r or statistical significance, and the rank correlation coefficient was: Spearman r = -0.294 ($p = 0.001$).

The odds ratio (OR) and relative risk (RR) of dependence of the occurrence of anxiety on variables of interest are shown in Table 2. Kaplan-Meier analysis showed a higher hazard of the occurrence of anxiety in female persons (Log Rank, $p = 0.024$) (Figure 3).

Table 3 shows Cox's regression analysis of the occurrence of anxiety depending on the degree of affection of the frontal lobe. It was established by means of Cox's analysis that the occurrence of anxiety depends on patient's gender. A higher risk for female persons was ascertained ($p = 0.033$). On the other hand, dependence of the occurrence of anxiety on frontal lobe affection was not obtained ($p = 0.277$). By inserting the same independent variables that were used in Table 3 (except for the frontal lobe variable) into Cox's model, we found that the risk of the occurrence of anxiety due to corpus striatum affection was 50.7% lower, but this difference was not statistically significant ($p = 0.223$).

The results of the Generalized Linear Model of the dependence of the intensity of anxiety manifestations (HRSA scores) on frontal lobe affection in the dominant hemisphere are shown in Table 4 and Figure 4.

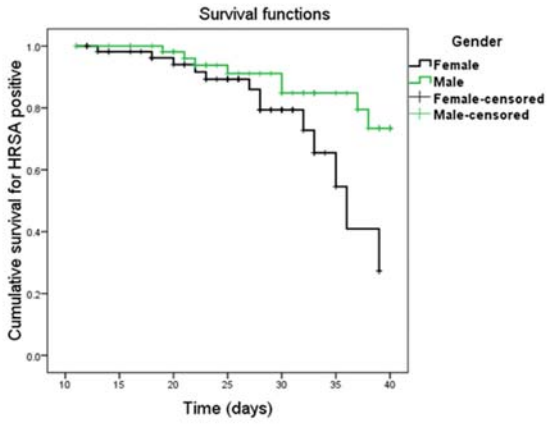

Fig. 3 – Kaplan-Meier hazard analysis of anxiety manifestations in patients with stroke depending on the patients gender. "Survival functions" is the common name for presented functions, since the initial tests were used to assess the risk of death from specific disease. The lower the curve of an event (in our example the occurrence of anxiety) for the modality of tested characteristics (gender of respondents), the higher the risk.
HRSA – Hamilton Rating Scale for Anxiety.

Table 2

Odds ratio and relative risk of anxiety occurrence in patients with stroke

Variable	Odds ratio (OR)	Relative risk (RR)	Fisher's exact test (2-sided)
Gender (female / male)	1.802	1.625	0.336
Detachment from parents (e.g. death of parent or divorce before age 18) (yes / no)	1.853	1.628	0.328
Hand-dominant hemisphere (yes / no)	2.163	1.903	0.153
Frontal lobe / Other forebrain segments	1.595	1.459	0.430
Striated body (yes / no)	0.552	0.606	0.425
Limbic lobe (limbic cortex, adjacent white matter, limbic nuclei) (yes / no)	0.844	0.868	0.999
Diencephalon (yes / no)	0.680	0.723	0.999
Hemorrhagic lesion (yes / no)	1.450	1.346	0.700

Table 3

Cox's regression analysis of anxiety occurrence in patients with stroke – affected frontal lobe

	Regression coefficient b	SE	df	*p*	Hazard ratio e^b (HR)	95.0% CI for HR	
						lower	upper
Gender (female / male)	1.008	0.474	1	0.033	2.739	1.082	6.932
Detachment from parents (yes / no)	0.183	0.577	1	0.751	1.201	0.387	3.725
Lesion area (mm²)	-0.00065	0.001	1	0.377	0.9993	0.998	1.001
Hand dominant hemisphere (yes / no)	0.579	0.492	1	0.239	1.784	0.681	4.676
Frontal lobe (yes / no)	0.566	0.521	1	0.277	1.762	0.635	4.889

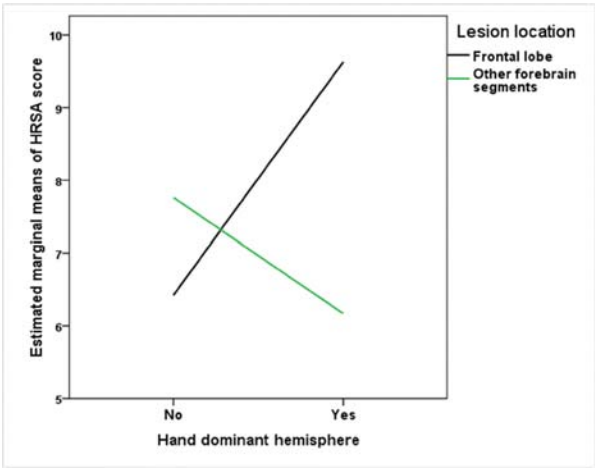

Fig. 4 – Estimated marginal means of Hamilton Rating Scale for Anxiety (HRSA) score. Figure shows estimated marginal means of HRSA score in case of simple or combined affectedness of the frontal lobe and hand dominant hemisphere. Simultaneous lesion of the frontal lobe and hand dominant hemisphere abruptly amplifies the value of HRSA score (p = 0.017).

The results of the Generalized Linear Model of dependence of the intensity of depression manifestations (HRSD scores) on frontal lobe affection and the size of cerebrovascular lesions are shown in Table 5 and Figure 5.

By applying the Generalized Linear Model we established that greater frontal lobe lesions (in relation to lesions of other regions of the forebrain) are associated with a smaller decrease in the intensity of manifestations of depression (p = 0.001). In case of damages of other regions of the forebrain, the regression coefficient was b = -0.000650, whereas in case of damage of the frontal lobe the regression coefficient increases and has the value b = 0.000463 (Table 5). Using the formula ln(Y) = 1.9369 - 0.000650 · [lesion area (mm²)] + 0.000463 · [lesion area (mm²) · frontal lobe (No = 0; Yes = 1)] in case of the lesion size of 218.72 mm² and damage of the other regions of prosencephalon, the predicted value of the mean of response of HRSD score was: $Y = e^{1.794732}$, that is, 6.018 (Euler's number, e = 2.71828), while in case of frontal lobe damage by the same lesion size the HRSD score is somewhat higher: $Y = e^{1.895999}$, that is, 6.659. An insight into the effect of the interaction frontal lobe * lesion area is gained by comparing the previously obtained values with HRSD score for greater lesions. For the lesion size of 1211.52 mm², HRSD score was Y = 3.156 due to damage of the other regions of prosencephalon, whereas in the case of frontal lobe it amounts to Y = 5.531, which is proportionally much greater than in the previous case 75.3% : 10.7% (Figure 5).

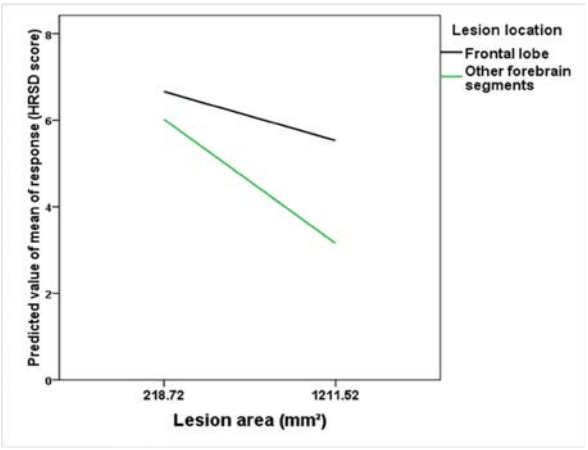

Fig. 5 – Interaction: frontal lobe * lesion area. The figure portrays significantly lesser decrease of Hamilton Rating Scale for Depression (HRSD) score when the frontal lobe is affected.

Table 4

Generalized linear model of dependence between anxiety manifestations [Hamilton Rating Scale for Anxiety (HRSA) scores] and frontal dominant lobe lesions

Parameter	Tests of model effects Generalized linear model: gamma with log link robust estimator		
	Wald chi-square	df	*p*
Intercept	833.110	1	< 0.001
Lesion area (mm²)	12.787	1	< 0.001
Frontal lobe * Hand dominant hemisphere	10.186	3	0.017

Dependent variable: HRSA score.
Model: intercept, lesion area (mm²), frontal lobe *hand dominant hemisphere.

Table 5

Generalized linear model of dependence between depressive manifestations [Hamilton Rating Scale for Depression (HRSD) scores] and the size of frontal lesions

Parameter	Regression coefficient b	SE	95% Wald confidence interval		Hypothesis test		
			Lower	Upper	Wald chi-square	df	*p*
Intercept	1.9369	0.075170	1.7896	2.0843	663.959	1	< 0.001
Lesion area (mm²)	-0.000650	0.000120	-0.000886	-0.000415	29.317	1	< 0.001
[Frontal lobe = Yes] *Lesion area (mm²)	0.000463	0.000134	0.000201	0.000726	11.969	1	0.001
[Frontal lobe = No] *Lesion area (mm²)	0*						

***Set to zero because this parameter is redundant.**

In the example dependence analysis of the intensity of depression symptoms on the size of lesions and affection of the frontal lobe (Table 5), we point out to authors that the value of correlation of the assessed parameters: lesion area with frontal lobe (Yes) * lesion area was 0.622. By using the general linear model with a transformed fourth root dependent (HRSD score) and an independent (lesion area) variable in order to satisfy the assumptions of the model (normality and homogeneity of variances), the observed power of the lesion area parameter was 0.967, while the observed power of the parameter frontal lobe (Yes) * lesion area amounted to an acceptable 0.790. By using linear regression with the stated transformed fourth root variables, collinearity between independent variables (lesion area and frontal lobe (Yes) * lesion area), that is, variance inflation factor was: VIF = 1.163, whereas the Condition Index = 12.634. The general linear model and multiple linear regression with transformed variables confirmed the results of the generalized linear model.

Discussion

Incidence of anxiety in patients with stroke

The frequency of anxiety in patients with cerebrovascular accident to a great extent depends on the design of the study, that is, the time of observing the patients as well as the psychometric tests used thereby. De Wit et al. [14] established the prevalence of anxiety two months after stroke in 25% of patients. This prevalence was 22% four and six months after the occurrence of stroke. Similarly, Aström [15] found the generalized anxiety disorder (GAD) in the early stages after stroke (the first three months) in 28% of the patients. Leppävuori et al. [16] describe the frequency of GAD three to four months after stroke of 20.6%. Observed over a longer period (3–5 years after stroke), the frequency of anxiety disorder does not differ significantly, amounting to about 20% [17].

By contrast to the mentioned studies, we stress that in our research we investigated the frequency of anxiety according to the HRSA criteria, and not the diagnostics of anxiety disorder (for instance, according to ICD-10 or DSM-IV). The frequency of anxiety 11–40 days after the occurrence of stroke amounted to 17.8%. We explain the differences in the frequency of anxiety in our research (17.8%) in relation to the one found in other studies [14, 15] (25% and 28%, respectively) in terms of different design of those studies, that is, later observation period (2–3 months after stroke), diagnosing anxiety by means of other measurement scales (for example, by using "Hospital Anxiety Scale"), as well as the presence of poorer somatic state of patients in our study due to a shorter period of recovery after stroke. A relatively low frequency of anxiety in our study is explained by the characteristics of HRSA that attaches greater significance to somatic equivalents of emotions, but also to the specific milieu our study was done in, which improves patients' psychosomatic state (patient health care involving rehabilitation Z50). There also arises the question of the validity of the study methodology in the acute phase of stroke (the first three months), given that the presence of anxiety symptoms is necessary to last as long as six months for diagnosing the generalized anxiety disorder by DSM-IV criteria [15].

Correlation analysis between intensity of anxiety manifestations and the size of cerebrovascular lesions

Interestingly, the literature on this subject is scarce. Sharpe et al. [17] exclude the connection between the size of lesion and the intensity of anxiety, but this study was done with patients three to five years after stroke. On the other hand, we examined this connection in the subacute phase, that is, 11–40 days after stroke. In our study the linear determination coefficient for the area of the biggest cross-section of cerebrovascular lesions and the intensity of anxiety manifestations (HRSA scores) was $R^2 = 0.066$ ($p = 0.005$). Due to a significant deviation of the basic assumption of the linear model – normality of the observed HRSA score values, as well as because of the presence of more severe heteroscedasticity (at lower values of the lesion area), the rank correlation coefficient was calculated. Spearman's coefficient was $r = -0.297$ ($p = 0.001$). A negative correlation between the lesion size and the intensity of anxiety manifestation was confirmed in this way. We explained a lower intensity of anxiety manifestations in patients with a greater area of cerebrovascular lesions by the activation of repair mechanisms due to poorer somatic state of patients in the subacute phase of stroke. From the viewpoint of evolutionary psychology and works of some researchers, depression-withdrawal has a defence character with the aim of saving organism energy [18]. In a similar way, greater lesions of the brain might also activate defence mechanisms associated with stopping the anxiety and energy saving. A high frequency of mild anxiety in our study (71.4% of the total anxiety) speaks in favour of this. One of the reasons for the indifference of patients with greater strokes, which is in accordance with the James-Lange theory of emotions, is the malfunctioning of peripheral sensitive/proprioceptive innervations (due to neurological deficits) that act as the antecedent of emotions. Although this concept was abandoned in favour of the Papez-MacLean theory of emotions, Damasio [19] explains the emergence of consciousness by means of these peripheral mechanisms. In an earlier research, we used these mechanisms to explain a lower intensity of depression manifestations (HRSD scores) due to greater cerebrovascular lesions of the brain (Spearman's $r = -0.263$, $p = 0.004$) [20].

Gender and anxiety in patients with stroke

The results anxiety dependence on gender differ in the literature. Schultz et al. [21] describe a higher frequency of anxiety and vulnerability of female and younger persons, whereas De Wit et al. [14] deny these differences. In our study, the risk for female persons (OR) was 1.802 times higher in relation to male persons, but this difference was not statistically significant ($p = 0.336$). However, a statistically important risk for women was determined by using the Kaplan-Meier model (Log Rank, $p = 0.024$) (Figure 3).

Side of hemispheric lesion and anxiety occurrence in patients with stroke

Results are contradictory with respect to the affected side of hemisphere. Aström [15] and Castellanos-Pinedo at al. [22] point to a higher frequency of anxiety disorder in persons with right-hemisphere lesions, whereas Williams [23] stress the affection of the left hemisphere. Schramke et al. [24] indentify a more pronounced distress in patients with left-sided lesions by using the Beck Anxiety Inventory. In our study we evaluated the dependence of anxiety occurrence on the damage of the motor-dominant hemisphere because of a greater operating-functional deficit of patients (affected dominant hand) which could have an impact on the occurrence of anxiety. A higher risk due to the affection of the dominant hemisphere was found, but it was not statistically significant (OR = 2.163, $p = 0.153$).

Morpho-anatomical localization of lesions and anxiety occurrence in patients with stroke

Sharpe et al. [17] exclude the connection between the localization of lesions and the intensity of anxiety. On the other hand, Tang et al. [25] suggest that right frontal acute infarcts may play a role in the development of post-stroke anxiety symptoms (OR = 4.44, $p = 0.002$). Robinson and Starkstein [26] associate the connection of major depression and the generalized anxiety disorder with cortical lesions, and isolated depression with subcortical lesions. Knutson et al. [27] in patients with penetrating brain injuries indicate anxiety to lesions of limbic areas and temporal lobe. When it comes to the affection of the prosencephalon structures in our study, the risk of the occurrence of anxiety is higher with the frontal lobe damage (OR = 1.595), while being lower in the case of corpus striatum and lobus limbicus (corpus striatum: OR = 0.552; lobus limbicus: OR = 0.844), but these differences are not statistically significant ($p > 0.05$). Although not statistically significant (Cox's model), the affection of basal ganglia is associated with 50.7% lower hazard of anxiety occurrence, which does not speak in favour of the thesis that the lesions of these anatomical structures are the cause of native emotional disorders in Parkinson's and Huntington's disease. The tendency of the development of anxiety due to the affection of the frontal lobe in the dominant hemisphere (Table 3) indicates the importance of cognitive functions in the etiopathogenesis of anxiety disorders, for instance, mistakes in making conclusions, anticipation and similar. A small number of positive cases of anxiety in our study represents one of the deficiencies of the risk parameters (OR and HR), which has an impact on the values of inferential statistics. In order to compensate for this and substantially increase the quantity of information as well as the examined sample (to n = 118), we processed data by means of the generalized linear model. Due to the earlier stated properties of empirical values of HRSA score, we used a subclass of the model: gamma with log link – robust estimator. This model showed a dependence and higher intensity of manifestations of anxiety due to frontal lobe lesions in the dominant hemisphere ($p = 0.017$) (Table 4, Figure 4). However, it is necessary to stress that the simple summation effect, was not determined in this case, but an interaction i.e. boosting effect of the frontal lobe and hand dominant hemisphere damages on the occurrence of anxiety manifestations. This effect is similar, for example, to the boosting effect of alcohol and benzodiazepines on the respiratory depression. We should also bear in mind that the persons with lesions in the dominant hemisphere suffer and anticipate a greater neurological operating-functional deficit given that the dominant hand is affected, which makes it the case that the stated interaction is not necessarily a consequence of the lateralization of the functions of the frontal lobe in the cerebral hemispheres. That cognitive functions play an important part in etiology of anxiety speaks in favour of the study [28] which associates deterioration of anxiety with better mentality. On the other hand, Starkstein and Tranel [29] stress that damage of ventromedial prefrontal cortex diminishes anxiety and thought concern for the future. Therefore, we recommend that impact of frontal lobe lesions on anxiety should be further investigated.

Frontal lobe lesions and depression

Some authors mention left anterior lesions, that is, lesions of the cerebral hemispheres that are closer to the frontal pole in the etiopathogenesis of post-stroke depressive disorders [30–35]. Tham et al. [36] have highlighted pathology of white matter in prefrontal brain region. On the other hand, other authors [37–39] exclude the connection between post-stroke depressions and left anterior lesions of the hemispheres, whereas Finset et al. [40] make connection between depression and deep retrorolandic lesions. We ascertained in our study that the greater frontal lobe lesions (in relation to lesions of other regions of the forebrain) are associated with a smaller decrease in the intensity of depression manifestations ($p = 0.001$) (Table 5, Figure 5). We had not noticed this in the previous study [20] given that we had not used the generalized linear model. The interaction frontal lesion * lesion area in the case of depression and the interaction frontal lesion * hand dominant hemisphere in the case of anxiety are precisely what proves the working hypothesis.

The frontal lobe is the seat of evolutionary younger and higher cortical functions: abstract thinking, judgement and attention [1]. Even though some authors [41] point out that humans and large primates have an equal size of the frontal cortex as the common characteristic, they do not exclude evolutionary upgrade and a higher degree of interconnectivity between the frontal areas in humans. In this sense, the frontal lobe damage and its connection with emotional disorders (depression and anxiety) which was confirmed in our study indicate the significance of evolutionary younger central nervous system structures and their relation to regulation of emotional behaviour in man.

Strength and limitations of study

As a limitation of our study we state the small number of positive cases of anxiety (HRSA positive), which makes it the case that the odds ratio (OR) and Cox's model analysis results are not definitive. In the example of the analysis of the dependence of the intensity of depression symptoms on the lesion size and the affection of the frontal lobe, we rec-

ommend increasing the sample due to a relatively high correlation of the assessed parameters, in order to ensure reproducibility of significant results.

The applicability of our study is limited to the population of patients in the subacute phase of stroke (11–40 days), given that poorer somatic state had a significant impact on the results. The advantages would be seen in a detailed statistical analysis in which a greater number of statistical models were used, and thereby in disclosure of hidden interactions that prove the dependence of emotional disorders in man on the lesions of specific brain regions.

Conclusion

Frontal lobe lesions are associated with changes in the emotional behaviour of patients with stroke. The results of this study indicate the significance of phylogenetically/evolutionary younger structures of the central nervous system for the regulation of emotional behaviour of man. Therefore, it is necessary to undertake proper early psycho/pharmacotherapy in the vulnerable group of patients.

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ORIGINAL ARTICLE

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The significance of adiponectin as a biomarker in metabolic syndrome and/or coronary artery disease

Značaj adiponektina kao biomarkera metaboličkog sindroma i/ili koronarne bolesti

Sanja Stojanović*, Marina Deljanin Ilić*, Stevan Ilić*, Dejan Petrović*, Svetlana Djukić†

***Institute for Treatment and Rehabilitation "Niška Banja", Niš, Serbia; †Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia**

Abstract

Background/Aim. Adiponectin exerts profound protective actions during insulin resistence or prediabetes progression towards more severe clinical entities such as metabolic syndrome and/or cardiovascular disease. Since hypoadiponectinaemia contributes to the pathophysiology of the metabolic syndrome and coronary artery disease the level of circulating adiponectin may be an early marker of cardiovascular events. The aim of this study was to determine the relationships between serum adiponectin levels and parameters of both insulin sensitivity and obesity in patients with the metabolic syndrome and/or coronary artery disease, as well as to assess predictive value of adiponectin serum levels as a biomarker of these entitetis. **Methods.** The study included 100 patients with metabolic syndrome and/or coronary artery disease with different degree of insulin resistance and healthy, normoglycemic individuals. The control group comprising healthy, normoglycemic individuals was used for comparison. Serum level of adiponectin, fasting glucose, fasting insulinemia Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index and anthropometric parameters were determined in all the subjects. Adiponectin was measured by using the ultrasensitive ELISA method. Insulinemia was measured by the radioimmunoassay (RIA) method. The presence of glycemic disorders was assessed on the basis of oral glucose tolerance test (OGTT). **Results.** Adiponectin level was inversely correlated with age (ϱ = - 0.015), parameters of both obesity (R = 0.437; $p < 0.001$) and insulin resistance (R = 0.374; $p < 0.01$). Decreasing in the level of adiponectin was strongly implicated in the development of insulin resistance. Most importantly, a statistically significant rapid decrease in adiponectin was in the prediabetic stages ($p < 0.01$). The predictor value of adiponectin was 1,356.32 ± 402.65 pg/mL. **Conclusions.** The obtained resultats suggest that adiponectin may be a useful marker in identification of individuals with risk of developing metabolic syndrome and coronary artery disease, as well as a predictor of prediabetes.

Key words:
adiponectin; biological markers; metabolic syndrome x; coronary disease.

Apstrakt

Uvod/Cilj. Adiponektin ispoljava snažno zaštitno dejstvo u toku progresije insulinske rezistencije ili predijabetesa u ozbiljnije kliničke entitete, kao što su metabolički sindrom i/ili kardiovaskularne bolesti. Pošto hipoadiponektinemija doprinosi patofiziologiji metaboličkog sindroma i koronarne bolesti nivo cirkulišućeg adiponektina može biti rani marker kardiovaskularnih događaja. Cilj ove studije bio je da se utvrdi povezanost nivoa adiponektina i parametara insulinske rezistencije i gojaznosti kod bolesnika sa metaboličkim sindromom i/ili koronarnom bolešću, kao i da se proceni prediktivni značaj nivoa adiponektina kao biomarkera ovih entiteta. **Metode.** Studija je uključila 100 bolesnika sa metaboličkim sindromom i/ili koronarnom bolešću različitog stepena insulinske rezistencije i zdrave ispitanike sa normalnom glikoregulacijom. Nivo serumskog adiponektina, glukoze našte, insulinemije našte, HOMA-IR indeksa i antropometrijski parametri određivani su kod svih ispitanika. Adiponektin je meren korišćenjem ultrasenzitivnog ELISA metoda. Insulinemija je određivana radioimunoesej (RIA) metodom. Prisustvo glikemijskih poremećaja procenjeno je na osnovu testa oralne glukozne tolerancije (OGTT). **Rezultati.** Nivo adiponektina bio je u inverznoj korelaciji sa starošću i parametrima insulinske rezistencije i gojaznosti. Sniženja nivoa adiponektina bila su udružena sa rastućim stepenom insulinske rezistencije. Najvažnije, statistički značajan pad nivoa adiponektina bio je u stadijumu predijabetesa ($p < 0,01$). Prediktorska vrednost nivoa adiponektina iznosila je 1,356,32 ± 402,65 pg/mL. **Zaključak.** Dobijeni rezultati ukazuju na to da adiponektin može biti koristan marker u identifikaciji osoba sa povećanim rizikom od razvoja metaboličkog sindroma i koronarne bolesti, kao i prediktor predijabetesa.

Ključne reči:
adiponektin; biološki pokazatelji; metabolički sindrom x; koronarna bolest.

Correspondence to: Sanja Stojanović, Institute for Treatment and Rehabilitation, "Niška Banja", Niš, Serbia. Bulevar Nemanjića 105/8, 18000 Niš, Serbia, Phone.: +381 18 221 193, 063 445 481, E-mail: sanjastdr@gmail.com

Introduction

Dysfunctional adipose tissue links obesity to cardiovascular disease by secreting a multitude of bioactictive adipokines with detrimental effects on the cardiovascular system. However, adipocytes secret a unique vasculoprotective adipokine – adiponectin [1, 2].

Adiponectin is a hormone secreted primarily from the mature adipocytes and circulates at a concentration significantly higher in metabolically healthy individuals of normal body weight. Recent studies have shown that the levels of adiponectin are in a statistically more significant inverse correlation with visceral than with total adipose tissue, indicating that the concentration of serum adiponectin is not determined only by the amount of adipose tissue, but also by its distribution [3, 4].

Interestingly, the part of the human adiponectin chromosome 3 (3q27) contains the "quantitative trait locus" (QTL) with a strong influence on the phenotypes of hypertension, prediabetes and metabolic syndrome [5, 6].

In physiological conditions, adiponectin has a protective role in the maintenance of insulin sensitivity, anti-inflammatory role in the prevention of pro-inflammatory response to numerous cytokines, vasculoprotective role in the maintenance of the vasculature in vasodilating condition and inhibition of proliferation of smooth muscle cells of blood vessels. Adiponectin realizes the primary effect of insulin sensitizer in the skeletal muscles and the liver, through inhibition of lipid synthesis and glyconeogenesis, maintaining glucose, triglycerides and free fatty acids in blood by means of adiponectin receptor AdipoR and molecules AMPK, PPAR-α and other unknown signaling pathways [7–9]. Since hypo-adiponectinaemia contributes to the pathophysiology of the metabolic syndrome (MS) and coronary artery disease, the level of circulating adiponectin may be an early marker of cardiovascular events.

In recent years, adiponectin as hormone has attracted the attention of many researchers due to its characteristics, it is easy to measure, stable in the circulation, and its circulatory concentrations inversely correlated with other potential cardiovascular markers such as other adipokines (leptin and resistin) and lipids.

Method

This cross-sectional study included 100 patients with metabolic syndrome and/or coronary artery disease with different degree of insulin resistance (IR) and healthy, normo-glycemic individuals (50 men and 50 women), aged 40–75 years. Blood for venous blood samples (10 mL), collected from all individuals, after an overnight 12 hour fast, was drawn from the antecubital vein between 8.30 and 9.30 *am*. Serum was separated and then quickly stored at -70°C for biochemical analyses. Determination of the concentration of hormone adiponectin was done at the Center for Molecular Medicine of Stem Cell, Faculty of Medical Sciences, University of Kragujevac.

The criteria for non-inclusion were: patients with associated diseases of the digestive and renal systems (malabsorption syndrome, liver and renal insufficiency), acute infections in the past three months, neoplastic diseases, diabetes mellitus.

All examinees were measured for anthropometric characteristics: body mass (kg), body height (cm), body mass index (BMI) as the ratio of body mass and the height square (kg/m2), waist circumference (cm), hip circumference (cm), waist/hip ratio (WHR), and body fat percentage (FAT %).

Determination of the total quantity of adipose tissue (Whole body fat/DEXA) was performed on the DXA (dual. energy X-ray absorptiometry scan) device Densitometer Hologic Dyscavery and shown in percentage (%); obesity was defined according to the following values: up to 40 years for men > 22% and women > 35%, over 40 years for men > 25% and women > 38% [10].

Insulinemia was determined in the Laboratory for Radioactive Isotopes, at the Department of Nuclear Medicine, Clinical Center "Kragujevac", using the diagnostic kit radioimmunoassay – RIA INSULIN (CIS). The reference range for insulin was 4.3 mU/L to 19.9 mU/L and intra- and interassay coefficients of variation were 4.5% and 5.0 %.

Based on the oral glucose tolerance test (OGTT), assessment of the existence of glycemic disorders was performed. Glycoregulation disorders were determined according to the current WHO classification [11].

Determination of the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index as an parametar of insulin resistance and HOMA-β index as an indicator of the function of pancreatic β-cells were determined according to homeostasis model assessment formula [12]. There are no standardized reference values for the HOMA-IR index due to conflicting values of different ethnic groups [13]. Therefore, based on the obtained values HOMA-IR index, the patients were grouped in the terciles. The highest tercile of the obtained values have 27% higher risk of developing a cardiovascular event than those in the lowest tercile of the insulin resistance.

The adiponectin concentration in serum was determined by commercial ELISA (Enzyme-linked immunosorbent assay) kit specific for human adiponectin (Human Adiponectin Duo Set ELISA Development kit, R & D systems, USA) [14]. Based on the measured values of the standards, a standard curve was created, and then the values for each individual sample were calculated. All samples were measured in triplicate. The measuring range of the method was 62.5–4000 pg/mL and intra- and interassay coefficients of variation were 4.5% and 5.0%.

For more detailed analysis of the correlation between adiponectin level and insulin resistance in accordance with the natural course of MS and IR stages and for determination of the adiponectin levels in IR stages, depending on glucose tolerance and values of blood insulin, the patients were divided into four groups:. group 1 – normal glucose tolerance and normoinsulinemia; group 2 – normal glucose tolerance and hyperinsulinemia; group 3 – patological glucose tolerance and hyperinsulinemia; group 4 – patological glucose tolerance and normoinsulinemia and/or hyperinsulinemia.

Data regarding coronary artery diseases were taken under coronarographic findings from the patients' medical records. Significant coronary artery stenosis was defined as >

50% reduction of absolute lumen diameter of major epicardial arteries or their major branches.

Statistical analysis was performed using descriptive and analytical methods. The mean values of the parameters are shown as the arithmetic mean (x) ± standard deviation (SD). The differences in the mean values of adiponectin levels and parameters of metabolic syndrome and coronary artery disease among groups were determined by variance analysis (ANOVA), while the correlation of adiponectin levels with these parameters was determined using multiple regression analysis and Pearson's (r) and Spearman's correlation coefficient (ρ).

All statistical analyzes were performed for the statistical significance level of $p < 0.05$.

Results

This study included 50 men and 50 women, average age 53.19 ± 15.0 years.

The results showed that adiponectin level inversely correlated with age (ρ = - 0.015; $p > 0.05$) and was significantly higher in the female patients ($r = 0.208$; $p < 0.05$).

The adiponectin levels showed a negative correlation with obesity parameters (R = 0.437, $p < 0.001$); the most significant impact on the adiponectin level had waist circumference (Figure 1) and waist/hip ratio WHR (r = - 0.203; $p < 0.05$), while BMI (r = - 0.138) and FAT(%) (r = - 0.020) did not have a significant impact on the level of adiponectin.

A correlation between the adiponectin level and parameters of IR [fasting glycemia (OGTT I), glycemia in the 120th minute (OGTT II), insulin, HOMA-IR and HOMA-β] was found in the whole study group, using the Pearson's correlation coefficient (Table 1). The results showed a negative, relatively weak correlation, but highly significant ($p < 0.01$) with OGTT I (r = - 0.292) and HOMA-IR (r = - 0.259), and a significant correlation with insulin (r = - 0.238, $p < 0.05$), and in the second part of Table a significant and relatively strong correlation between the IR parameters.

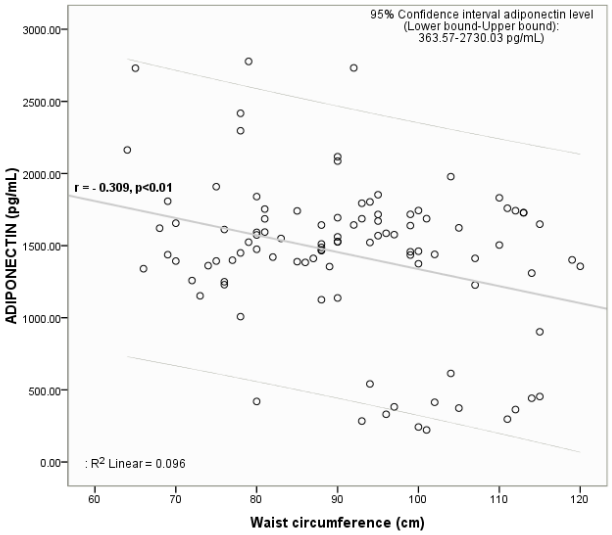

Fig. 1 – Relationship between adiponectin level and waist circumference.

A correlation between the adiponectin level and IR parameters, their relationships and a significance in the prognostic set, using multiple regression analysis are shown in Table 2.

A correlation between the adiponectin level and IR parameters was moderate and significant (R = 0.374, $p < 0.01$). In the prognostic set of IR parameters in the model, the most significant impact on the level of adiponectin ($p < 0.05$) had OGTT I (β = - 0.312) and insulin (β = - 0.211). Pearson and partial correlation coefficients are shown in the Correlations column.

The one-way analysis of variance was used to determine whether there were any significant differences between adiponectin levels in the groups of patients with the different IR stages. There was a statistically significant difference between adiponectin levels between the first and the second groups ($p < 0.01$). There was no statistically significant difference between the other groups of patients. Comparing to the stage of normal glucose regulation, in the first stage of "pre-

Table 1

Correlation (Pearson's coefficient) between adiponectin level and parameters of insulin resistance

Parameters	Adiponectin	OGTT I	OGTT II	Insulin	I N D E X	
					HOMA-IR	HOMA-β
OGTT I	- 0.292**	-	-	-	-	-
OGTT II	- 0.069	0.595***	-	-	-	-
Insulin	- 0.238*	0.353***	0.242*	-	-	-
HOMA-IR	- 0.259**	0.526***	0.342**	0.970***	-	-
HOMA-β	- 0.062	- 0.068	- 0.023	0.585***	0.480***	-

***p < 0.05; **p < 0.01; ***p < 0.001; OGTT – oral glucose tolerance test; HOMA-IR – homeostasis model assessment of insulin resistance.**

Table 2

Correlation between the adiponectin level and parameters of insulin resistance

MODEL	*R*	R^2 Square change	Sig.	Predictors	Unstandar. coefficient B	Standard. coefficient	Sig.	Correlations	
								Zero-order	Part
Adipo-nectin *(Backward)*	0.374**	0.140	0.002	(constant)	2536.065		.000		
				OGTT I	- 137.731	- 0.312*	.013	- 0.292**	- 0.239
				OGTT II	- 37.177	- 0.174	.144	- 0.069	- 0.139
				Insulin	- 175.899	- 0.211*	.044	- 0.238*	- 0.193

***p < 0.05; **p < 0.01; OGTT – oral glucose tolerance test.**

diabetes", a condition of normal glucose tolerance and hiperinsulinemia (Figure 2), showed an increase in insulin and a sudden decrease in adiponectin level. In the second stage, the impaired glucose tolerance, hyperglycemia was maintained, while glycemia in the 120th min continuously increased, continued in the final stage of insulin resistance, also with fasting glycemia, which was constantly increasing, until the end of this stage maintained hyperglycemia at a high level. The levels of adiponectin, after a sharp decrease in the first stage of increased fasting glycemia, rapidly increased in the second stage, as induced response to an increase in glycemia and the impaired glucose tolerance, and in the final stage of insulin resistance it continued increasing and maintained the achieved level, significantly below the initial values, in terms of high hyperglycemia and insulin resistance.

A correlation between adiponectin levels and parameters of insulin resistance was negative. In the normal weight patients there was a significant correlation ($p < 0.01$) with insulin ($r = - 0.287$) and HOMA-IR index ($r = - 0.299$), and less significant with the OGTT I ($r = - 0.266$, $p < 0.05$), while in the obese patients there was no significant correlation.

In view of the importance of cardiovascular risk in metabolic syndrome, the incidence of coronary artery disease was determined in different phases of its developement. In the stages during metabolic syndrome when metabolic decompensation is manifested, and the appearance of condition of normal glucose tolerance and hiperinsulinemia, relative incidence of coronary artery disease increases compared to the initial condition. In the first group 30% of patients had coronary artery disease, in the second one 36%, in the third one 24%, and in the fourth one 12%.

Discussion

With the emergence of obesity and development of MS, the process of deregulation of adipocytes leads to of intracellular function disorders, and the results are insulin resistance at the level of adipose tissue, increased production of adipokines, free fatty acids, and other inflammatory mediators in the form of insulin resistance, particularly in skeletal muscles and liver. Insulin resistance further affects the cells of vascular membrane, which further increases the risk of cardiovascular diseases [15].

Over the last decade through many clinical studies [16, 17], as well as this one, reported that the level of adiponectin paradoxically decreases in the states of MS, MS-associated diseases and coronary artery diseases (CAD), as well as in acute phases of CAD [18], as a reaction to an abnormal inflammatory response. Due to the inhibitory impact of adiponectin on the production of TNF-α and other proinflammatory cytokines, the expression of adhesion molecules, and the growth-factor-induced proliferation of smooth muscle cells, are significant mostly in the early stages of atherosclerosis and acute exacerbations. The protective effects of adiponectin are often associated with a proven reduction of inflammation and endothelial dysfunction, which are in the basis of MS and CAD occurrence, which leads to the conclusion that hypoadiponectinaemia, haemodynamic vascular abnormalities, as well as the complications of atherosclerosis are consequently correlated.

The results of this study, as well as the study of Wang et al. [19] show that in middle-aged patients serum levels of adiponectin in relation to gender, are more significant in men with CAD, whereas in women they are more significant in the group with MS and prediabetes, which explains that there is male predisposition towards the development of coronary artery disease, whereas in women towards metabolic disorders, due to the up-regulation of estrogen to adiponectin in premenopausal women. Adiponectin level in men is significantly lower than in women due to the inhibitory effects of testosterone on its production.

The results of recent studies [16, 20], and the results of this research show that the patients with CAD and MS have the

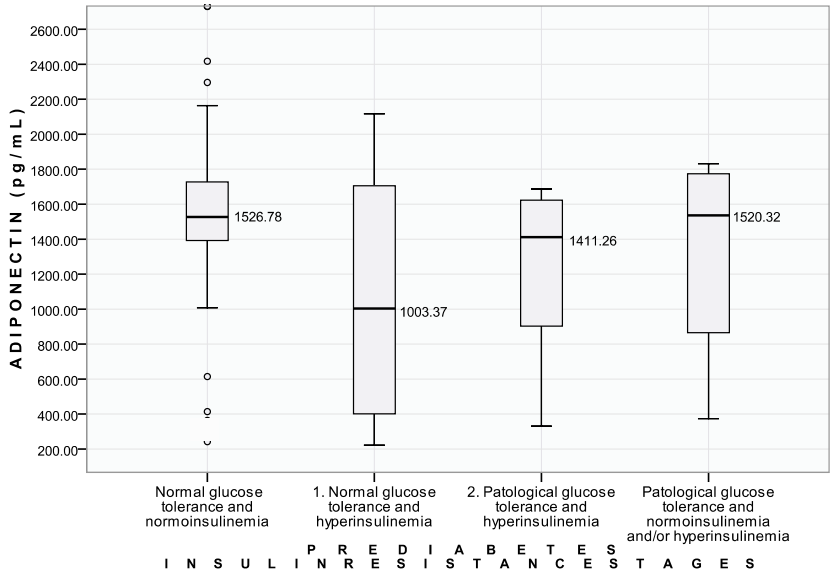

Fig. 2 – Adiponectin serum level according to insulin resistance.

lowest levels of adiponectin, higher in the patients with MS then in the patients with CAD; the highest level of adiponectin is in healthy examinees, and women have higher levels of adiponectin than men. The reason for significantly lower serum levels of adiponectin in patients with MS and CAD should not be looked for in the combined pathogenesis of the diseases, but in common genetic predisposition by mediation of transcription factors (PPAR-γ, FOKS), which with the external risk factors leads to the expression of both conditions [20].

Although MS and atherosclerosis in MS include lower levels of inflammation in their pathogenesis, their molecular pathogenetic mechanisms are different. Clinically manifested MS immediately results in a decrease of adiponectin levels, since it is in the stage of adiponectin decompensation, when endothelial cells do not have enough signals for the secretion of adiponectin from adipose tissue. Probably the early stage of MS, or just prior to MS, is accompanied by increasing levels of adiponectin in a so-called compensation phase, while later results in a sharp decrease, when the levels of adiponectin signalling are worn out. During the formation of atherosclerotic plaques, macrophages, not yet converted into the foamy cells secrete pro-inflammatory cytokines leading to the inhibitory impact on the expression of adiponectin and destablisation of atherosclerotic plaque. In a stable form of multiple coronary artery disease, where the adiponectin levels are slightly increased, the state of the mild adiponectin resistance is maintained, as opposed to the sudden decrease in the adiponectin level created in the acute coronary syndrome.

The results show that the level of adiponectin significantly decreases with the increase of nutrition/obesity level, especially visceral adipose tissue, as evidenced by recently published studies [21, 22], while the total amount of adipose tissue does not show any significant effect.

Among the obesity parameters waist circumference is the most significant showing strongest correlation with the level of adiponectin, thus confirming the clinical significance of its measurements.

It is important to note that large adipocytes, which are particularly contained in visceral adipose tissue, create less antiinflammatory adiponectin, and more proinflammatory cytokines and adipokines, supporting vicious cycle of low level chronic inflammation [23].

Persons with normal weight, but increased amount of visceral adipose tissue, have an increased risk of developing MS and CAD. On the other hand, obese, but metabolically healthy persons may have an increased insulin sensitivity, and thus the protecting effect of metabolic diseases associated with obesity over combined and partly crossed adiponectin-insulin signaling pathways [24].

Inverse association of adiponectin level with parameters of insulin resistance is confirmed by many studies [25, 26], of which the Shams et al. [25] study, with similar study sample by gender and age, in patients with CAD, shows a significant negative correlation ($p < 0.01$) with insulin level ($r = - 0.192$) and HOMA-index ($r = - 0.216$), and Mohan's study [27] a significant negative correlation of adiponectin level with fasting glucose and HOMA-IR index ($p < 0.001$).

In terms of hypoadiponectinaemia, MS, and chronic inflammation, it comes to signalization disorders in insulin and adiponectin ways and their receptors, and subsequent formation of insulin resistance. Disorders in adiponectin and insulin signalization lead to the occurrence of metabolic and vascular disorders, including endothelial dysfunction and metabolic syndrome.

The precise correlation of hypoadiponectinaemia and damaged signalization on insulin signalling pathway is not fully understood. However, there are several mechanisms of negative correlation between adiponectin level and parameters of insulin resistance: damaged signalization under the influence of TNF-α, disorder of the constituent components of insulin receptors, disordered function of APPL protein, down-regulation of genes PAPR-γ and PAPR-α and decreased expression of adiponectin receptor (AdipoR), as well as the disorder of all the other signalling molecules (AMPK, Akt, PKB, p38 MAPK) and the secondary messengers of insulin/adiponectin cascade pathways at the transcriptional and postranslational level [7–9].

Regardless of the cause of prediabetic state, it appears that changes in adiponectin level may validly reflect on the severity and stage of the IR evolution, as demonstrated by the results of this study, as well as by the results of other ones obtained in prediabetic patients [28], wherein the statistical significance was higher compared to non-diabetic patients.

Bearing in mind the aforementioned, adiponectin could be a representative marker of the early stages of insulin resistance, even in chronic cardiovascular damage. However, there are still too small number of studies dealing with the relationship of adiponectin level and insulin sensitivity in pre-diabetes, in order to determine the extent of change in adiponectin level as a routine clinical biomarker.

Conclusion

It can be emphasized that this study supports the hypothesis that adiponectin plays the central role in maintaining the functioning of adipose tissue, insulin sensitivity and energy consumption of the body.

Bearing in mind that the serum concentration of this significant hormone reflects early changes in the development of insulin resistance and prediabetes leading to the progression and acceleration of the creation of atherosclerotic lesions, adiponectin might be a significant and independent clinical marker of metabolic syndrome and coronary artery disease.

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Experimental closure of gunshot wounds by fibrin glue with antibiotics in pigs

Zatvaranje prostrelne rane primenom fibrinskog lepka sa antibiotikom u eksperimentu na svinjama

Nebojša Djenić*†, Milan Višnjić†, Saša Dragović*, Vladmila Bojanić†, Zoran Bojanić†, Dragan Djurdjević‡§, Boris Djindjić†, Miloš Kostov*§

***Department of General Surgery, Military Hospital, Niš, Serbia; †Faculty of Medicine, University of Niš, Niš, Serbia; ‡Institute for Medical Research, Military Medical Academy, Belgrade, Serbia; §Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

Abstract

Background/Aim. Gunshot wounds caused by the automatic rifle M70AB2 (AK-47) 7.62 mm, after the primary surgical management, were closed with delayed primary suture during the next four to seven days. This period coincides with the fibroblastic phase of wound healing. Fibrin glue is used as a local hemostatic and as a matrix for the local dosed release of antibiotics. Antibiotics addition to fibrin glue resulted in continuous diffusion into the surrounding next 4 to 7 days. The aim of this study was to create the preconditions for gunshot wounds closing without complications by the application of fibrin glue with antibiotics 24 h after primary surgical treatment. **Methods.** A total of 14 pigs were wounded in the gluteofemoral region by the bullet M67, initial velocity of 720 m/s. All wounded animals were surgically treated according to the principles of the war-surgery doctrine. Seven wounds were closed with primary delayed suture four days after the primary surgical treatment (traditional approach). Fibrin glue with antibiotics was introduced in seven wounds during the primary surgical treatment and primary delayed suture was done after 24 h. The macroscopic appearance and the clinical assessment of the wound were done during the primary surgical treatment and during its revision after 24 h, as well as histopathological findings at the days 4 and 7 after wounding. **Results.** Gunshot wounds caused by the automatic rifle M70AB2 (AK-47) 7.62 mm, and treated with fibrin glue with antibiotics after primary surgical management, were closed with primary delayed suture after 24 h. In further wound evolution there were no complications. **Conclusion.** Uncomplicated soft-tissue wounds caused by an automatic M70AB2 rifle may be closed primarily with delayed suture without the risk of developing complications if on revision, 24 h after primary surgery, there were no present necrotic tissues**,** hematoma, and any signs of infection when fibrin glue with antibiotics (ceftriaxone and clindamycin) was applied. The use of this method should be limited to individual and strictly controlled cases in civil practice for now.

Key words:
wounds, gunshot; wound closure techniques; fibrin tissue adhesive; anti-bacterial agents; surgical procedures, operative; treatment outcome; swine.

Apstrakt

Uvod/Cilj. Prostrelna rana naneta projektilom iz automatske puške M70 AB2 (AK-47) 7,62 mm nakon primarne hirurške obrade zatvara se primarno odloženim šavom u periodu 4–7 dana. Ovaj period se poklapa sa fibroblastnom fazom u procesu zarastanja rana. Fibrinski lepak se upotrebljava kao lokalni hemostatik i kao matriks za lokalno dozirano oslobađanje antibiotika. Dodatak antibiotika fibrinskom lepku dovodi do kontinuiranog delovanja narednih 4 do 7 dana. Cilj rada bio je da se primenom fibrinskog lepka sa antibiotikom 24 časa nakon primarne hirurške obrade stvore preduslovi za zatvaranje rane bez komplikacija. **Metode.** Ukupno 14 svinja ranjeno je u eksperimentu u gluteofemoralnom predelu projektilom M67, početne brzine 720 m/s. Sve ranjene životinje hirurški su obrađene po principima ratnohirurške doktrine. Sedam rana zatvoreno je konvencionalnim pristupom primarno odloženim šavom 4. dana od primarne hirurške obrade. Sedam rana obrađeno je primarno hirurški i unet je fibrinski lepak sa antibiotikom, a zatim zatvoreno primarno odloženim šavom nakon 24 časa. Prikazan je makroskopski izgled rane procenjen klinički u toku primarne hirurške obrade i na njenoj reviziji nakon 24 časa, kao i patohistološki nalaz mišića, četvrtog i sedmog dana od ranjavanja. **Rezultati.** Kod mekotkivnih prostrelnih rana izazvanih automatskom puškom M70AB2, koje su nakon primarne hirurške obrade tretirane fibrinskim lepkom sa antibioticima (cef-

Correspondence to: Nebojša Djenić, Department of General Surgery, Military Hospital, Bulevar dr Zorana Djindjića bb, Niš, Serbia.
E-mail: djani1508@yahoo.com

triakson i klindamicin) i zatvorene 24 časa kasnije primarno odloženim šavom, u daljem toku nije došlo do razvoja komplikacija. **Zaključak.** Nekomplikovane mekotkivne prostrelne rane izazvane projektilom iz automatske puške M70AB2 tretirane fibrinskim lepkom s antibioticima mogu se zatvoriti odloženim šavom bez rizika od komplikacija, ako one ne budu prisutne 24 h od postavljanja lepka. Primenu ove metode za sada bi trebalo ograničiti na pojedinačne i strogo kontrolisane slučajeve u civilnoj praksi.

Ključne reči:
rana vatrenim oružjem; rana, zatvaranje, tehnike; tkivni lepkovi, fibrinski; antibiotici; hirurgija, operativne procedure; lečenje, ishod; svinje.

Introduction*

The principles of gunshot wounds treatment have been changing during the evolution of surgery. In 1898, the German surgeon Paul Leopold Friedrich [1] (1864–1916) carried out the experiment proving all gunshot wounds primarily contaminated with bacteria. He also stated that bacteria were still found no deeper than 1–2 mm from the edge of wound after six hours and only after that period they penetrated into the deeper layers of the tissue. These findings resulted in two very important conclusions: first, that wound should be surgically treated within a 6-hour interval, and second, that excision can sterilize wound within this period. Modern principles of primary surgery (PS) of wounds were developed from Le Dran-Desault-Larreys' concept. The delayed primary suture (DPS) is the legacy of the World War Two, and it was used 4–10 days after PS, regardless the bacterial wound culture test. The results of surgical treatment have been significantly improved by a widespread application of penicillin since 1943, but the protection of war wound with antibiotics cannot replace surgical treatment [2–5]. Active approach to war wound in Serbia leads back to Dr. Mihailo Petrović. In Balkan wars (1912–1913) injuries caused by small caliber bullet were treated conservatively using antiseptic solution of carbolic acid. Because of the increased number of injuries caused by projectiles with high initial velocity, Dr. Petrović introduced active approach to wound treatment leaving them open and applying debridement [6–11]. Projectiles with high initial velocity (speed exceeding 750 m/s) inflict specific wounds, as the consequence of the particular shooting mechanism. While penetrating through the tissue, a projectile directly affects it creating "the zone of direct traumatic necrosis". The side shock wave by the indirect action around the channel of the projectile causes "the zone of molecular concussion". The edge between the areas with functional and irreversible circulatory disturbances is not clear nor definite for some time [3, 4, 12–16].

Fibrin glue (FG) is a two-component biological system with local hemostatic, adhesive and sealing effects and it is also used as a matrix (carrier) for local dosed release of antibiotics. The component 1 of the fibrin glue is made of fibrinogen, coagulation factor XII, fibronectin and plasma. FG component 2 includes thrombine, calcium ions and exogenous antifibrinolitics when needed. There are commercial preparations of FG and they have higher concentrations of fibrinogen. The application of FG, obtained from the plasma of individual donors, is associated with the risk of viral infections transmission, while using a unit of blood/plasma [17–22].

Primary surgical treatment and systemic use of antibiotics within the optimal time period have an indispensable role in excision of devitalized muscle tissue, removal of possible bacteria contained in it and in infection prevention.

Application of FG with antibiotics (FGA) after primary surgical treatment if properly done prevents both postoperative hematoma in wound and the development of local microorganisms.

FGA reduces the development of secondary complications and improves the basic biological reactions in the tissue repair processes.

In this study soft-tissue wounds were inflicted by the automatic rifle M70AB2 (AK-47) 7.62 mm in pigs. The aim of the study was to create the preconditions for primary delayed wounds closure without complications 24 h after the primary surgical treatment, and the usage of FGA.

Methods

The research was carried out on 14 experimental animals after having been approved by the Ethics Committee of the Faculty of Medicine in Niš, No. 01-2066-3, 2010. Before the experiment, all animals were examined by the veterinarian and proved to be healthy. The pigs were wounded by the bullet M67 (diameter 7.62 × 39 mm, weight 8 g, copper-jacketed, lead-core, flat-base) [23]. The model of gunshot injuries applied by the Swedish authors, and modified in the experimental investigations at the Military Medical Academy in Belgrade [16, 24–26], was performed. The male Yorkshire-Landrace breed pigs, 3.5 months old, weighing 32–39 kg, were used. The experimental induction of soft tissue wounds with channel length over 100 mm in the gluteofemoral region is possible at that age of animals. The animals were fasted for 12 h before being injured. The pigs were divided into two groups, with seven animals *per* each group: the group A (PS was made by traditional approach) and the group B (treated with FGA).

Diazepam [2 mg/10 kg body weight (BW), Bensedin®, Galenika, Serbia] was given in premedication. Animals were introduced into anesthesia by intramuscular (*im*) administration of 0.05 mg/kg BW acepromazine maleate (Combistress®, VanaGes.mH, Austria) 20 minutes before injuring. Ketamine chlorohydrate (Laboratorio Sanderson SA, Chile) was given (*im* 0.5 mg kg/BW) 15 min after the application of acepromazine maleate. All anesthetized animals were breathing spontaneously. Then their backs were turned down with slightly suspended and well exposed rear right leg laterally positioned toward the shooter.

A professional shooter with an automatic rifle M70AB2 fired a projectile M67, which had an initial speed of 720 m/sec from the distance of 5 m at the military shooting range. The initial entrance speed remains at initial high velocity levels [26]. Sho-

*This study is a part of the first author's dissertation

oting was done two times in a 10-day interval and each time seven animals were wounded. The skin of the pigs was not washed, shaven, nor disinfected before firing. Packing of wounds and a compressive bandage were applied for temporary bleeding control [26–29].

The animals were housed in separate cages at the Center for Biomedical Research of the Faculty of Medicine in Niš. We followed the principles of gunshot wounds treatment according to the traditional approach recommended by the International Red Cross and the war-surgery doctrine [3, 4, 12–16, 30]. PS was performed 4–12 h after wounding under general anesthesia. All animals were given preoperatively 1 g of ceftriaxone [30–36].

The assessment of the wound severity was made according to the Red Cross Wound Classification [37, 38]. PS is performed using aseptic techniques. The skin around the entrance and exit of wounds was shaved. Washing of the skin surrounding wounds was carried out with foam and povidone-iodine solution. The surgical field was surrounded with sterile compresses. The length of the wound channel and the size of the wound entrance and exit were measured. The clinical appearance of the wound during PS and clinical evaluation of the effectiveness of PS at revision, were presented as the macroscopic parameters of necrosis, infection and bleeding. Necrosis was assessed as: 0 – absent, 1 – minimal (0–2 mm), 2 – moderate (2–5 mm) and 3 – marked (more than 5 mm) clinical signs. The infection intensity was marked as following: 0 – absent, 1 – minimal (clear exudates), 2 – moderate (blurred exudates) and 3 – marked (abundant purulent exudates with ammonia smell). The level of hemorrhage was registered as: 0 – absent, 1 – minimal (smaller blood clots in lumen of the wound channel or between the muscles), 2 – moderate (bigger blood clots in the wound and between the muscles), and 3 – marked (open large blood vessels and injury channel filled with large blood clots).

Primary surgical treatment was performed according to the existing principles for the management of the soft tissue wounds, with the access from the entrance and the exit and in layers from the surface to the depth of the wound. A longitudinal incision of the tissue, long enough for a good access and exploration of the whole wound channel, was performed.

The skin was excised about 3–4 mm away from the wound edge. In the cases with the skin torn into strips and clearly avascular and necrotic, the approach was more radical. The separated and torn layers of fascia were excised. The estimation of muscle vitality was performed on the basis of the "4 C's" criteria: color, consistency, contractility, and capacity to bleed [3, 4, 30, 39].

The devitalized muscles were radically excised. The hemostasis was achieved by ligation of the blood vessels without the use of electrocautery. The wound was thoroughly rinsed several times with the solution of hydrogen diluted with saline. The mass of excised devitalized tissue was measured. After the revision was completed, a drain was inserted through the injury channel [16, 27, 28]. In animals from the group A, the wound was left open, covered with several layers of loose gauze in order to prevent the secondary contamination, while the gauze was fixed with leucoplast. In animals from the group B, after the drain insertion, the wound was instilled with 1 g of ceftriaxone and 600 mg of clindamycin, and then the two components of FG were applied. The openings of the wounds were closed by the primary suture, covered with gauze and fixed with leucoplast [27, 28].

The aim of wound revision is clinical assessment of the efficiency of PS after 24 h.

From the clinical aspect efficiently treated wounds were those that on revision looked clean, dry, with a little fibrin without signs of infection, without exudates and secretion retention, without large clots and edema, with light hyperemia, without signs of necrosis or with necrosis foci not deeper than 2 mm from the surface of the wound.

The wound channel was open through the entire length in order to detect the presence of necrotic tissue, bleeding or infection signs. A drain was placed through the wound channel after the completion of wound revision in animals from the group A. The wound was left open, covered with several layers of loose gauze and fixed with leucoplast. The wound entrance and exit were closed with primary delayed suture on the day 4.

FGA was applied again in the surgically treated wound after drain insertion in animals of the group B and the wounds were closed. After 24 h, while changing bandages, the drains were removed from all wounds. The plaster of Paris immobilization was not applied, so the wounds were disinfected every day and the bandage with gauze were fixed with leucoplast and 1 g of ceftriaxone was administered up to the postoperative day 7.

Muscle biopsy samples for histopathological examination under the light microscopy, were taken during wound revision on the day 4 and 7. Framed tissue blocks were cut into tissue layers 5 μm thick, and were stained with classical hematoxylin-eosin (H & E) method, as well as with the special method for mucopolysaccharide staining, method periodic acid-Schiff (PAS), for mucoproteins staining and Masson's trichrome stain for showing collagen. The aim of histological examination of muscle tissue preparation was to estimate the degree of necrosis, inflammatory reaction and bleeding intensity and to mark it semiquantitatively as: 0 – absent, 1 – minimal, 2 – moderate and 3 – marked. As efficiently PS treated wounds were classified those with the presence of necrosis marked with 0 or 1, meaning that there were no necrotic changes or they were spread up to 2 mm in the slice. The inefficiently treated wounds were marked with 2 and 3, where the necrosis spread from 2–5 mm or more respectively. The degree of inflammatory reaction and intersti-tial hemorrhage were used for estimation of the difference in the efficiency of wound treatment between the group A and the group B.

Results

Data relevant for wound ballistics

All wounds were recorded as perforating soft tissue injuries. The assessment of severity according to the Red Cross Wound Classification showed the second or the third level of tissue damage, i.e. a high transfer of kinetic energy. Gunshot channel lengths varied from 100 to 180 mm (Table 1).

Table 1

Ballistic parameters of wounds

No.	Code of the animal	Exit wound size (mm)	Exit wound surface (cm^2)	Wound channel length (l) (mm)	Mass of necrotic tissue (m) (g)	m/l (g/mm)
1	2558	15 × 30	4.5	140	42.94	0.306714
2	2589	15 × 30	4.5	140	28.77	0.2055
3	2582	20 × 30	6	180	60.64	0.336889
4	2587	20 × 25	5	100	35.89	0.3589
5	2594	20 × 30	6	100	47.29	0.4729
6	230	30 × 70	21	110	111.22	1.011091
7	2559	40 × 50	20	180	62.05	0.344722
8	2553F	20 × 30	6	100	30.6	0.306
9	2592F	15 × 15	2.25	160	133.41	0.833813
10	2554F	25 × 25	6.25	130	23.93	0.184077
11	2557F	35 × 40	14	100	43.34	0.4334
12	225 F	40 × 50	20	100	58.27	0.5827
13	237 F	40 × 50	20	100	72	0.72
14	235 F	40 × 90	36	100	103.15	1.0315

The dimensions of all exit wound openings are given in Table 1. The largest exit openings were found in wounds number 6 and 14, which showed the maximum mass of devitalized tissue, related to 1 mm length of the wound channel (m/l).

The clinical patterns of the wound

The primary surgery of the wound

The data obtained in this study were related to the extent and severity of the injuries in the affected tissues. The skin injury was assessed through the characteristics of the entrance and the exit wound openings (Figure 1). The entrance wound openings were of regular round shape skin defects with the diameter of 8 × 8 mm. All edges of the hole were bruised with the contusion zone 2–3 mm wide.

The exit openings were irregular, star-like lacerations, with strips of ischemic skin, detached from the subcutaneous tissue, requiring excision. The skin injury was wider at the exit than at the entrance, with bruise size up to 10 mm from the edge of the wound, which sometimes spreaded into the intracutaneous hematoma. Skin vitality estimation was easily made and a narrow zone of clearly mashed and avascular skin straps was excised.

The differences in the clinical aspect of necrosis in muscles during PS of the wound are shown in Table 2.

Table 2

The differences in the clinical aspect of necrosis during primary surgery (PS) and after 24 h

Necrosis (extent)	Animals (n)			
	during PS		after 24 h	
	A	B	A	B
0	0	0	4	3
1	4	5	3	4
2	3	2	0	0
3	0	0	0	0
Total	7	7	7	7

A – Animals with wounds closed traditionally; B – Animals in which fibrin glue with antibiotics was introduced in wounds and PS and primary delayed suture was done after 24 h. Extent: 0 – absent; 1 – minimal; 2 – moderate; 3 – marked.

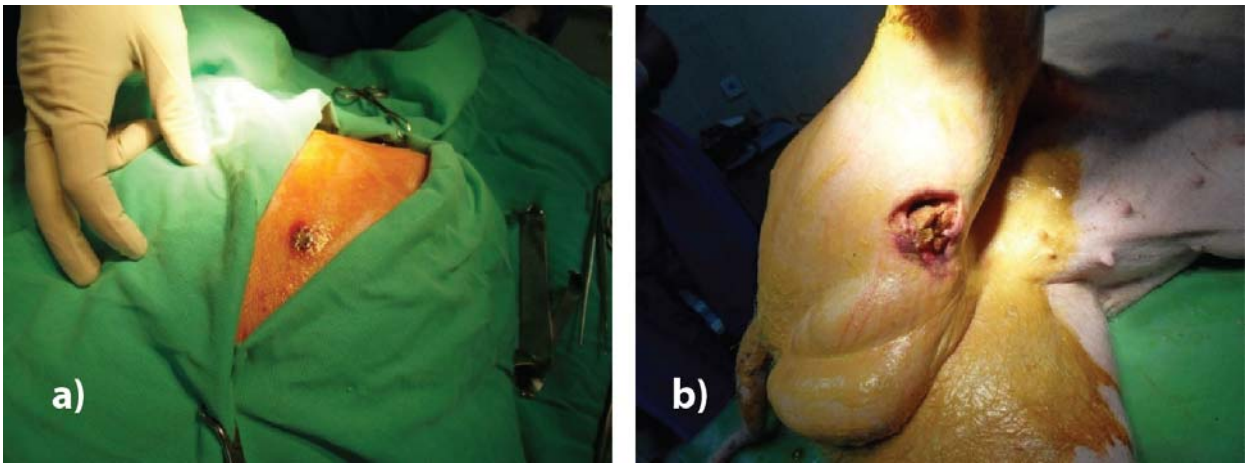

Fig. 1 – a) The entrance wound and b) exit wound (gauze packing).

The muscles showed different degrees and extent of the injuries. The wound channels were filled with blood clots and necrotic tissue caused by tissue contusion due to direct bullet action (Figure 2). There was a circular zone of the devitalized muscle tissue around the wound channel. Devitalized muscle was of dark red color, not elastic consistency without contractility on touch with a pair of pincers and without capillary bleeding on the surface section. The line between the zone with clear signs of devitalization and the region of healthy muscle was quite clearly visible (Figure 3).

Fig. 2 – Wound channel filled with blood clot and necrotic tissue.

Fig. 3 – The completed primary surgery with clear zone of healthy muscles.

The numerous smaller or larger foci of hemorrhage were also found in muscles. A mass of devitalized excised tissue during PS was shown in the Table 1 for all wounds. This Table clearly presents the devastating effect of a bullet by measuring and comparison of the size of exit hole, the length of the wound channel and the mass of the excised devitalized tissue. A femoral blood vessel was injured and ligated in only one case. Drains were placed after PS through all the wounds. The wounds in the group B were covered with FGA along wound channel and primarily closed (Figure 4).

Thrombin and fibrinogen solutions were loaded into a double-barreled syringe that allowed them to mix and combine and injected in the wound channel.

Wound revision

All of 14 PS treated wounds underwent revision 24 h. The whole wound channel is longitudinally open that gave the possibility to well overview the clinical appearance of the wound.

The differences in the clinical aspect of necrosis after 24 hours are shown in Table 2.

The results of PS in all wounds fulfilled the clinical requirements, so the wounds from both groups were assessed as effectively treated (Table 2). Since there were no or minimal signs of necrosis, hemorrhage and infections (0–1) in animals from the group B, FGA was applied again, then the drain was placed and wounds were closed primarily (Figure 5).

Fig. 4 – Application of fibrin glue with antibiotics after primary surgery.

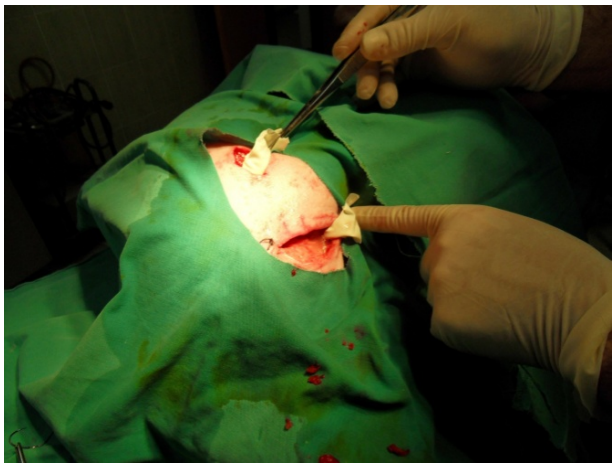

Fig. 5 – After wound revision a drain was placed and fibrin glue with antibiotics applied.

The drain was removed 24 h after revision. Later, during the experiment, the pigs were in good general condition, with body temperatures within the normal range and taking food and water normally. All animals survived the first 7 days after the injury.

Light microscopy

Light microscopy of tissue samples from the revision 24 h after PS showed bleeding of low to moderate degree,

absent or minimally spread necrosis of muscle tissue, while the degree of inflammatory reaction was usually minimal to moderate both in the group A and B. There were no statistically significant differences after 24 h between the groups in the degree of necrosis, bleeding and inflammation.

In samples taken on the postoperative day 4 with and without FGA, a moderate inflammatory reaction was registered with mixed cellular composition, composed of lymphocytes and macrophages, and less often of polymorphonuclears, while necrosis was absent or poorly spread. The young granulation tissue was found, rich in blood vessels, as well as the fibroblasts and the fibrocytes with the initial production of immature collagen of a moderate degree.

On the postoperative day 4 in the group B, acellular eosinophilic homogeneous material was observed, which represented unabsorbed residues of the fibrin glue, located on the surface of the wound or in the shape of small irregular islands, surrounded by the collagen connective tissue. The mononuclear inflammatory cells, type of lymphocytes and macrophages, were little to moderately present, while the connective tissue cells, fibroblasts and fibrocytes were prevalent. The thick and dense collagen fibers arranged in the form of bundles, as well as the young granulation tissue with numerous blood vessels were observed (Figure 6).

The differences in bleeding after 4 days are shown in Table 3.

The statistically significant differences between the groups A and B after 4 days were found in the degree of bleeding and fibroblast reaction (Table 3) ($p < 0.01$), whereas the differences in the degree of necrosis and inflammation, as well as in the produced amount of collagen and the number of giant cells were not statistically significant.

The signs found on the postoperative day 7 in to group B were: mature collagen connective tissue, lymphocytes and the remains of unabsorbed fibrin glue with strong giant cell reaction around the foreign body (Figure 7).

Table 3

The differences in histopathological findings on the days 4 and 7 after wounding

Histopathological findings (extent)	Animals (n)		
	A	B	Total
Bleeding on the day 4*			
0	0	7	7
1	7	0	7
Fibroblast reaction on the Day 4†			
1	2	0	2
2	5	5	10
3	0	2	2
Collagen amount on the Day 7‡			
2	6	3	9
3	1	4	5

For abbreviations see under Table 2. *Fisher's exact test, $p < 0.01$. † Somers'd 2.84, $p < 0.01$.‡ Somers'd 2.1, $p < 0.05$.

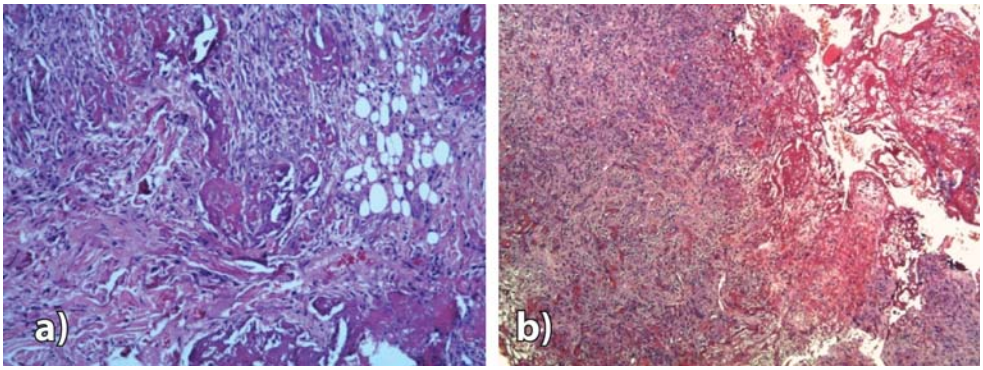

Fig. 6 – a) The inlets of fibrin glue and bunched thick collagen fibers, and b) The fibrin glue and young granulation tissue with the numerous blood vessels (hematoxyllin and eosin, × 100).

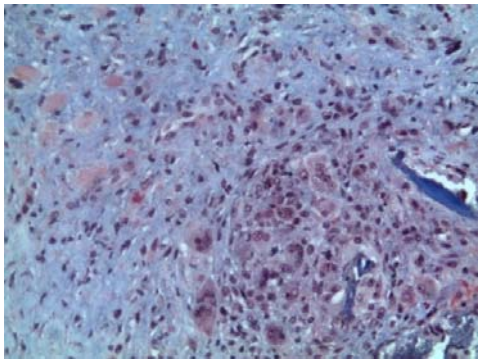

Fig. 7 – The presence of mature collagen connective tissue, lymphocyte and giant cell reaction around unabsorbed fibrin glue remains (Masson trichrome, × 200).

Statistically significant differences after 7 days of surgery between the groups A and B were found in the amount of the produced collagen, while the differences in the degree of necrosis, inflammation, and fibroblast reaction were not statistically significant (Table 3).

Discussion

More than 70% of all firearm injuries in modern wars and civil practice are injuries of extremities, and 60% of them are soft-tissue injuries [30, 35].

These injuries most often belong to the second degree of urgency. This implies that surgery may be postponed for a certain period of time, without the fear of developing of adverse consequences because of the delay, if properly treated [4, 40].

Soft-tissue gunshot wounds of extremities are characterized by the high morbidity and low mortality. Their high incidence is the problem for the second and third military level (echelon) of care [31, 32]. The basic war surgery principles of aggressive resuscitation, early and thorough debridement, short-duration damage-control surgical procedures, and rapid evacuation were critical in reduction of wound infection rates [35, 36]. Nowadays the frequency of this kind of injuries is increasing because of criminal and terroristic activities. A high incidence of soft tissue injuries and the difficult conditions for the surgical treatment raise the question: how to shorten hospital treatment and provide better surgical approach. The timing of wound closure is important. Delayed primary closure is wound closure performed within 4–7 days after the injury. The aim of DPS is to close the wound during the fibroblastic phase of wound healing. This occurs between the days 3 and 6 following injury [15].

The original approach to treatment of gunshot wounds is applied in this experiment.

There are no data in the medical literature about the experimental monitoring of the healing process of shot wounds after PS treatment to the full healing, as well as the data about experimental application of FGAs within PS treatment. The available works only show the effectiveness of FG as hemostatic in war wounds [41–44]. Fibrin sealants have several advantages. They speed up the formation of a stable clot; they can be applied to very small blood vessels and to areas that are difficult to reach; they lower the risk of postoperative inflammation or infection; they provide slow-release delivery of medications (antibiotics) to tissues exposed during surgery and they are conveniently absorbed by the body during the healing process.

Greco et al. [45] presented that the delivered amount of each drug was enough to maintain the minimal inhibitory concentration until the day 4 of culture for the most of antibiotics, resulting in a prolonged release of the drug. Kram et al. [46] presented that the addition of antibiotics to fibrin sealant clots resulted in continuous diffusion of antibiotics into the surrounding for up to 5 to 7 days. The antibacterial effects of fibrin sealant clots with antibiotics were significantly higher compared to fibrin sealant clots without antibiotics. In addition, the presence of fibrin sealant clots with antibiotic resulted in a reduction in bacterial growth [46].

Fibronectin consisted in FG can locally support creation of fibrin polymers, adhesion of fibroblasts and reepithelization of the tissue, and that speeds up the wound healing process [18, 41–44]. Personal war experience and experimental works are the source of the idea how to improve the surgical approach and the treatment of the wounds caused by a projectile of a high initial velocity [16, 18, 26, 47].

The M70AB2 automatic rifle has the initial velocity of projectile M67 of 720 m/s, which is close to the initial high velocity level. For this reason and with the intention to avoid bone damage, shooting was done from the distance of 5 m [26]. Projectile M67 after causing short straight channel (9 cm ± 3 cm) starts to destabilize. The shapes and dimensions of the exit wound are directly dependent on the quantity of the energy transferred from the projectile, which is proportional to the length of the wound channel and the degree of destabilization of the bullet [23, 48, 49]. The wound channel with the length over 100 mm is characterized by large devitalization, which may be measured by the amount of excised tissue. The mass of devitalized tissue is increasing in proportion to the length of wound channel. The basic knowledge about ballistic gunshot wounds, as well as about destructive power of certain weapons, is needed to be known by the surgeon in order to assess the nature and extent of injury and to apply the most efficient treatment. Our study confirmed that the applied primary surgery of the wounds induced with a shot with a M70AB2 rifle was fully effective [16, 23, 26, 47].

The margins of the irreversible circulatory disturbances become clearly recognized only after several hours from injury, and they are ideal limits for the removal of necrotic tissues during primary surgery. The signs of devitalization are much clearer after six than after one hour from injury. There are no significant differences in the extent of the irreversible changes between 6 and 12 h. The pathological changes after 24 h were more clearly expressed than after 12 h, but there was no increase of a necrotic zone. After 24 h an additional necrosis can be developed only as a result of the impaired circulation or an infection [50]. For that reason, primary closure was performed after 24 h in the group B.

Histopathologically no statistically significant differences in the degree of necrosis, inflammation and bleeding were registered between the groups A and B, after 24 h. There were significant differences in the degree of bleeding and fibroblast reaction between the groups A and B after 4 days and in the amount of produced collagen after 7 days. This suggests a local protective and general stimulating effect of FGA.

We gave 1 g of ceftriaxone preventively to all animals because of its broad spectrum of antimicrobial activity and the simplicity of application. The use of antibiotics can prevent infection and local metabolic disturbances, limit local tissue destruction, and reduce the amount of necrotic tissue in penetrating gunshot wounds of soft tissue [30, 31, 51]. Systemic use of antibiotics is necessary before starting primary surgical therapy that is a part of the Definitive Surgical Trauma Care in the days that follow [51, 52].

Conclusion

Primary surgical treatment of wounds in this study represents the basis for successful treatment. Uncomplicated soft-tissue wounds caused by an automatic M70AB2 rifle may be

closed primarily with delayed suture without the risk of developing complications if on revision, 24 h after primary surgery, there were no present necrotic tissues, hematoma, and any signs of infection when fibrin glue with antibiotics (ceftriaxone and clindamycin) was applied.

The use of this method should be limited to individual and strictly controlled cases in civil practice for now. The application of this method in echeloned military field medical systems dealing with casualties needs further scientific confirmations.

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Analysis of over-the-counter medicines use among nursing students

Analiza uzimanja lekova bez recepta među studentima zdravstvene nege

Suzana Mlinar, Rosanda Rašković Malnaršič

Faculty of Health Science, University of Ljubljana, Ljubljana, Slovenia

Abstract

Background/Aim. The use of over-the-counter (OTC) medication is widespread among the adult and student populations in Slovenia. The aim of the study was to analyse the prevalence of OTC medicines use among nursing students with respect to sociodemographic characteristics. **Methods.** A total of 241 nursing students in the Faculty of Health Sciences, Ljubljana, were included in the cross-sectional study. A questionnaire was created for the purposes of the study. Statistical analysis was performed with SPSS 20. Descriptive statistics, *t*-test and the contingency coefficient were calculated. Statistical significance was set at the p-value of < 0.05. **Results.** The study showed that the use of prescription drugs was significantly higher in women ($p = 0.029$), students living in rural areas ($p = 0.005$) and students who described themselves as being of bad health ($p = 0.008$). At the same time, a third of the respondents had been taking one OTC medicine within the last month; those taking several prescription drugs commonly administered several over-the-counter medicines ($p = 0.027$). Women used OTC medicines to treat pain and fever ($p = 0.001$), respiratory issues ($p = 0.015$), and fungal infections ($p < 0.000$) more often than men. OTC medicines were also used to treat minor mental health issues by a higher proportion of respondents over 21 years of age ($p = 0.005$) and women ($p < 0.000$), while over-the-counter medicines for treating skin conditions were more frequently used in rural areas ($p = 0.006$). **Conclusion.** Nursing students tend to use OTC medicines on their own accord, receiving instructions for safe use with their purchase, which points to adequate promotion of safe use of medications in Slovenia. Men's assessment of their personal health tends to be better than that of women, who also use medication more frequently. A connection between poor health and a higher incidence of the use of OTC medicines was established. The use of OTC medicines to treat minor mental health issues increased with age and was more typical of women. The results obtained demonstrate the importance of personal control over the safe use of medicines, motivation for personal good health and a healthy lifestyle.

Key words:
nonprescription drugs; questionnaires; students; medical staff; socioeconomic factors; decision making.

Apstrakt

Uvod/Cilj. Uzimanje lekova bez recepta veoma je rašireno među odraslima i među studentima u Sloveniji. Cilj ove studije bio je da se prouči prevalencija uzimanja lekova bez recepta među studentima zdravstvene nege u odnosu na njihove sociodemografske karakteristike. **Metode.** Ova unakrsna studija obuhvatala ja ukupno 241 studenta medicinske nege sa Fakulteta medicinskih nauka u Ljubljani. Za potrebe ove studije sastavljen je upitnik. Statistička analiza podataka urađena je pomoću SPSS 20. Uradili smo deskriptivnu statistiku, *t*-test i izračunali koeficijent kontingencije. Statistička značajnost stavljena je na *p*-vrednost manju od 0,05. **Rezultati.** Studija pokazuje da je uzimanje lekova na recept značajno učestalije među ženama ($p = 0.005$) i među studentima koji su naveli da su lošijeg zdravlja ($p = 0.008$). Istovremeno, trećina ispitanika uzimala je jedan lek bez recepta tokom prethodnog meseca, a oni koji su pili nekoliko lekova na recept obično su uzimali po nekoliko lekova bez recepta ($p = 0.027$). Žene su uzimale lekove bez recepta za ublažavanje bola i groznice ($p = 0.001$), kao i gljivičnih infekcija ($p < 0.000$) mnogo češće nego muškarci. Veći broj ispitanika starijih od 21 godine (muškarci: $p = 0.005$; žene: $p < 0.000$), takođe, uzimali su lekove bez recepta protiv manjih psihičkih smetnji, dok su lekove bez recepta za lečenje kožnih oboljenja češće uzimali oni iz seoske sredine ($p = 0.006$). **Zaključak.** Među studentima zdravstvene nege postoji tendencija uzimanja lekova bez recepta za koje dobijaju uputstvo prilikom kupovine. Ovo ukazuje na potrebu odgovarajućeg promovisanja bezbednog uzimanja lekova u Sloveniji. Muškarci bolje procenjuju svoje sopstveno zdravlje nego žene koje, takođe, mnogo češće uzimaju lekove. Utvrđena je veza između lošeg zdravlja i veće incidencije uzimanja lekova bez recepta. Uzimanje lekova bez recepta protiv manjih psihičkih smetnji povećava se sa godinama i tipičnije je za ženski pol. Dobijeni rezultati ističu važnost lične kontrole bezbednog uzimanja lekova, brige o ličnom zdravlju i zdravom životu.

Ključne reči:
lekovi bez recepta; upitnici; studenti; kadar, medicinski; socioekonomski faktori; odlučivanje.

Correspondence to: Rosanda Rašković Malnaršič, Faculty of Health Science, University of Ljubljana Zdravstvena pot 5, 1 000 Ljubljana, Slovenia. Phone: +838 1 300 11 63. E-mail: rosanda.malnarsic@zf.uni-lj.si

Introduction

Health is a dynamic process that involves constant changes; however, it is necessary to take into account the fact that sometimes environmental stimuli (physical, psychological) are so strong that can cause illness [1]. Health is an important human value. If healthy, we want to do our best to maintain or even strengthen it; if sick, we endeavour to recover as soon as possible and as much as possible. Since prehistoric times, we have tried to help ourselves when ill, namely, to use all the resources at our disposal, in particular those that are efficient from our point of view. And the same is true today; now individuals with deteriorating health first try to help themselves, by means of over-the-counter (OTC) medicines [2]. OTC medicines are drugs sold directly to consumers without prescriptions and are safe to use and labelled for use without the supervision of a healthcare professional [3]. In Slovenia, OTC medicines are available in pharmacies and specialised stores, they are also available on the Internet; in general, they are the most commonly purchased in pharmacies [4, 5].

Use of OTC medicines is an individual choice that is likely to be made in the event of milder symptoms that do not need treatment provided by a physician [6]. An individual decides to take them with reference to the expressed symptoms, as well as general knowledge and personal experience in the use of such products [2]. Taking OTC medicines to relieve health problems without consulting your physician, with the advice of a pharmacist or other health workers, constitutes a form of self-medication [7]. It can last for three to seven days and cannot be longer than seven days [7]. It is an effective method for the treatment and relief of minor health problems, such as flu, colds, coughs, sore throat, headaches and muscle pain [8].

OTC medicines only provide benefits for a user when taken properly. Users often falsely believe that two tables provide twice as much benefit as one [9]. In taking OTC medicines, there is a greater likelihood that a user will not be provided with any instructions from a healthcare professional, compared to the use of prescription drugs [9]. Thus, users of OTC medicines should be informed about the benefits and risks of use of such products [8]; an important role thereof should be assumed by pharmacists, physicians and nurses. Risks associated with the use of OTC medicines or side-effects may occur even if used correctly; in fact, taking OTC medicines increases the likelihood of drug-drug interactions, including interactions with alcohol [9]. Using OTC medicines may affect the individual's consistency in treatment and thus its quality of life [3]. Self-medication is a form of self-help and also an important part of individual behaviour and the perception of health maintenance [10]. It is therefore important that self-medication is designed on the basis of expert advice to the user regarding correct, safe and efficient treatment [11] and moreover, characterised by strict adherence to the instructions for the safe use of such drugs. In Slovenia, the use of OTC medicines among the adult population is widespread (84.0%), in particular among students and the economically active population; women are also more likely to take OTC medicines and vitamins and minerals than men [5]. Some authors note that OTC medicines are used more frequently by women than men, as well as the better educated population with a higher income, white men and elderly persons [12–14]. In 2008 at the University of Ljubljana, 92.8% of health care students and 91.9% of non-healthcare students undertook self-medication. Among the health care students of the University of Ljubljana, there was a significant increase in self-medication among students in the previous academic year compared to the first academic year [4]. Due to the widespread use of OTC medicines, it is important that users get appropriate and professional information on the safe use of such products for self-medicating minor health problems before taking OTC medicines. This is of particular importance since it is known that students rarely visit a physician and often purchase OTC medicines in pharmacies [4].

The aim of the study was to analyse the prevalence of the use of OTC medicines among nursing students of the Faculty of Health Science, Ljubljana, in relation to sociodemographic characteristics.

Method

The cross-sectional study included nursing students of the Faculty of Health Science, a first-cycle programme in the 2012/13 academic year. The students were not divided according to the different years of study. The respondents who attended lectures on pharmacology in the first semester of the program, were eligible for the study. Thus, the sample represents the direction of healthcare students, irrespective of secondary education. Students were informed about the purpose of the study and their anonymity was assured.

Data was collected using an anonymous questionnaire. The study was conducted from February to April 2013. The survey questionnaire was designed for the purpose of this study; the choice of questions was based on the findings of previous studies [4, 5, 15, 16]. The questionnaire included sociodemographic data, personal assessment of health status, frequency of use of prescription and non-prescription drugs, the decision to use OTC medicines, received instructions on safe use, symptoms and the decision to purchase OTC medicine. The Cronbach's alpha coefficient was used to calculate the questionnaire's consistency, with the resulting value of 0.682. A pilot study was not carried out. Instructions for filling out the questionnaire were enclosed. A total of 65.8% of the correctly filled-out questionnaires were included in the analysis.

A statistical analysis of the data was carried out using SPSS v. 20 (SPSS Inc., Chicago, Ill., USA). Descriptive statistics was calculated, *t*-test performed in order to determine differences according to gender and contingency coefficient to identify differences due to place of residence, age and current health status assessment. A p value ≤ 0.05 was considered statistically significant.

Results

The sample included 241 nursing students, aged 18 to 30 years, mean age 20.43 ± 1.67 years. Among them were 17.4% males and 82.6% females. Other sociodemographic data is de-

scribed in Table 1. The study did not find any significant differences between healthcare (Secondary Nursing School) and non-healthcare (Grammar School or Other Vocational Secondary Schools) respondents by secondary education.

Table 1

Sociodemographic characteristics of the study population (n = 241)

Variables	Participants	
	n	(%)
Gender		
men	42	(17.4)
women	199	(82.6)
Secondary education		
Secondary Nursing School	156	(64.7)
Grammar School	41	(17.0)
Other Vocational Secondary Schools	44	(18.3)
Place of residence		
countryside	130	(53.9)
suburb	44	(18.3)
city/town	67	(27.8)
Age (years)		
18–20	153	(63.5)
21–30	88	(36.5)

Less than half of the respondents (45.6%) assessed their current health status as very good, 24.1% as good and 20.3% as excellent (Table 2). The average value of health assessment from 1 to 5 was 3.74 (SD = 0.97), which mean a tendency towards very good health. Men assessed their health status better than women ($p = 0.050$). No significant differences in assessing the health status by place of residence and age was observed.

In the previous year, 70.5% respondents visited their physician due to health problems that was statistically significant for those who rated their health as poor or satisfactory ($p = 0.011$).

In the previous month, 57.7% of the respondents had not been treated with prescription drugs; 31.1% had been treated using one prescription drug, and 7.5% with two different prescription drugs (Table 3). In the previous month, more women than men ($p = 0.029$), more of those who lived in rural areas and in the suburbs ($p = 0.005$) and those who assessed their health status as worse ($p = 0.008$) had administered prescription drugs. The use of several prescription drugs

Table 2

Personal assessment of the current health status by participants

Assessment of the current health status	Participants		Gender		Place of residence		Age		Secondary education	
			t	*p*	CC	*p*	CC	*p*	CC	*p*
	n	(%)	2.177	0.050	0.191	0.328	0.152	0.223	0.191	0.336
Excellent	49	(20.3)								
Very good	110	(45.6)								
Good	58	(24.1)								
Satisfactory	16	(6.6)								
Poor	8	(3.3)								

$p \leq 0.05$ was considered statistically significant; CC – contigency coefficient.

Table 3

Taking prescription drugs and over-the-counter (OTC) medicines by students in the previous 30 days

Variables	Participants		Gender		Place of residence		Age		Secondary education		Assessment of health	
	n	%	CC	*p*	CC	*p*	CC	*p*	CC	*p*	CC	*p*
Different prescription drugs taken in the previous month			0.207	0.029	0.289	0.005	0.106	0.600	0.171	0.512	0.346	0.008
none	139	(57.7)										
one	75	(31.1)										
two	18	(7.5)										
three	5	(2.1)										
four	4	(1.7)										
Different OTC medicines taken in the previous month (n)			0.116	0.509	0.173	0.494	0.129	0.399	0.173	0.489	0.350	0.006
none	104	(43.2)										
one	81	(33.6)										
two	36	(14.9)										
three	17	(7.1)										
four	3	(1.2)										
Different OTC medicines taken compared to two years ago (n)			0.175	0.055	0.128	0.674	0.104	0.453	0.091	0.920	0.252	0.173
a larger	21	(8.7)										
a smaller	50	(20.7)										
approximately the same	123	(51.0)										
I do not know	47	(19.5)										
OTC medicines taken compared to two years ago (frequency)			0.086	0.613	0.173	0.494	0.122	0.303	0.115	0.777	0.164	0.877
more often	25	(10.4)										
less often	50	(20.7)										
the same	122	(50.6)										
I do not know	44	(18.3)										

$p \leq 0.05$ was considered statistically significant; CC – contigency coefficient.

in the previous month correlated with the concomitant use of several OTC medicines ($p = 0.027$).

In the previous month 43.2% of the respondents used no OTC medicines, 33.6% one and 14.9% two different OTC medicines (Table 3). Respondents who had been taking several different OTC medicines in the previous month assessed their health status as worse ($p = 0.006$).

Approximately the same number of OTC medicines were taken by 51% of the respondents as they did two years ago (50.6%); 20.7% used a small number and 20.7% less often than two years ago. In the previous month, 8.7% of the respondents used a greater number of OTC medicines and 10.4% more often than two years ago. There was a trend toward statistical significance for the use of more OTC medicines in women than men in the previous month ($p = 0.055$), compared to two years ago (Table 3).

When purchasing OTC medicine, 76.3% respondents received an explanation on safe use. A total of 68.5% of the respondents decided themselves to use OTC medicines, 12.9% on the basis of physician's recommendation, 7.9% on the basis of pharmacist's recommendation and 10.8% for other reasons (Table 4). Those who assessed their health status as poor or satisfactory were more often advised to use OTC medicines by a physician or by a pharmacist compared to those who assessed their health status as good or satisfactory ($p = 0.026$).

In the previous six months, 79.3% of the respondents used OTC medicines for pain and fever relief (significantly more women than men, $p = 0.001$), 44.8% for respiratory problems (significantly more women than men, $p = 0.015$) and 41.9% of respondents for better overall health. OTC medicines for skin problems were significantly more likely to be used by respondents who lived in rural areas and suburbs ($p = 0.006$) and those for ease of psychological disorders were significantly more likely to be used by individuals of 21 years and more ($p = 0.005$) and women ($p < 0.001$); drug use for fungal infections was statistically significant for women ($p < 0.001$). Respondents who rated their health status as poor had statistically significant problems with fungal infection ($p = 0.011$), allergies ($p = 0.048$) and minor mental health issues ($p < 0.001$) and those who rated their health status as good suffered from respiratory problems ($p < 0.001$) (Table 5).

In the previous six months only 4.1% respondents had not used any OTC medicines, 34.9% used one drug, 27.0% two different drugs and 18.3% three different OTC medicines (Figure 1). The majority of the respondents (68.9%) purchased OTC medicines in the event of suffering from health problems, others (14.9%) because they had such products at their disposal before suffering from health problems and the rest (12.0%) to be safe from the possible occurrence of seasonal diseases. Given the sociodemographic information, no

Table 4

Person advising use of over-the-counter medicine

The type of person	Person		Gender		Place of Residence		Age		Secondary education		Assessment of health	
	n	(%)	t 0.941	*p* 0.350	CC 0.158	*p* 0.401	CC 0.045	*p* 0.920	CC 0.150	*p* 0.479	CC 0.296	*p* 0.026
Physician	31	(12.9)										
Pharmacist	19	(7.9)										
Own decision	165	(68.5)										
Other	26	(10.8)										

$p \leq 0.05$ was considered statistically significant; CC – contigency coefficient.

Table 5

Taking over-the-counter medicines within the previous six months to facilitate health problems

Health status			Gender		Place of residence		Age		Secondary education		Assessment of health	
	n	%	t	*p*	CC	*p*	CC	*p*	CC	*p*	CC	*p*
Pain and fever	191	(79.3)	-3.622	0.001	0.118	0.183	0.079	0.217	0.025	0.929	0.183	0.080
Respiratory problems	108	(44.8)	-2.490	0.015	0.081	0.447	0.077	0.233	0.090	0.371	0.279	0.000
Allergies	24	(10.0)	-0.745	0.459	0.135	0.106	0.051	0.431	0.118	0.182	0.196	0.048
Indigestion	49	(20.3)	-0.230	0.819	0.115	0.201	0.083	0.196	0.083	0.436	0.182	0.083
Minor mental health issues	14	(5.8)	-3.871	0.000	0.122	0.161	0.177	0.005	0.077	0.490	0.284	0.000
Fungal infections	16	(6.6)	-4.161	0.000	0.093	0.349	0.029	0.651	0.057	0.672	0.226	0.011
Skin problems	28	(11.6)	-1.163	0.249	0.200	0.006	0.205	0.001	0.138	0.097	0.168	0.136
Better general well-being	101	(41.9)	-0.552	0.583	0.074	0.514	0.089	0.165	0.043	0.801	0.190	0.062

$p \leq 0.05$ was considered statistically significant; CC – contigency coefficient.

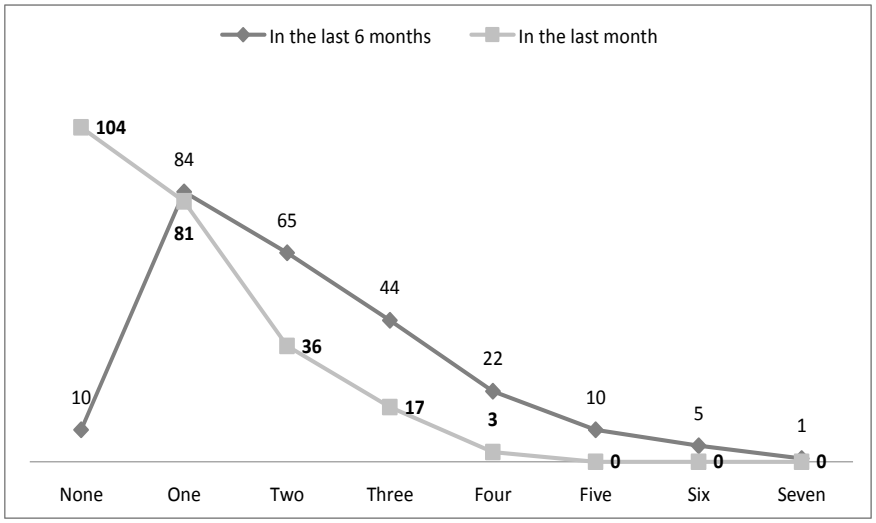

Fig. 1 – Comparison of taking different over-the-counter medicines in numbers of students.

statistically significant differences were found in the decision on when to purchase an OTC medicine.

Discussion

The results of this study suggest that 65.9% of nursing students, the average age of 20 years, assessed their health as excellent and very good, of which men assessed their health status as being better compared to women. A third of the Brazilian students assessed their health status as excellent and one in three consulted a physician in the previous year [17]. Half of Americans aged 18 years assessed their health status as excellent and very good and among them 74% of people consulted a physician [15]. In the previous year more than two-thirds of the respondents consulted a physician, among them all those who assessed their health status as worse. The link between the self-assessment of health status and using OTC medicines is well known, poorer self-perceived health status promotes the use of OTC medicines [18]. A study of self-medication and drug use among adult Slovenian showed that in 2008, 70.5% of the population aged 18 years or more had experienced health problems [5].

In the previous month, more than half of the respondents used prescription drugs and a third used one prescription drug. Among them women and people living in rural areas statistically dominated. In the previous month, 43.2% of the respondents did not take any OTC medicines and one third of the respondents used one OTC medicine. A total of 38.6% of medicine and pharmacy students of the University Zagreb used vitamins and minerals [19]. Among adult Slovenians, 84% used OTC medicines and vitamins and minerals [5]. We found that half of the respondents estimated their use of OTC medicines in the previous month at the same level as two years ago. Among adult Americans, there is a very small percentage of people who do not take drugs at all, more than a third use an OTC medicine and a third a prescription drug, with half of the people stating that their consumption of OTC medicines is about the same as three years ago [15].

An important benefit of OTC medicines is that they are intended for milder symptoms that require immediate treatment, such as an acute headache for which the immediate use of over-the-counter painkillers is recommended and their efficacy is increased if immediately consumed [20]. In case of migraine, headache treatment in the first hour since the onset of pain significantly reduces the duration and strength compared to beginning the treatment after one hour [20]. For the effective relief of symptoms, an OTC medicine consumer should ensure the safe and effective use of such products [21]. Therefore, OTC medicine consumers should receive relevant information about the product, they should be provided with a range of products so they can choose the most relevant for their health status and expressed symptoms [21]. A total of 76.3% of the respondents indicated they had been provided with clarification on the safe use of OTC medicines. Advice from pharmacists on using OTC medicines lead to better treatment outcomes [21]. A pharmacist is usually the first professional person that an individual may turn to when considering self-medication [3]. The pharmacist plays the important role in providing advice and assistance to consumers and should thus ensure that a product properly addresses the symptoms [22]. The important role is also assumed by the physician, who should be aware of the self-medication of each patient. Among many tasks a physician should provide is guidance on the safe and rational use of OTC medicines [3]. However, in our study 68.5% respondents decided themselves to use OTC medicines, 12.9% on the basis of their physician's advices, 7.5% on the basis of pharmacist's advice.

We found that physicians significantly provide advice on OTC medicines to those who assessed their health status as worse and pharmacists to those who assessed their health status as better. Taking into account the results of this study, it can be concluded that physicians and pharmacists are consistently engaged in advising consumers on the consumption of OTC medicines. Many individuals choose to use OTC medicines after consulting their physician or a pharmacist,

but their final decision is autonomus [23]. Therefore, OTC medicine consumers need to be educated for responsible self-medication and the safe use of drugs. The use of OTC medicines is useful, users describe it as easy to use, safe, convenient, at your "fingertips" and easily and quickly accessible [24]. The decision on self-medication is also based on knowledge of OTC medicines and confidence in their own abilities to undertake a proper decision [17, 23]. Brazilian health care students are more likely to opt for self-medication compared to non-health care students [17].

The study found that women are significantly more likely to use OTC medicines to relieve pain and fever, respiratory problems, fungal infections and minor mental health issues. More respondents aged 21 years or more and a statistically significant larger number of women used OTC medicines to relieve minor psychological problems; and significantly more respondents living in rural areas used OTC medicines to relieve skin problems. Women more often used OTC medicines [14], in particular painkillers, compared to men [25]. Individuals older than 30 years, employed and in a relationship are in general more inclined to self-medicate; authors indicate the male gender and having children as protection factors against self-medication [17].

The participants in this study most often purchased OTC analgesic and antipyretic drugs, drugs for respiratory problems and drugs to improve general well-being. Surprisingly, the study conducted in North Carolina in 2003 also observed the use of the same OTC medicines to treat minor health problems [26]. Even students of the University of Zagreb frequently self-medicated due to respiratory problems [19], while Brazilian students used OTC drugs for fever, menstrual cramps, muscle pain and coughs [17]. Since OTC medicines quickly and effectively help with disease problems, respondents often purchase them in the event of health problems that are already expressed.

Three important limitations of the study should be noted. The first is a small sample; as we included nursing students from only one Slovenian faculty, the results cannot be generalised to all Slovenian nursing students or the occupational group of nurses. The questionnaire did not include questions on the experience of stress and the rate thereof. Since we observed that using OTC medicines increases with age, it would be reasonable to determine the level of stress and its connection to the administration of OTC medicines. Also, the questionnaire did not include questions on taking the prescription contraceptive pill and questions about taking OTC medicines exclusively for menstrual cramps (pain, nausea).

An important finding of this study is that nursing students who used multiple prescription drugs in the previous month assessed their health status as poor and used several different OTC medicines, as well. The decrease in the number of different drugs used by respondents in the previous six months compared to the previous month was statistically significant – probably at the expense of prescription drugs or due to the implementation of the survey in the spring months. Half of the respondents used about the same number and at the same frequency of OTC medicines in the previous month as they did two years ago. This is a good indicator of the rational use of OTC medicines among nursing students; in fact, drugs are more accessible today. Self-control over the safe use of drugs and motivation for health and healthy lifestyle are of paramount importance.

Conclusion

Based on the obtained results, we can conclude that in Slovenia the safe use of drugs is ensured as most of the respondents received an explanation about the safe use of OTC medicines. We observed that men felt healthier and women were more likely to take drugs. We found that the use of OTC medicines to relieve minor psychological problems increased significantly with age and it was typical of women. Therefore, it would be worth exploring the relationship between stress and taking OTC medicines in the future.

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Copper and zinc concentrations in atherosclerotic plaque and serum in relation to lipid metabolism in patients with carotid atherosclerosis

Koncentracija bakra i cinka u aterosklerotskom plaku i serumu u odnosu na metabolizam lipida kod bolesnika sa karotidnom aterosklerozom

Nebojša M. Tasić*, Danijela Tasić*, Petar Otašević*, Mirjana Veselinović[†], Vladimir Jakovljević[‡], Dragan Djurić[§], Djordje Radak*

***Dedinje Cardiovascular Institute, [§]Institute of Physiology "Richard Burian", Faculty of Medicine, University of Belgrade, Belgrade, Serbia; [†]Department of Rheumatology, Internal Medicine Clinic, Clinical Centre Kragujevac, [‡]Department of Physiology, Faculty of Medicine, University of Kragujevac, Kragujevac, Serbia**

Abstract

Background/Aim. Some oligoelements are now investigated as possibly having a role in atherosclerosis. The aim of this study was to compare the concentrations of copper and zinc in the serum and carotid plaque and parameters of lipid metabolism in patients with different morphology of carotid atherosclerotic plaque. **Methods.** Carotid endarterectomy due to the significant atherosclerotic stenosis was performed in 91 patients (mean age 64 ± 7). The control group consisted of 27 patients (mean age 58 ± 9), without carotid atherosclerosis. Atherosclerotic plaques were divided into four morphological groups, according to ultrasonic and intraoperative characteristics. Copper and zinc concentrations in the plaque, carotid artery and serum were measured by atomic absorption spectrophotometry. **Results.** Serum copper concentrations were statistically significantly higher in the patients with hemorrhagic in comparison to those with calcified plaque (1.2 ± 0.9 μmol/L *vs* 0.7 ± 0.2 μmol/L, respectively; p = 0.021). Zinc concentrations were statistically significantly lower in plaques of the patients with fibrolipid in comparison to those with calcified plaques (22.1 ± 16.3 μg/g *vs* 38.4 ± 25.8 μg/g, respectively; p = 0.024). A negative significant correlation was found for zinc and triglycerides in the serum in all the patients (r = -0.52, p = 0.025). In the control group we also demonstrated a positive significant correlation for low-density lipoprotein cholesterol and copper in the serum (r = 0.54, p = 0.04). **Conclusion.** The data obtained in the current study are consistent with the hypothesis that high copper and lower zinc levels may contribute to atherosclerosis and its sequelae as factors in a multifactorial disease. Further studies are necessary in order to conclude whether high concentration of copper and zinc in the serum could be risk factors for atherosclesrosis.

Key words:
zinc; copper; risk factors; arteriosclerosis; carotid stenosis; lipids.

Apstrakt

Uvod/Cilj. Neki oligoelementi sada se istražuju zbog moguće uloge u aterosklerozi. Cilj ovog istraživanja bio je da se uporede koncentracije bakra i cinka u serumu i karotidnom aterosklerotskom plaku i parametri metabolizma lipida kod bolesnika sa različitim morfologijama karotidnog plaka. **Metode.** Karotidna endarterektomija zbog klinički napredovale ateroskleroze urađena je kod 91 bolesnika starosti 64 ± 7 godina. Kontrolnu grupu činilo je 27 bolesnika prosečne starosti 58 ± 9 godina, bez prisutne karotidne ateroskleroze. Prema morfologiji, aterosklerotski plakovi su podeljeni u četiri grupe na osnovu ultrazvučnog nalaza i intraoperativnih karakteristika. Spektrofotometrijski su određivane koncentracije bakra i cinka u aterosklerotskom plaku, zidu karotidne arterije i serumu. **Rezultati.** Koncentracija bakra u serumu bila je statistički značajno viša kod bolesnika koji su imali hemoragičan plak u odnosu na bolesnike sa kalcifikovanim karotidnim plakom (1,2 mL ± 0.9 *vs* 0,7mL ± 0,2; p = 0,021). Kod bolesnika sa fibrolipidnim plakom koncentracija cinka u samom plaku bila je statistički značajno niža u odnosu na koncentraciju cinka u kalcifikovanom karotidnom plaku (22,1 ± 16,3 μg/g *vs* 38,4 ± 25,8 μg/g; p = 0,024). Kod svih bolesnika u studiji pronađena je negativna korelacija između serumske koncentracije cinka i vrednosti triglicerida u serumu (r = -0.52, p = 0.025). U kontrolnoj grupi serumska koncentracija bakra bila je u pozitivnoj korelaciji sa lipoproteinima holesterola male gustine (r = 0,54, p = 0,04). **Zaključak.** Rezultati dobijeni u ovoj studiji podudaraju se sa pretpostavkom da visoke koncentracije bakra i cinka u serumu mogu doprineti razvoju ateroskleroze i njenih kliničkih manifestacija. Potrebna su dalja klinička istraživanja koja bi dokazala da visoke koncetracije bakra i cinka u serumu mogu biti faktori rizika od nastanka i razvoja ateroskleroze.

Ključne reči:
cink; bakar; faktori rizika; arterioskleroza; aa. carotis, stenoza; lipidi.

Correspondence to: Nebojša M. Tasić, "Dr Aleksandar D. Popović" Cardiovascular Research Center, Dedinje Cardiovascular Institute, Milana Tepića 1, 11 040 Belgrade, Serbia. Phone: +381 11 3601 669, Fax: +381 11 2666 445. E-mail: nebtsa@yahoo.com

Introduction

The positive correlation of elevated low-density lipoprotein (LDL) cholesterol serum levels and inverse correlation of high-density lipoprotein (HDL) cholesterol levels with the atherosclerotic process is a mainstay for research activities regarding pathophysiology and treatment of atherosclerotic clinical manifestation [1].

In recent years investigators have focused their research activities on the role of several trace elements in the pathogenesis of atherosclerosis. Among trace elements, copper and zinc are well-documented as important modulating factors in cardiovascular homeostasis. Special research interest has been given to the relationship between copper and zinc serum and tissue concentrations and lipid status. Several studies pointed out a significant role of copper in LDL oxidation, however the exact mechanism is not yet understood [2]. Copper has the pro- and anti-atherogenic (dual) effect [3]. Some experimental studies showed a positive correlation of copper deficiency with high total cholesterol levels and atherosclerotic changes on coronary arteries [4]. Low copper intake with copper defficiency and high dietary zinc/copper ratio leads to the increase in total serum cholesterol and development of atherosclerotic coronary disease [5]. Other studies implied that high copper serum levels could lead to the endothelial damage and the beginning of the atherosclerotic process [6].

Zinc is an essential trace element for the normal membrane structure and function of important enzymes [7]. Studies reported that low zinc concentration may have an important role in the pathogenesis of atherosclerosis [8]. The effect of high zinc concentration on endothelial cells is not yet understood. Some evidence suggest that zinc can act as an endogenous protective factor against atherosclerosis by inhibiting the oxidation of LDL by cells or transition metals and protect the cell against cell-destabilizing agents such as inflammatory cytokines and polyunsaturated lipids [9].

Through the interaction with endogenic basic fibroblast growth factor zinc potentiates endothelial and vascular smooth muscle cell proliferation which is an important part of intimal hyperplasia during the atherosclerotic process [10].

Diversity and heterogeneity of atherosclerotic plaques implies that the atherosclerotic process is dynamic, complex and continuous. Numerous factors influence the morphology and growth pattern of different atherosclerotic plaques.

The aim of this study was to compare the concentrations of copper and zinc in the serum and carotid plaque and parameters of lipid metabolism in patients with different morphology of carotid atherosclerotic plaques.

Methods

The study group consisted of 118 subsequent patients (mean age 63 ± 8 years; 54.2% males) admitted to the Department of Vascular Surgery at Dedinje Cardiovascular Institute in Belgrade for carotid surgery. Carotid endarterectomy due to significant atherosclerotic stenosis and symptoms of cerebrovascular insufficiency was performed in 91 patients (mean age 64.2 ± 6.9 years, 62.6% males). The control group consisted of 27 patients (mean age 58.4 ± 9.3 years, 25.9% males) without carotid atherosclerosis which were operated due to the symptomatic kinking and coiling of carotid artery.

All the patients underwent neurological, cardiological and vascular surgeon exam, carotid artery ultrasound measurements and were treated surgically. Blood samples were drawn from all the patients before the surgery and sera were analyzed for the concentrations of lipid parameters (total cholesterol, HDL- and LDL-cholesterol and triglycerides), copper and zinc.

Tissue samples, atherosclerotic plaques from the atherosclerotic patients and normal carotid tissue from the control group patients were analyzed for the concentrations of copper and zinc. In the atherosclerotic subjects, atherosclerotic plaques were divided into four morphological groups, namely fibrolipid, hemorrhagic, ulcerated, and calcified plaque, according to ultrasonic and intraoperative characteristics [11].

The study was approved by the Ethics Committee of the Dedinje Cardiovascular Institute. Written consent was obtained from all the patients before they entered the study.

Total cholesterol concentration in the serum was determined by the reaction with cholesterol-oxidase by using a diagnostic kit by Abbott. HDL-cholesterol in the serum was determined with the direct method by using a diagnostic kit by Abbott. Triglycerides in the serum were estimated by the reaction with glycerol-oxidase by using a diagnostic kit Abbott. LDL cholesterol was calculated according to the obtained levels of other lipid parameters noted above.

Copper and zinc concentrations in plaques, carotid artery tissue and the serum were estimated by means of flame atomic absorption spectrophotometry (AAS) using the Varian AA-5 instrument under the conditions recommended by the producer (acetylene/air flame, wavelenght: $\lambda Cu = 324.75$ nm, $\lambda Zn = 213.86$ nm).

Data are presented graphically as a box-plots, showing the mediana, minimum and maximum values. Differences in quantitative variables were assessed using the Mann-Whitney *U* test. The associations between the groups were analyzed by the Spearman's correlation. A p-value < 0.05 was considered statistically significant. Data were analyzed by SPSS version 10 (SPSS Inc, Chicago, Illinois, USA).

Results

Our patients cohort exhibited most of the classic atherosclerosis risk factors (Table 1). The percentage of hypertensive, smoking subjects was higher in the patients with carotid plaque than in the controls ($p < 0.05$). The presence of diabetes mellitus showed statistically significant differences between the patients and the controls ($p < 0.05$).

Atherosclerotic subjects were divided into four groups according to different morphology of analyzed atherosclerotic plaques [11]. The group I (22 patients; 18.6%) represented the patients with fibrolipid atherosclerotic plaques. The group II included the patients with hemorrhagic plaques (8 patients; 6.8%), the group III included the patients with ul-

Table 1

Baseline characteristics of the patients with carotid atherosclerosis (the study group) and the control group of patients

Variable	Study group (n = 91)	Control group (n = 27)	*p* values
Age (years), $\bar{x} \pm SD$	64.2 ± 6.9	58.4 ± 9.3	$p > 0.05$
Hypertension	90.1	59.3	$p < 0.05$
Smokers	67	40.7	$p < 0.05$
Diabetes mellitus	26.4	14.8	$p > 0.05$
Famiily history	49.4	55.6	$p > 0.05$
Carotid artery bruit	90.1	33.3	$p < 0.05$

Results are given as percentage of patients

cerated plaques (31 patients; 26.3%), and the group IV included the patients with calcified atherosclerotic plaques (30 patients; 25.4%). The patients from the control group, without significant atherosclerotic changes in carotid artery, constituted the group V (27 patients; 22.9%).

Values of the distribution of HDL- and LDL-cholesterol between the different plaques morphology groups and controls are presented in Figures 1 and 2, respectively.

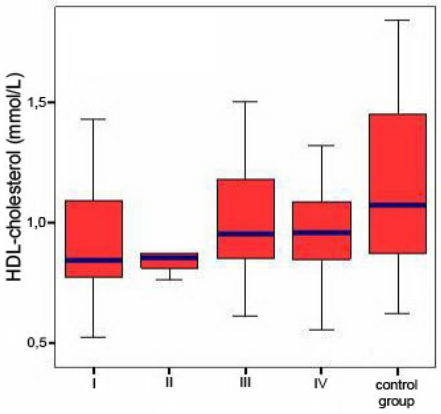

Fig. 1 – Average high-density lipoprotein (HDL) cholesterol values in patients with different morphological groups of atherosclerotic plaques. Groups of patients: I – with fibrolipid atherosclerotic plaque; II – with hemorrhagic plaque; III – with ulcerated atherosclerotic plaque; IV – with calcified atherosclerotic plaque.

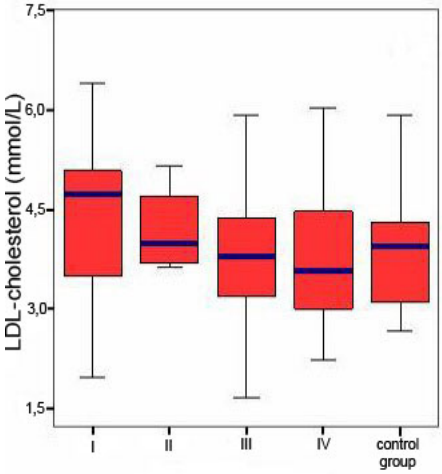

Fig. 2 – Average low-density lipoprotein (LDL) cholesterol values in patients with different morphological groups of atherosclerotic plaques (for explanation see under Figure 1)

The distribution of copper concentration in plaque, carotid tissue and the serum are shown in Figures 3 and 4, respectively. Our study revealed a statistically significantly higher average serum copper concentration in the patients with hemorrhagic plaque (the group II) compared to the patients with calcified (the group IV) atherosclerotic plaques (1.2 μg/g ± 0.9 *vs* 0.7 μg/g ± 0.2, respectively; $p < 0.05$). The average values of copper concentrations in morphologically different atherosclerotic plaques and normal carotid tissue were similar.

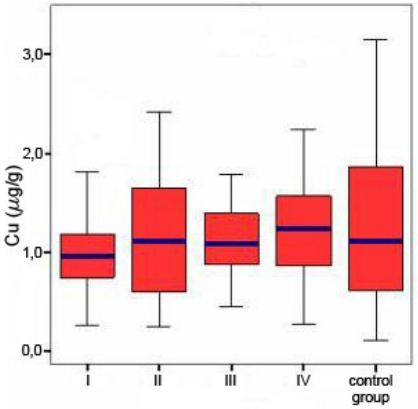

Fig. 3 – Average plaque copper concentration in the different groups of patients (for explanation see under Figure 1).

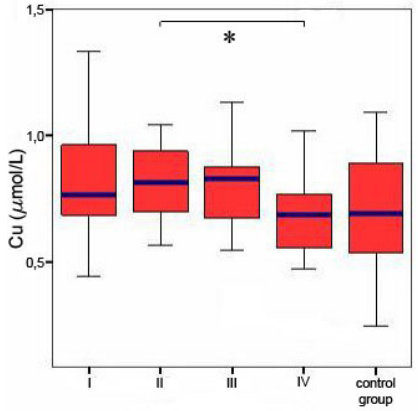

Fig. 4 – Average serum copper concentration in the different groups of patients.

***p < 0.05 (statistically significant difference between the groups II and IV). (for explanation see under Figure 1)**

No significant difference in the serum zinc concentrations was found among the groups (Figure 5). We found a statistically significantly lower average zinc plaque concen-

tration in the patients with fibrolipid plaque (the group I) in comparison to the patients with calcified (the group IV) plaques (22.1 ± 16.3 μg/g *vs* 38.4 ± 25.8 μg/g respectively; $p = 0.024$), (Figure 6).

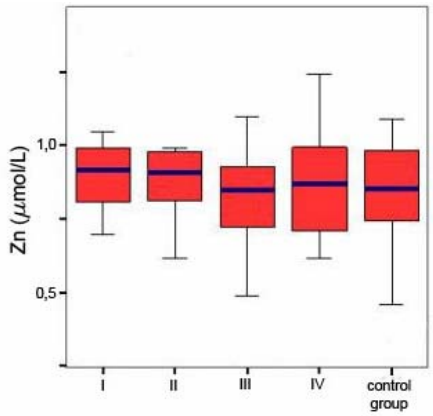

Fig. 5 – Average serum zinc concentration in the different groups of patients (for explanation see under Figure 1).

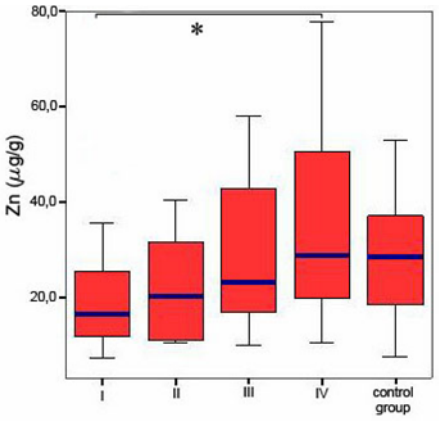

Fig. 6 – Average plaque zinc concentration in the different groups of patients.

***p < 0.05 (statistically significant difference between the groups II and IV) (for explanation see under Figure 1)**

The negative and significant correlation coefficients were calculated for zinc and triglycerides in the selected patients ($r = 0.52$, $p = 0.025$) (data not showed).

In the control group we also demonstrated positive significant correlation coefficient for LDL-cholesterol and copper in the serum ($r = 0.54$, $p = 0.04$) (data not showed).

Discussion

Metal ions have been proposed as causative agents in a number of diseases, including atherosclerosis. The involvement of transition metals in atherosclerosis is controversial. Experimental studies have reported elevated levels of iron and copper in diseased human arteries but have often used methods that release metal ions from proteins [12]. Elevated levels of copper were also detected in carotid lesions [13]. Other studies do not support the hypothesis that elevated metal ion levels may be a major causative factor in aggravated atherosclerosis [14].

Copper can promote low-density lipoprotein oxidation and the formation of macrophage-derived foam (lipid-laden) cells *in vitro* [15]. These elevated metal ion levels may therefore affect plaque stability and propensity to rupture.

Our study revealed a significantly higher copper concentration in the serum of patients with complicated hemorrhagic plaque in comparison to the morphological group IV (calcified plaque). There are no consistent data in the literature regarding the relationship between serum copper concentrations and the severity of atherosclerotic process [16].

An Iranian study revealed a significantly higher serum copper concentration in patients with coronary heart disease of both sexes compared to controls [17].

Diaz Romero et al. [18] determined equivalent serum copper values in patients with coronary heart disease and controls. Correspondingly to our results Alissa et al. [19] revealed no significant difference in both copper and zinc serum concentrations between the patients with established atherosclerosis and controls.

Our investigation showed a similar average copper concentration in atherosclerotic plaques and normal carotid tissue. The previous study of our group reported significantly higher copper concentrations in patients with ulcerated plaque in comparison to copper concentration in normal carotid tissue [20]. Serum copper concentrations are also associated with other coronary risk factors, including body mass index, levels of physical activity, serum HDL-cholesterol and C-reactive protein (CRP) [21].

Polish authors [22] in their investigations demonstrated higher copper concentration in atherosclerotic plaques of patients with peripheral arterial disease in comparison to controls.

Our results show a trend of higher serum zinc concentration in the patients with fibrolipid and hemorrhagic plaques than in the patients in other morphology groups of atherosclerotic plaques. We found no significant difference between serum zinc levels in atherosclerotic and normal subjects. We found a significantly lower average zinc plaque concentration in the patients with fibrolipid plaques in comparison to the patients with calcified plaques. Stadler et al. [23] found elevated levels of zinc in human atherosclerotic advanced lesions carotid and abdominal artery compared to healthy tissue or early lesions. The control subjects had no significantly higher plaque zinc levels compared to noncalcified atherosclerotic plaque patients which is in accordance with our earlier investigation [20].

Recent studies reported lower zinc serum levels in patients with different clinical form of atherosclerosis compared to controls and suggested that protective effect of zinc accumulation in atherosclerotic plaque could be associated with lesion calcification [23, 24]. Patients with the established coronary artery disease had significantly higher serum CRP and lower serum zinc compared to both patients without coronary artery disease and healthy controls [16]. Zinc deficiency has been associated with the development of atherosclerosis [23].

The presented higher proportion of male subjects in atherosclerotic groups and female subject in patients with

kinking and coiling carotid artery is in accordance with the results of earlier studies [25]. The atherosclerotic subjects in our study had higher LDL cholesterol and lower HDL cholesterol serum levels in comparison to the control subjects. Previous studies also demonstrated a significant positive correlation of LDL values and negative correlation of HDL values with the severity of the carotid atherosclerotic process [26]. The investigators from the "Tromso study" reported a strong positive correlation of HDL values and reduction of atherosclerotic plaque progression, higher plaque echogenity and in some cases a reduction in plaque size [27].

We found a significant negative correlation between serum zinc concentration and triglycerides. Serum copper concentrations were positively associated with fasting serum triglycerides in Iranian patients [16]. In the control group we demonstrated a positive significant correlation of LDL cholesterol and copper concentration in the serum.

Human studies investigating the effect of copper and zinc status on cholesterol metabolism are very limited [28]. In an adult African-American community females taking either zinc supplements had higher HDL-cholesterol values than nonsupplementing females [29].

Serum zinc and copper levels were both significantly lower in individuals with normal versus high levels of LDL-cholesterol in a large Iranian population sample [30]. One of the theory implies that hypercholesterolemia and endothelial damage lead to peroxynitrite formation, decrease in pH and increase in copper ion release from ceruloplasmin. This process enhance LDL oxidation and plaque growth. Several studies failed to confirm a significant correlation between oligoelements and lipoprotein fractions [28]. Iranian authors [17] reported no significant relationship between copper and zinc serum concentration and different lipoprotein fractions in atherosclerotic patients.

Conclusion

Overall, the data obtained in the current study are consistent with the hypothesis that copper and zinc may contribute to atherosclerosis and its sequelae as factors in a multifactorial disease. Registered differences in oligoelements between different morphological groups should be further evaluated in clinical settings of different oligoelements intake.

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Children's health risk assessment based on the content of toxic metals Pb, Cd, Cu and Zn in urban soil samples of Podgorica, Montenegro

Procena rizika po zdravlje dece na osnovu sadržaja toksičnih metala Pb, Cd, Cu i Zn u gradskom zemljištu na teritoriji Podgorice, Crna Gora

Boban Mugoša*, Dijana Djurović*, Aleksandra Pirnat*, Zorica Bulat†, Snežana Barjaktarović-Labović‡

***Institute of Public Health, Podgorica, Montenegro; †Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia; ‡Health Center Bar, Bar, Montenegro**

Abstract

Background/Aim. Due to their low tolerance to pollutants and hand-to-mouth pathways the health risk is very high in children's population. The aim of this study was to evaluate risk to children's health based on the content of heavy metals in urban soil samples from Podgorica, Montenegro. This study included the investigation of several toxic metals such as Pb, Cd, Cu and Zn in soil samples from public parks and playgrounds. **Methods.** Sampling was conducted in a period October-November, 2012. Based on cluster analysis, soil samples were divided into two groups related to similarity of metal content at examinated locations: the group I – near by recreational or residential areas of the city, and the group II – near traffic roads. Concentration of toxic metals, in urban soil samples were determined by a graphite furnace atomic absorption spectrometry (Pb and Cd) and by inductively coupled plasma optical emission spectrometry technique after microwave digestion. Due to exposure to urban soil, non-cancerogenic index hazardous index (HI) for children was estimated using 95th percentile values of total metal concentration. The value of the total (ingestion, dermal and inhalation) HI is calculated for maximum, minimum and the average concentration of metals for children. **Results.** Mean concentrations of Pb, Cd, Cu and Zn in the surface layer of the studied urban soils were 85.91 mg/kg, 2.8 mg/kg and 52.9 mg/kg and 112.5 mg/kg, respectively. Samples from group II showed higher metal content compared to group I. Urbanization and traffic are the main sources of pollution of the urban soils of Podgorica. Most of the samples (93.5%) had a high Pb content, 12.9% of the samples had a higher content of Cd, while Cu and Zn were within the limits prescribed by national legislation. At one location the level of security for lead is HI = 0.8 and very closed to maximum acceptable value of 1. It is probably the result of intensive traffic near by. **Conclusion.** All metals investigated showed relatively higher concentrations at sites that were close to industrial places and high ways. The mean concentrations of Pb and Zn and maximum concentrations of Pb, Cd, and Zn were higher than presented values in the National Regulation.

Key words:
metals, heavy; soil; risk assessment; health; child.

Apstrakt

Uvod/Cilj. Zbog niske tolerancije na zagađivače i puta prenošenja ruke-usta, rizik od narušenja zdravlja je veoma visok u dečjoj populaciji. Cilj ovog rada bio je da se izvrši procena rizika po zdravlje dece na osnovu sadržaja teških metala u uzorcima gradskog zemljišta u Podgorici, Crna Gora. Ovo istraživanje je obuhvatilo određivanje koncentracija nekoliko toksičnih metala kao što su Pb, Cd, Cu i Zn u uzorcima zemljišta javnih parkova i dečijih igrališta. **Metode.** Uzorkovanje je sprovedeno tokom oktobra i novembra 2012. godine. Klaster analizom uzorci zemljišta podeljeni su u dve grupe na osnovu sličnosti sadržaja metala na ispitivanim lokacijama: grupa I – uzorci sa mesta u rekreativnim ili stambenim delovima grada i grupa II – uzorci iz parkova i igralište blizu saobraćajnice. Koncentracija metala u uzorcima urbanog zemljišta određena je primenom atomske apsorpcione spektrometrije pomoću grafitne kivete (Pb i Cd) i tehnike optičke emisione spektroskopije sa induktivno kuplovanom plazmom (Cu i Zn) nakon mikrotalasne digestije. Na osnovu stepena izloženosti uticaju gradskog zemljišta, nekancerogeni indeks opasnosti (*hazard index* – HI) za decu izračunat je na 95. procentu vrednosti ukupne koncentracije metala. Vrednost za ukupni (ingestioni, dermalni i inhalacioni) HI izračunata je za maksimalne, minimalne i srednje koncentracije ispitivanih metala za decu. **Rezultati.** Srednja koncentracija toksičnih metala u uzorcima površinskog sloja zemljišta iznosila je 85,91 mg Pb/kg tla, 2,8 mg Cd/kg tla, 52,9 mg Cu/kg tla i 112,5 mg Zn/kg tla. Sadržaj metala bio je značajno veći u uzorcima zemljišta iz grupe II nego u uzorcima grupe I. Ovo jasno ukazuje na izražen i uočljiv uticaj urbanizacije, a naročito saobraćaja, na zagađenje zem-

Correspondence to: Dijana Djurović, Institute of Public Health, Džona Džeksona bb, 81 000 Podgorica, Montenegro, Phone: + 382 67 266 459, Fax: + 382 20 243 728, E-mail: dil@t-com.me

ljišta. Većina uzoraka (93,5%) imala je povećan sadržaj Pb, 12,9% uzoraka imalo je povećan sadržaj Cd, dok je sadržaj Cu i Zn bio u granicama propisanim nacionalnom regulativom. Na jednoj lokaciji vrednost za nivo bezbednosti za olovo bio je HI = 0,8, vrlo blizu maksimalne prihvatljive vrednosti koja iznosi 1, što je verovatno posledica intenzivnog saobraćaja u neposrednoj blizini ispitivane lokacije.

Zaključak. Ispitivani metali imali su više koncentracije na mestima u blizini industrijske zone i autoputa. Prosečne koncentracije Pb i Zn, kao i maksimalne koncentracije Pb, Cd i Zn bile su više od vrednosti propisanih nacionalnom regulativom.

Ključne reči:
metali, teški; zemljište; rizik, procena; zdravlje; deca.

Introduction

Continual urbanization and industrialization induces metals emissions into the terrestrial environment which may greatly influence human health [1]. Samples of soils become a very good diagnostic tool of environmental conditions that influence human health [2, 3]. Chemical composition of soil has been conducted in many studies during the last ten years. Special attention has been devoted to studies on urban park playgrounds. Dermal contact, ingestion and inhalation are the main route of exposure to toxic metals in urban environment [2, 4]. A high concentration of toxic metals in urban soils is an important source of human metal intake. Possibility of exposure to adverse effects of soil ingestion is higher in children than adults [2]. Urban children mainly come in contact with soil in parks and playgrounds. A significant amount of toxic metals children could ingest from soil, dust and air [5]. Due to their low tolerance to pollutants and hand-to-mouth pathways the health risk is very high in this population [6, 7]. So, the control of potentially harmful substances in soil is of high importance and has to be kept at low level in the areas frequented by children [8].

As heavy metals are nondegradable and there is no known homeostasis mechanism for them, any high level of this pollutant may affect the human health affecting the normal functioning of organs, liver, kidney, central nervous system, bones, etc, or acting as cofactors in other diseases [9, 10].

The aim of this study was to evaluate risk assessment to children's health based on the content of toxic metals in urban soil samples of Podgorica, Montenegro. This study included the investigation of several toxic metals such as Pb, Cd, Cu and Zn in surface soil samples from public parks, playgrounds and kindergartens of Podgorica. Children health risk due to children´s toxic metal exposure from urban soil according to hazardous indices (His) was estimated.

Method

Sampling and analysis

This study presents concentrations of four toxic metals, Pb, Cd, Cu and Zn, in surface soil samples from the city's playgrounds in public parks, playgrounds and kindergartens of Podgorica, the capital of Montenegro. A total of 31 parks and playgrounds from the different location of the city were studied. Sampling was conducted during October and November, 2012. Samples of approximately 500 g weight, from top 10 cm layer, within 20 × 20 cm of surface soil, consisting of three sub-samples, were taken and mixed to obtain a bulk composite sample at each playground. Sampling was conducted near by playground equipment such as swings, slides, etc. Stainless trowel was used for sampling and samples are transferred to the laboratory in plastic bags. Stones and foreign objects were hand-removed, and the samples were air-dried for several days. After drying at room temperature samples were gently crushed and sieved to 2 mm and 1.0 ± 0.01 g was weighed for analysis. Microwave acid digestion based on US EPA 3052 method was used for sample preparation. The concentrations of Pb and Cd were determined by a graphite furnace atomic absorption spectrometry (GFAAS) (240Z AA Agilent Technologies-Netherlands) and Cu and Zn by inductively coupled plasma-optical emission spectrometry (ICP-OES) (AMETEC-SpectroArcos, Germany).

Reagents and standards

All chemicals used through the study were analytical grade chemicals. There was no further purification for preparation of all reagents and calibration standards. Deionized ultra pure water was used with conductivity < 1μS/cm. Certified metal stock solution of 1,000 mg/L (J.T. Baker) by successive dilution with deionized water was used for preparing standards for calibration. Each sample was carried out in triplicate.

Data analysis and risk assessment

Statistical package (SPSS 17.0 for Windows) was used for statistical data analysis. This software uses the upper limit of the 95 percent confidence interval (95 percent upper confidence limit – UCL) for the mean concentrations for risk estimation. For evaluation of the similarity of sampling sites with respect to contribution of metals in urban soils, cluster analysis (CA) was applied [11]. Hierarchical CA was performed using the Ward´s method and Euclidean distances as a measure of similarity and the results are showed in a dendrogram. Before applying CA, the normality of all metals was checked using Shapiroe-Wilk´s normality test ($p < 0.05$). In this study prior to CA all the data were log-transformed to reduce the influence of high values.

Input parameters (toxicity values) for estimation have been taken USEPAs exposure parameters [12, 13]. Children could be exposed to contaminants from soil *via* three different pathways oral intake ($I_{ingestion}$), inhalation intake ($I_{inhalation}$) and through skin exposure (I_{dermal}) [13]. Based on this fact noncancer risk assessment in this study was estimated. For intake estimation *via* each exposure pathways the following equitations were used.

$$\text{Intake}_{\text{ingetion}} = \frac{C \times \text{IngR} \times EF \times ED}{BW \times AT} \times 10^{-6} \quad (1)$$

where, C – concentration of a contaminant in soil (mg/kg), IngR – ingestion rate of soil (mg/day) = 200 [14, 15], EF – exposure frequency (days/year) = 360 [13], ED – exposure duration (years) = 6 [16], BW – average body weight (kg) = 20.3 [17], AT – averaging time (days) = ED*365 [13]

$$\text{Intake}_{\text{inhalation}} = \frac{C \times \text{InhR} \times EF \times ED}{PEF \times BW \times AT} \quad (2)$$

where,
InhR – inhalation rate (m^3/day) = 7.6 [18], PEF – particle emission factor = 1.36×10^9 m^3/kg [16].

$$\text{Intake}_{\text{dermal}} = \frac{C \times SA \times SAF \times ABS \times EF \times ED}{BW \times AT} \times 10^{-6} \quad (3)$$

where,
SA – surface area of the skin that contacts the soil (cm^2) = 2,800 [16], SAF - skin adherence factor for soil (mg/cm^2) = 0.2 [16], ABS – dermal absorption factor (chemical specific) = 0.001(for all metals) [18–20].

In this study, the body weight of 20.3 kg was taken from World Health Organization – WHO reference value [17]. After the three exposure pathways $\text{intake}_{\text{ingestion}}$, $\text{intake}_{\text{inhalation}}$ and $\text{intake}_{\text{dermal}}$ were calculated, hazard quotient (HQ) and HI based on non-cancer toxic risk can be calculated as follows [13]:

$$HQ = \frac{\text{Intake}}{RfD} \quad (4)$$

$$HI_{ex}P = \sum HQ_{ex}P \quad (5)$$

where, exP are different exposure pathways, respectively.

Reference dose (RfD) (mg/kg/day) is an estimate value of the daily exposure, maximum permissible risk, to the human population, including sensitive subgroups (children) during a lifetime. The values of RfD are showed in Table 1 [19].

Table 1

Values for reference doses (RfDs)

Metals	$RfD_{ingestion}$	RfD_{dermal}	$RfD_{inhalation}$
Cu	4E-02	1.2E-02	4E-0
Pb	3.5E-03	5.25E-04	3.5E-03
Cd	1E-03	1E-05	1E-03
Zn	3E-01	6E-02	3E-01

In this study it was assumed that after inhalation, all toxicants bonded to particular matter will have similar health effect as if they are ingested. It was assumed that absorption factor for inhalation and ingestion is 100 and this value was used in this study [12, 19–21].

Each HQ for different pathways could be calculated and summed to generate HI (Eq.5). If the value of HI < 1, there is no significant risk of noncancerogenic effect. But if the HI > 1, there is probability of occurrence of noncancerogenic effect and it will be increased if HI increases [22].

Results

Based on metal concentrations after cluster analysis, urban soils, collected from 31 locations, were classified into two groups and results are presented in Figure 1. The group I con-

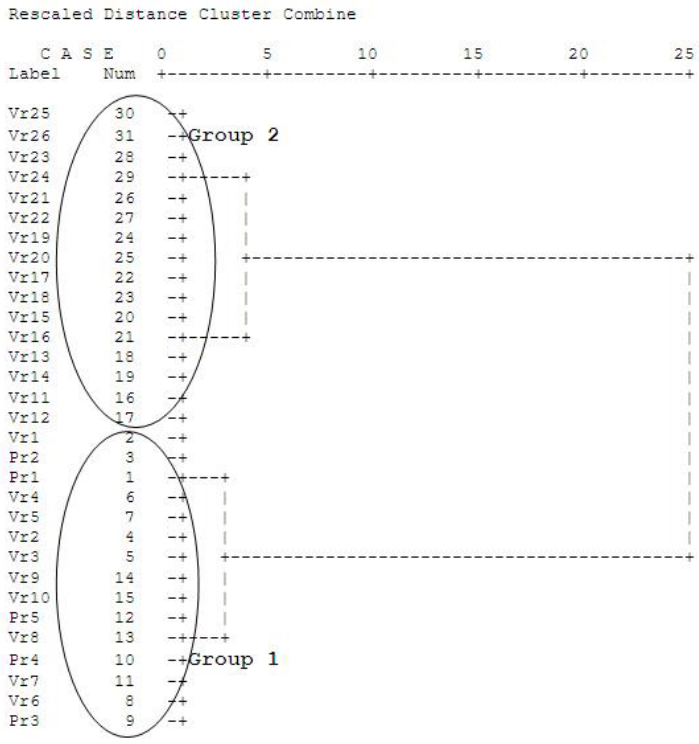

Fig. 1 – Dendrogram showing clustering of monitoring sites. (for explanation see under Table 2).

sisted of samples Vr1, Pr2, Pr1, Vr4, Vr5, Vr2, Vr3, Vr9, Vr10, Pr5, Vr8, Pr4, Vr7, Vr6, and Pr3 from locations that were near recreational or residential places. The group II consisted of samples Vr25, Vr26, Vr23, Vr24, Vr21, Vr22, Vr19, Vr20, Vr17, Vr18, Vr15, Vr16, Vr13, Vr14, Vr11, and Vr12 and these sites were near traffic roads and some small building materials facilities such as Vr11 location. Descriptive statistics of the two groups are shown in Table 2. Figure 1 shows the dendogram of clustering of monitoring sites.

All mean metal concentrations in the group II were higher, except of Cd, compared to the group I. The mean concentration of Pb in the group II was 30% higher than in the group I, while the mean Zn concentration in the group II was 50% higher than in the group I. There is no significant difference between mean concentrations of Cd and Cu in these two groups.

Correlation analysis

Pearson's correlation analysis was applied for each group to analyze the relationships of metal concentrations, and the results are showed in Table 3. Pb, Cd, Cu and Zn were among significantly positively correlated with each other in the group II. Cu and Zn showed very strong positive correlation (0.85) indicating that also natural source together with traffic and industry contribute to contamination. In the group I, there were no statistically significant correlations among metals, and this might be due to natural content and lower pollution of these sites. Concentrations of Cd and Zn were negatively correlated, but not statistically significant, probably indicating different sources of pollution by these two metals.

The obtained results of noncancerogenic children health risk, based on metal concentrations in urban soils and exposure by three different pathways (ingestion, inhalation and dermal) are shown in Table 4. The results for HI for Pb at all investigated locations are showed in Figure 2. In soil sample at the location Vr11 HI for Pb was 0.8 and it is very close to the upper limit of the safe level [1]. HI for Pb (0.68) at the location Vr22, in children was also lower than the upper limit of the safe level [1]. HI for Pb at the 29 investigated locations is lower than 0.4. A high Pb concentration in urban soil

Table 2

Metal concentrations of urban soils (mg/kg) in two group sites in Podgorica and different regulations for metal concentrations in urban soil

Groups	Cu		Pb		Cd		Zn	
	($\bar{x}$ ± SD)	(min-max)	($\bar{x}$ ± SD)	(min-max)	($\bar{x}$ ± SD)	(min-max)	($\bar{x}$ ± SD)	(min-max)
Group I	51.82± 20.0	9.95 – 99.3	75.99 ± 17.75	23.65–109.23	2.85 ± 0.60	0.89–3.55	84.6±50.36	15.00–236.54
Group II	53.73±21.18	3.57–87.6	98.19 ± 60.25	41.10–285.50	2.77 ± 0.51	1.59–3.65	125.59±77.81	41.59–383.96
National regulations [23]	100		50		3		300	
Residental/ recreational intervention limits	120		100		2		150	

Group I – urban soil samples from locations near recreational and residental places;
Group II – urban soil samples form sites near traffic roads

Table 3

Correlation among concentrations of investigated metals in two group sites of Podgorica

Sites	Cu	Pb	Cd	Zn
Group 2*				
Cu	1	0.58*	0.79†	0.85†
Pb	0.58*	1	0.73†	0.39
Cd	0.79†	0.73†	1	0.69†
Zn	0.85†	0.39	0.69*	1
Group 1*				
Cu	1	0.07	0.44	0.47
Pb	0.07	1	0.10	0.18
Cd	0.44	0.10	1	-0.10
Zn	0.47	0.18	- 0.10	1

***for explanation see under Table 2**

Table 4

Non-carcinogenic (three exposure pathways) risk for children (95% UCL)

Metal	$HQ_{ingestion}$			$HQ_{inhalation}$			HQ_{dermal}			HI_{ex}		
	min	max	mean	min	max	mean	min	max	mean	min	max	mean
Pb	0.07	0.8	0.24	1.8E-06	2.3E-05	7E-06	1.2E-03	1.5E-04	4.5E-03	0.071	0.8	0.25
Cd	8.6E-03	3.5E-02	2.7E-02	2.4E-07	1.0E-06	7.6E-07	2.4E-03	1.0E-02	7.6E-03	0.011	0.05	0.035
Cu	9E-04	2.4E-02	1.3E-02	2.5E-08	7.0E-07	3.5E-07	8.1E-06	2.3E-04	1.2E-04	0.001	0.024	0.015
Zn	6.4E-04	1.2E-02	3.4E-03	1.4E-08	3.5E-07	9.5E-08	7.0E-06	1.7E-04	5.0E-05	0.0006	0,024	0,004

HQ – hazard quotient; HI – hazard index; UCL – upper confidence limit.

at the location Vr11 is the result of the vicinity of building materials facility and intensive traffic. Noncancer lead risk (HI) for children at the examined locations of Podgorica is shown in Figure 2.

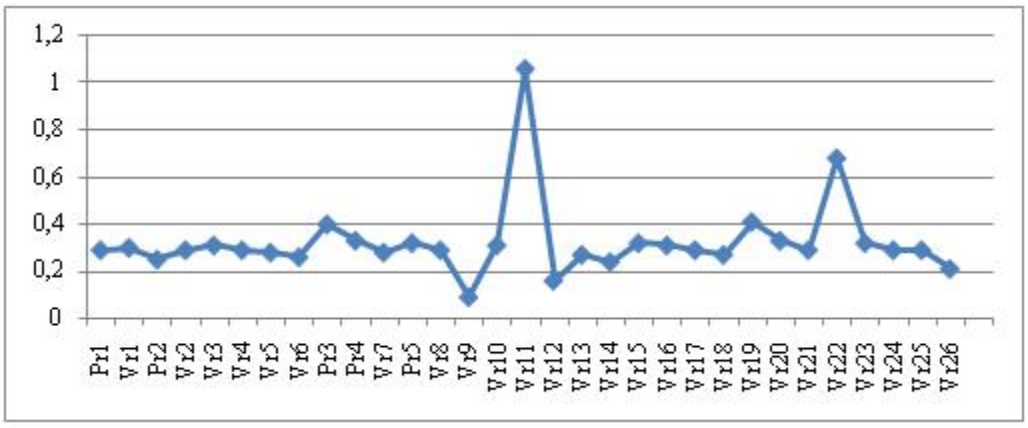

Fig. 2 – Noncancer risk for lead (HI) for children at examined locations of Podgorica, Montenegro.
Group I (Vr1, Pr2, Pr1, Vr4, Vr5, Vr2, Vr3, Vr9, Vr10, Pr5, Vr8, Pr4, Vr7, Vr6, Pr3) – samples from locations that were near recreational and residential places;
Group II (Vr25, Vr26, Vr23, Vr24, Vr21, Vr22, Vr19, Vr20, Vr17, Vr18, Vr15, Vr16, Vr13, Vr14, Vr11, Vr12) – samples from locations that were near traffic rods.

Discussion

Metals concentrations were compared with maximum allowed concentrations (MAC) values, recommended by the National Regulation [23] and the Italian intervention criteria for soils (the residential/ recreational intervention limits fixed by the Italian Environmental Law DM 471/99) [24]. According to the National Regulation, the mean concentration of Pb in both groups was higher than the prescribed value. The mean concentrations of Cd exceeded the Italian residential/recreational intervention limits. The maximum concentrations of Pb and Cd in urban soils, in both groups exceeded the National Regulation limits and Italian residential/recreational intervention limits. In the group II the maximum Zn values were above the National Regulation limits and residential/recreational intervention limits, while in the group I they were above residential/recreational intervention limits. Higher concentration of all investigated metals in the group II could be explained to its proximity to traffic roads and some industrial locations. Podgorica has been under high urbanization in the past few decades. In the study areas, there were no specific pollution sources of toxic metals, because of that, the toxic metal contamination of the soils was derived from continuous urbanization and development, which can influence human health in the contaminated area. It is important to emphasize that the main road that connects south and east part of Montenegro, goes through the city center, with very intensive and heavy traffic. It is common practice to compare the mean concentration of toxic metals in some urban soils from different urban cities [1]. It can be concluded that the existing level of Cd and Cu soil contamination in Podgorica is significantly higher than comparable levels in some other cities over the world. Zinc concentrations vary from city to city, while Pb content is the lowest except in the city of Madrid, Spain.

The major path of children exposure to urban soil that adverse human health by Pb, Cd, Cu and Zn is ingestion, followed by dermal exposure. Contribution of inhalation exposure to HI is the smallest. For noncancer risk HI for children decreases in order Pb > Cd > Cu > Zn. So, Pb exposure to the urban soil in Podgorica may also pose health threat, specially, to young children. The daily ingestion rates of soil by children was calculated to be between 39 and 270 mg/day [25]. Because of its negative effects on the children's central nervous system, monitoring of Pb content in soil is of great importance [26]. Many neurological and developmental disorders may be observed in children's population due to the long period of exposure and ingestion of certain amounts of Pb from contaminated soil, such as anemia, kidney damage, colic, muscle weakness and brain damage [19, 21, 27]. Ingestion of small quantity of Pb from dust may be harmful for blood, development, behavior and intellectual functioning, as well [27, 28].

Because of such threats to children's health it is necessary to take action to decontaminate locations where HI is below the safe level, but high enough to adverse children's health during a long-time exposure. The potential health risk from Cd and Cu is low while potential health risk from Zn is the least for children's population.

Conclusion

Based on the content of toxic metals, Pb, Cd, Cu and Zn, in urban soils, playgrounds and parks, in Podgorica, Montenegro and after cluster analysis all metals showed relatively higher concentrations at sites that were close to industrial places and highways clearly indicating the influence of rapid urbanization and industrialization in the last few decades. The mean concentration of Pb and maximum concentrations of Pb, Cd and Zn were higher than the prescribed value in the National Regulation. The highest risk is associated with soil particle ingestion and the noncarcinogenic health risk for children was very high at two locations. Because of such threats to chil-

dren's health it is necessary to take action to decontaminate these sites and prevent and protect children's health. This integrated approach based on statistical and non-cancerogenic probabilistic risk analysis may help in the decision making process in every growing urban and industrial region.

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Tacrolimus concentration/dose ratio as a therapeutic drug monitoring strategy: the influence of gender and comedication

Odnos koncentracije i doze takrolimusa kao strategija terapijskog monitoringa leka: uticaj pola i komedikacije

Nemanja Rančić*†, Viktorija Dragojević-Simić*†, Neven Vavić†‡, Aleksandra Kovačević*†, Zoran Šegrt†§, Biljana Drašković-Pavlović†‖, Momir Mikov¶

***Center for Clinical Pharmacology, ‡Center for Transplantation of Solid Organs, §Sector for Treatment, ‖Institute for Medical Investigation, Military Medical Academy, Belgrade, Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia; ¶Institute for Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia**

Abstract

Background/Aim. A combination of tacrolimus and other drugs such as corticosteroids has been commonly used immunosuppressive regimens. On the other hand, there is a growing body of evidence that male and female may differ in their response to the equal drug treatment. The aim of the study was to estimated the use of tacrolimus concentration/dose (C/D) ratio for the assessment of the influence of gender differences and comedication on tacrolimus exposure in renal transplant recipients. **Methods.** This prospective case series study included 54 patients, in which the unit of monitoring was outpatient examination (1,872) of the renal transplant patients. The patients were monitored in the period 2010–2014, starting one month after the transplantation. Tacrolimus trough concentrations (TTC) were measured by chemiluminescence microparticles immunoassay. **Results.** TTC and the tacrolimus C/D ratio were significantly lower in the females comparing with the males. Contrary to the males, in the females a significant increase of the tacrolimus daily dose (TDD) *per* body weight and TTC, along with the corticosteroid dose increase, was not accompanied by any significant changes in the tacrolimus C/D ratio; in different corticosteroid doses faster elimination of tacrolimus was found with the exception of the doses > 0.25 mg/kg. In the patients treated with proton pump inhibitors, mainly with pantoprazole TDD *per* body weight and TTC were significantly higher, while the tacrolimus C/D ratio was significantly lower compared to the patients without this treatment. In the patients treated with calcium channel blockers, TDD *per* body weight was significantly lower (particularly with amlodipine) while the tacrolimus C/D ratio was higher compared to the patients who were not treated by them. **Conclusion.** A lower tacrolimus exposure was detected in females in comparison to males. When gender differences were considered in the context of different corticosteroid doses, faster elimination of tacrolimus in the females was also seen, with the exception of the doses > 0.25 mg/kg. Tacrolimus exposure in the pantoprazole-treated patients was significantly less expressed, while in patients treated with CCB amplodipine the tacrolimus C/D ratio was significantly higher in comparison with the patients not treated with them.

Key words:
kidney transplantation; tacrolimus; immunosuppressive agents; drug therapy, combination; dose-response relationship, drug; sex.

Apstrakt

Uvod/Cilj. Kombinacija takrolimusa i drugih lekova kao što su kortikosteroidi, čest je imunosupresivni režim. S druge strane, raste broj dokaza da se muškarci i žene mogu razlikovati u odgovoru na lečenje istim lekom. Cilj ovog rada bio je da se proceni uticaj razlike među polovima i komedikacije na izloženost takrolimusu, uz pomoć odnosa koncentracije i doze (C/D odnos) takrolimusa kod bolesnika sa transplantiranim bubregom. **Metode.** Ispitivanje je sprovedeno kroz prospektivnu seriju od 54 bolesnika, gde je jedinica posmatranja bio kontrolni ambulantni pregled (1 872) bolesnika sa transplantiranim bubregom. Bolesnici su praćeni od 2010. do 2014. godine, a praćenje je započeto mesec dana nakon transplantacije. Minimalna koncentracija takrolimusa u krvi (*tacrolimus trough concentration* – TTC) merena je uz pomoć hemiluminiscentnog mikročestičnog imunoeseja. **Rezultati.** Odnos TTC i C/D bio je značajno niži kod žena nego kod muškara-

Correspondence to: Nemanja Rančić, Centre for Clinical Pharmacology; Faculty of Medicine of the Military Medical Academy, University of Defence; Crnotravska 17, 11 002 Belgrade, Serbia. E-mail: nece84@hotmail.com

ca. Za razliku od muškaraca, kod žena je nađeno značajno povećanje dnevne doze takrolimusa (TDD) po kg telesne težine i TTC, zajedno sa povećanjem doze kortikosteroida, koje nije bilo praćeno značajnim promenama odnosa C/D. Bolesnici koji su upotrebljavali inhibitore protonske pumpe (većinom pantoprazol), imali su značajno viši TDD po kg telesne težine i TTC, dok je odnos C/D bio značajno niži nego kod bolesnika bez ovog tretmana. Kod bolesnika koji su upotrebljavali blokatore kalcijumovih kanala (pogotovo amlodipina) TDD po kg telesne težine bio je značajno niži, dok je odnos C/D bio značajno viši nego kod bolesnika bez ovog tretmana. **Zaključak.** Rezultati pokazuju da su žene manje izložene takrolimusu nego muškarci. Kada su posmatrane polne razlike u odnosu na različite doze kortikosteroida, utvrđeno je brže eliminisanje takrolimusa kod žena, osim kada je doza kortikosteroida bila > 0,25 mg/kg. Izloženost takrolimusu u prisustvu pantoprazola bila je značajno manje izražena, dok je u prisustvu amlodipina bila značajno viša nego kod bolesnika koji nisu bili lečeni ovim lekovima.

Ključne reči:
transplantacija bubrega; takrolimus; imunosupresivi; lečenje kombinovanjem lekova; lekovi, odnos doza-reakcija; pol.

Introduction

Immunosuppressive therapy used to prevent liver, kidney or heart allograft rejection often includes tacrolimus, a calcineurin inhibitor. It is a potent agent, pharmacologically related to cyclosporine, but 10- to 200-fold more potent on a weight basis in T-cell immune function suppression. A combination of tacrolimus, mycophenolate mofetil and corticosteroids has been among the most commonly used immunosuppressive regimens, so far [1–4].

However, tacrolimus has a dose-dependent toxicity, as well as large intra- and inter-individual pharmacokinetic variability. Numerous factors which are supposed to contribute to the aforementioned are: gender, age, body mass index, albumin concentration, diarrhoea, corticosteroids and other comedication, food, hepatitis, diabetes, gene polymorphism, etc. [1, 2, 5, 6]. Additional reasons for tacrolimus pharmacokinetic variability include its poor dissolution, restricted absorption, strong affinity for erythrocytes (tacrolimus concentrations in whole blood is up to 30 times greater than in plasma) and hepatic impairment, which can be associated with a decrease of tacrolimus clearance and about 3-fold increase of its half-life [1, 2, 7].

Patient-tailored regimen requires the exploration of multiple clinical factors in order to determine their effects on tacrolimus pharmacokinetics [5, 6, 8–10]. For example, single-nucleotide polymorphism is found on the genes encoding for cytochrome P450 (CYP) 3A family, especially CYP 3A4 and CYP 3A5 members, responsible for the major route of tacrolimus metabolism, both in the liver and intestine. Moreover, new findings indicate that this also applies to P-glycoprotein (P-gp) efflux pump, as well. On the other hand, the inhibition and induction of CYP 3A-mediated metabolism of tacrolimus are regarded as the clinically most important drug-drug interaction mechanism. Drugs that inhibit this enzyme system, such as azoles, calcium channel blockers, macrolide, HIV-protease inhibitors, etc., may produce the increased tacrolimus blood concentrations [1, 7, 11, 12]. Quite the opposite, the inducers of CYP 3A may reduce its blood concentrations (carbamazepine, phenobarbital, nevirapin, rifampicin, St John's wort). Since oral prednisone is an integral component of most immunosuppressive regimens in solid organ transplantation, its potential interactions with tacrolimus are of special importance. It is mostly due to the common metabolic (CYP 3A) and transporter pathways (P-gp) of corticosteroids and tacrolimus [13].

There is a growing body of evidence that male and female may differ in their response to the equal drug treatment, as a result of their differences in drug pharmacokinetics [14, 15]. It is essential to understand these gender differences since they can result in a modified pharmacological response and may affect both drug effectiveness and safety. The research considering the difference in pharmacokinetic properties of tacrolimus between male and female patients is in progress [16–19].

Therapeutic drug monitoring (TDM) is very important for drugs with a narrow therapeutic index (NTI), for drugs with proven relationship between drug exposure, efficacy and adverse effects, and when samples for TDM are easily accessible. According to the revised European Medicines Agency Guideline on the Investigation of Bioequivalence, tacrolimus is NTI drug, with 90.00–111.11% acceptance criterion tightened for the area under the curve (AUC), while for C_{max}, 80.00–125.00% acceptance limits are still valid [20]. Tacrolimus is administered daily, divided in two doses, every 12 hours, and the dose adjustment is based on tacrolimus trough concentrations (TTC), which has been standard practice for many years [21]. Tacrolimus target levels in renal transplant recipients have been defined between 5 and 10 ng/mL without induction therapy, while with induction therapy between 7 and 10 ng/mL [7, 22, 23]. The importance of TDM can be seen from the fact that overexposure can be linked with significant tacrolimus toxicity [24], while underdosing is associated with an increased risk of kidney rejection [23, 25–27]. Since TTC are routinely monitored and the dose is adjusted based almost solely on trough measurements, the effects of the multiple factors affecting tacrolimus pharmacokinetics are not regarded in the consistent manner by various transplant centers. Therefore, trial and error approach to dosing is still a common everyday practice and needs a novel approach. On the other hand, although full dose interval area under the concentration-time curve (AUC_{0-12}) is generally considered the best marker for tacrolimus exposure, it has not been used as a routine method in the clinical settings, due to its complexity and the high cost of the procedure [28]. Quite recently, however, the tacrolimus concentration/dose (C/D) ratio, a relatively simply obtained TDM tool, has been suggested to be used to define tacrolimus exposure profile better [5].

The aim of the study was to estimate the use of tacrolimus C/D ratio for the assessment of the influence of gender differences and comedication on tacrolimus exposure in renal transplant recipients.

Methods

The study was designed as a prospective case series study, in which the unit of monitoring was outpatient examination recorded in the database of patients subjected to kidney transplantation in the Center for Solid Organ Transplantation of the Military Medical Academy, Belgrade, Serbia (the tertiary health care university hospital). The study group consisted of 54 patients subjected to renal transplantation. They were all monitored in the period from 2010 to 2014 (mean follow-up time was 636.70 ± 209.28 days), starting one month after the transplantation.

Transplantation protocol and concurrent medication

All the patients were treated in accordance with the established therapeutic protocol in the Center, as described in the earlier study [29]. After kidney transplantation, they were subjected to the triple-drug-therapy, including corticosteroids (methylprednisolone, prednisone), mycophenolate mofetil and tacrolimus (Prograf®, Fujisava, Japan). After renal transplantation, an induction therapy (anti-T lymphocyte globulin – ATG) was applied to 29 (53.7%) of our patients. ATG was administered intravenously (as a slow intravenous infusion) as a series of divided doses during the first post-transplant week (in a dose 2–4 mg/kg/day). On the day of transplantation, tacrolimus was introduced in the initial oral dose 0.1–0.3 mg/kg/day, divided into 12-h intervals [30]. The patients were given the dose of 500 mg of methylprednisolone, intravenously, on the day of the surgical intervention, before the transplantation itself; the next 2 days the dose was 250 mg/day, and then reduced to 125 mg/day in the following 2 days, followed by 3 days, in the dose of 1.5 mg/kg/day. During the second week after transplantation, the dose of 0.3 mg/kg/day of prednisone was administered orally; the same dosage was used until the end of the first month. The prednisone dose of 10 mg/day was prescribed until the end of the first year after transplantation, while 10 mg dose was recommended every other day, during the second year of treatment and later on. Mycophenolate mofetil was given orally, 1 g, twice daily, starting 2 days before the kidney transplantation. Three months after transplantation, mycophenolate mofetil dose was reduced to 500 mg, twice daily. After this dose reduction, mycophenolate mofetil was taken permanently.

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

Clinical data

Physical examination, biochemical analyses (complete blood count, haematocrit, C-reactive protein test, creatinine blood test, blood urea nitrogen test, blood glucose level, sodium test, potasium test, blood calcium test, plasma protein test, albumin blood test, aspartate aminotransferase test, alanine aminotransferase test, blood sedimentation rate, urine test, including urine culture test and cytology exam of urine) and other medical examinations (blood pressure, color Doppler ultrasonography of the graft with an assessment of resistance index of its interlobular artery) were performed.

Therapeutic drug monitoring

TDM involved tacrolimus daily dose (TDD), TDD *per* body weight, TTC and the tacrolimus C/D ratio. The tacrolimus C/D ratio is the ratio between C_0 or TTC and 24-h dose (D) normalized by patient's weight (mg/kg/day) [5].

All these parameters were used to investigate the influence of comedication and gender differences on TDD adjustment.

TTC were measured by chemiluminescence microparticles immunoassay (CMIA) (ARCHITECT i1000SR Abbott Laboratories; Abbott Park, Illinois, USA). The whole blood samples were taken 12 h after the evening dose, 10 min before the morning dose.

Three days after the transplantation, tacrolimus dose was adjusted depending on the whole blood TTC. The target concentration range was from 5 to 10 ng/mL during the first month after the renal transplantation, as recommended [22, 31], although some authors recommend the lower range i.e. from 3 to 7 ng/mL [22, 32, 33]. After the first month of transplantation, TTC have been recommended target concentration range from 6 to 10 ng/mL in the renal transplant recipients. If the TTC was greater than 10 ng/mL, the TDD was reduced, while if the TTC was less than 6 ng/ml, the TDD was increased.

In accordance with the previous findings which showed that prednisone dosage was a significant covariate influencing tacrolimus parameters [34], the patients were divided into 3 groups according to corticosteroid doses per body weight: the group with the doses < 0.15 mg/kg, the group with the doses from 0.15 – 0.25 mg/kg and the group with the doses > 0.25 mg/kg.

Statistical analysis

The complete statistical analysis of data was done with the statistical software package, PASW Statistics 18. All variables were presented as frequency of certain categories, while statistical significance of differences were tested by the χ^2-square test. Continuous variables were summarized as means ($\bar{x}$) and standard deviations (SD). Continuous variables were compared using Student's *t*-test for independent samples or Mann-Whitney *U*-test. Two-way between-groups analysis of variance was used in order to analyze both individual as well as joint infuence of fixed factors on dependent

variables. The normality of the data was assessed using Kolmogorov-Smirnov test. Ratios between TDD *per* body weight, TTC and the tacrolimus C/D ratio were tested by Pearson's coefficient correlation. All the analyses were estimated at $p < 0.05$ level of the statistical significance.

Principles of ICH Good Clinical Practice were strictly followed and ethical approval No 01/31-01-13 from the Ethics Committee was obtained for the study protocol No.910-1.

Result

Demographic characteristics of renal transplant patients are presented in Table 1. A total of 54 patients was subjected to kidney transplantation, 34 (63%) males and 20 (37%) females; the average recipient age was 40.46 ± 11.38 years. Body height, body weight and body mass index were significantly higher in men.

The total number of 1,872 outpatient examinations was performed during this follow-up (Table 2). The average TDD, TTC and the tacrolimus C/D ratio in renal transplant patients were significantly lower in females comparing with males (Table 2).

A very strong correlation between TDD *per* body weight and the tacrolimus C/D ratio was shown ($r = -0.700$, $p < 0.0001$). The correlation between TDD *per* body weight and TTC, as well as between TTC and the tacrolimus C/D ratio was weak ($r = 0.218$, $r = 0.257$, respectively).

Renal transplant patients' biochemical analyses are shown in Table 1. All biochemical parameters, such as haematocrit, blood urea nitrogen, creatinine and proteinuria, were significantly higher in males in comparison with females.

All the patients were treated with corticosteroid therapy. The average corticosteroid daily dose (CDD) was 14.40 ± 5.85 mg or 0.22 ± 0.09 mg/kg. The males were subjected to significantly higher CDD, expressed in miligrams (males 15.16 ± 6.37; females 13.29 ± 4.82; $p < 0.0001$), but it turned out that comparing with females, it was a significantly lower dose when expressed in mg/kg *per* body weight (males 0.21 ± 0.09; females 0.23 ± 0.09; $p < 0.0001$). In the groups of patients who received less than 0.15 mg/kg and 0.15–0.25 mg/kg of corticosteroids, the female patients were treated with significantly higher TDD *per* body weight in comparison to the males (Table 3 and Figure 1). However, in the group of patients who received more than 0.25 mg/kg of corticosteroids, the male patients were treated with significantly higher TDD *per* body weight in comparison with the females. In the males, along with the prednisone dose increase (> 0.25:0.15–0.25, > 0.25:< 0.15 and 0.15–0.25:< 0.15 mg/kg) both TDD *per* body weight and TTC increased significantly, while the tacrolimus C/D ratio decreased significantly (Table 3). However, in the females this significant increase of TDD *per* body weight and TTC, along with the corticosteroid dose increase, was not accompanied by any significant changes in the tacrolimus C/D ratio.

Out of 1,872 outpatient examinations in 1,407 (75.2%) (888 examination including males and 519 including females) were registered treatment with proton pump inhibi-

Table 1

Demographic characteristics and biochemical analyses of renal transplant patients according to gender

Parameter	Male [n = 34 (63%)]	Female [n = 20 (37%)]	Total [n = 54 (100%)]
	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Age, years	41.44 ± 11.73	38.80 ± 10.85	40.46 ± 11.38
Height, m	1.78 ± 0.06	1.65 ± 0.07**	1.74 ± 0.09
Weight, kg	72.38 ± 13.15	59.06 ± 8.91**	67.96 ± 13.47
Body mass index, kg/m^2	22.38 ± 3.31	19.97 ± 2.31**	21.49 ± 3.18
Haematocrit, L/L	0.40 ± 0.05	0.38 ± 0.05**	0.39 ± 0.05
Blood urea nitrogen, mmol/L	11.42 ± 18.58	8.17 ± 8.90**	10.03 ± 15.29
Creatinine, μmol/L	152.38 ± 55.13	108.54 ± 42.17**	133.64 ± 54.49
Proteinuria, g/24h	0.35 ± 0.30	0.19 ± 0.24**	0.30 ± 0.29

Statistically significant difference (males/females): **- $p < 0.01$; $\bar{x}$ - mean; SD – standard deviation.

Table 2

Average tacrolimus daily doses (TDD), TDD per body weight, tacrolimus through concentrations (TTC) and the tacrolimus concentration/dose (C/D) ratio in renal transplant patients: gender distribution

Parameter	Outpatient examinations by gender ($\bar{x}$ ± SD)		
	Male (n = 1,154; 61.6%)	Female (n = 718; 38.4%)	Total (n = 1,872; 100%)
Outpatient examinations *per* patient, n	33.94 ± 11.17	35.90 ± 10.78	34.67 ± 0.96
TDD, mg	5.56 ± 3.53	4.50 ± 2.31**	5.13 ± 3.13
TDD *per* body weight, mg/kg	0.075 ± 0.047	0.079 ± 0.041	0.077 ± 0.045
TTC, ng/mL	6.74 ± 2.31	6.26 ± 2.45**	6.54 ± 2.38
Tacrolimus C/D ratio, ng/mL/mg/kg/day	137.56 ± 102.50	100.45 ± 64.99**	121.78 ± 90.37

Statistically significant difference (males/females): **- $p < 0.01$; $\bar{x}$ – mean; SD – standard deviation.

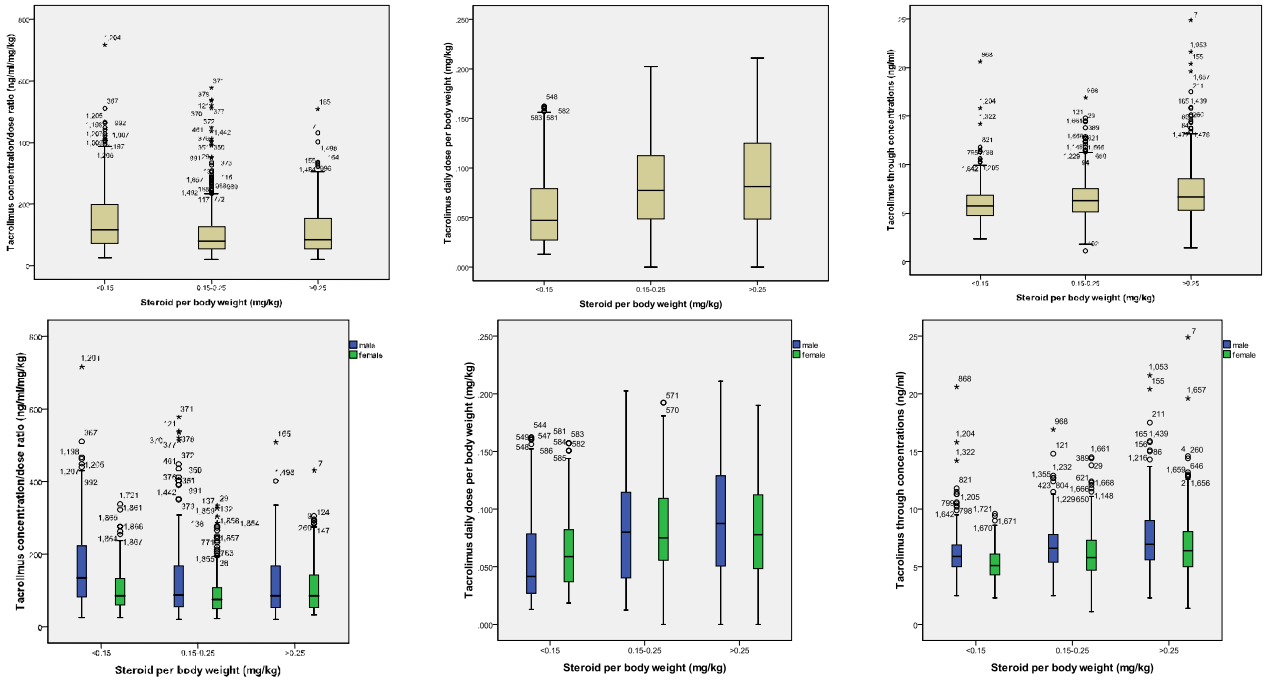

Fig. 1 – Impact of gender on distribution of tacrolimus daily doses *per* body weight, tacrolimus trough concentrations, and the tacrolimus concentration/dose ratio in renal transplant patients according to corticosteroid dose comedication.

Table 3

Gender distribution of tacrolimus daily doses (TDD) *per* body weight, tacrolimus trough concentrations (TTC), and the tacrolimus concentration/dose (C/D) ratio in renal transplant patients according to corticosteroid dose comedication

Gender	Corticosteroid dose (mg/kg)	TDD *per* body weight (mg/kg)	TTC (ng/mL)	Tacrolimus C/D ratio (ng/mL/mg/kg/day)
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Male + Female	< 0.15	0.058 ± 0.038	5.95 ± 1.85	147.58 ± 99.83
	0.15–0.25	0.081 ± 0.042#**	6.44 ± 2.12#**	106.86 ± 83.44#**
	> 0.25	0.090 ± 0.049	7.26 ± 2.88	111.59 ± 76.63
Male/Female	< 0.15	0.057 ± 0.039/0.064 ± 0.035##**	6.17 ± 1.89/5.28 ± 1.54##**	161.35±105.31/106.52 ± 66.45##**
	0.15–0.25	0.080 ± 0.046/0.081 ± 0.039##	6.69 ± 2.01/6.12 ± 2.24##**	129.15 ± 109.36/93.61 ± 59.16##**
	> 0.25	0.091 ± 0.050/0.087 ± 0.047##*	7.57 ± 2.87/6.93 ± 2.86##**	116.08 ± 81.54/106.82 ± 70.92##
Male	< 0.15	0.057 ± 0.039	6.17 ± 1.89	161.35 ± 105.31
	0.15–0.25	0.080 ± 0.046#**	6.69 ± 2.01#**	129.15 ± 109.36#**
	> 0.25	0.091 ± 0.050	7.57 ± 2.87	116.08 ± 81.54
Female	< 0.15	0.064 ± 0.035	5.28 ± 1.54	106.52 ± 66.45
	0.15–0.25	0.081 ± 0.039#**	6.12 ± 2.24#**	93.61 ± 59.16#
	> 0.25	0.087 ± 0.047	6.93 ± 2.86	106.82 ± 70.92

Statistically significant difference: *- $p < 0.05$ and **- $p < 0.01$; #- < 0.15/0.15–0.25/>0.25; ##- males/females; $\bar{x}$ – mean; SD – standard deviation.

tors. In these cases TDD *per* body weight and TTC were registered treatment with significantly higher compared to the patients without this treatment, while the tacrolimus C/D ratio was significantly lower in the renal transplant recipients whose treatment included one of the drugs from this group (Table 4). Considering gender, the ratio of these parameters was the same as in the whole patient population treated with proton pump inhibitors (Table 4). However, in males, TDD *per* body weight was significantly lower, while TTC and tacrolimus C/D ratio were significantly higher compared to female patients (Table 4). Considering various proton pump inhibitors, the patients who were treated with pantoprazole were given significantly higher TDD *per* body weight comparing with esomeprazole, while their TTC and the tacrolimus C/D ratio were significantly lower (Table 5).

Out of 1,872 outpatient examinations in 39.9% (551 including males and 195 including females) cases calcium channel blockers were registered. In these patients TDD *per* body weight was significantly lower compared to the patients without this treatment, while the tacrolimus C/D ratio was higher in the renal transplant recipients whose treatment included one of the drugs from this group (Table 4). Taking into account all the examined parameters (TDD *per* body weight, TTC and tacrolimus C/D ratio), when the comparison between the groups treated and not treated with calcium channel blockers was done, it turned out that there were no significant differences considering the males and the females (Table 6). In our patients, the tacrolimus C/D ratio was higher in the renal transplant recipients treated with amlodipine than in those treated with nifedipine (157.15 ± 118.11 *vs*

Table 4

Impact of comedication with/without proton pump inhibitors and calcium channel blockers on tacrolimus daily doses (TDD) *per* body weight tacrolimus trough concentrations (TTC), and tacrolimus concentration/dose (C/D) ratio in the renal transplant patients acording to gender

Gender	Comedication	TDD *per* body weight (mg/kg) $\bar{x} \pm SD$	TTC (ng/ml) $\bar{x} \pm SD$	Tacrolimus C/D ratio (ng/ml/mg/kg/day) $\bar{x} \pm SD$
Both	PPT	0.080 ± 0.045/0.066 ± 0.044**	6.66 ± 2.52/6.16 ± 1.83**	115.70 ± 86.24/140.09 ± 99.70**
Both	CCB	0.074 ± 0.043/0.078 ± 0.046*	6.62 ± 2.35/6.49 ± 2.40	136.00 ± 107.90/112.87 ± 76.11**
Males/Females	With PPI	0.078 ± 0.046/0.085 ± 0.041**	6.78 ± 2.42/6.46 ± 2.68**	130.44 ± 98.03/92.35 ± 55.86**
	With CCB	0.072 ± 0.045/0.080 ± 0.037**	6.71 ± 2.33/6.37 ± 2.41*	147.70 ± 116.94/103.96 ± 68.71**
Males/Females	Without PPI	0.065 ± 0.051/0.066 ± 0.038	6.52 ± 1.81/5.85 ± 1.80**	166.23 ±114.74/117.93 ± 78.61**
	Without CCB	0.078 ± 0.050/0.078 ± 0.043	6.77 ± 2.30/6.23 ± 2.46**	127.79 ± 85.33/99.33 ± 63.78**
Males	With/without PPI	0.078 ± 0.046/0.065 ± 0.051**	6.78 ± 2.42/6.52 ± 1.81	130.44 ± 98.03/166.23±114.74**
	With/without CCB	0.072 ± 0.045/0.078 ± 0.050	6.71 ± 2.33/6.77 ± 2.30	147.70 ± 116.94/127.79 ±85.33
Females	With/without PPI	0.085 ± 0.041/0.066 ± 0.038**	6.46 ± 2.68/5.85 ± 1.80**	92.35 ± 55.86/117.93 ± 78.61**
	With/without CCB	0.080 ± 0.037/0.078 ± 0.043	6.37 ± 2.41/6.23 ± 2.46	103.96 ± 68.71/99.33 ± 63.78

Statistically significant difference: *- $p < 0.05$ and **- $p < 0.01$; The same for: with/without comedication; $\bar{x}$ – mean; SD – standard deviation; PPI – proton pump inhibitors; CCB – calcium channel blockers.

Table 5

Relationship of tacrolimus daily doses (TDD) *per* body weight, tacrolimus trough concentrations (TTC), and the tacrolimus concentration/dose (C/D) ratio in the renal transplant patients without/with proton pump inhibitors pantoprazole or esomeprazole comedication

Proton pump inhibitors	TDD *per* body weight (mg/kg) $\bar{x} \pm SD$	TTC (ng/mL) $\bar{x} \pm SD$	Tacrolimus C/D ratio (ng/mL/mg/kg/day) $\bar{x} \pm SD$
Without	0.066 ± 0.044	6.16 ± 1.83	140.09 ± 99.70
Pantoprazole	0.081 ± 0.043**	6.62 ± 2.57**	112.27 ± 84.26**
Esomeprazole	0.075 ± 0.053	6.93 ± 2.08	145.81 ± 97.09

Statistically significant difference: **- $p < 0.01$; $\bar{x}$ – mean; SD – standard deviation.

Table 6

Relationship of tacrolimus daily doses (TDD) *per* body weight, tacrolimus trough concentrations (TTC), and the tacrolimus concentration/dose (C/D) ratio in the renal transplant patients without/with calcium channel blockers amlodipine or nifedipine, comedication

Calcium channel blockers	TDD *per* body weight (mg/kg)	TTC (ng/mL)	Tacrolimus C/D ratio (ng/mL/mg/kg/day)
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Without	0.079 ± 0.046	6.50 ± 2.40	112.85 ± 76.32
Amlodipine	0.066 ± 0.043**	6.68 ± 2.62**	157.15 ± 118.11**
Nifedipine	0.078 ± 0.043	6.57 ± 2.08	118.76 ± 93.67

Statistically significant difference: **- $p < 0.01$; $\bar{x}$ – mean; SD – standard deviation.

118.76 ± 93.67; $p < 0.001$), while TDD *per* body weight was lower in the patients treated with amlodipine than with nifedipine (0.066 ± 0.043 vs 0.078 ± 0.043; $p < 0.001$) (Table 6).

The influence of gender and comedication on TTC, as well as the tacrolimus C/D ratio was investigated by using two-way between-groups analysis of variance. When the dependent variable was TTC, statistically significant individual influence of gender, comedication with proton pump inhibitors and corticosteroid groups were established ($p = 0.002$; $p = 0.003$; $p < 0.0001$, respectively), while their joint influence was not significant. On the other hand, when tacrolimus C/D ratio was considered, individual influence of all already mentioned independent variables was also significant ($p < 0.0001$; $p < 0.0001$; $p < 0.0001$, respectively). Whenever the influence of corticosteroid groups associated with any other investigated independent variables was estimated, a significant influence on the tacrolimus C/D ratio was found (with gender, $p = 0.001$; with proton pump inhibitors, $p = 0.044$; with calcium channel blockers, $p < 0.0001$; with gender + proton pump inhibitors, $p = 0.005$; with gender + calcium channel blockers, $p = 0.001$; proton pump inhibitors + calcium channel blockers, $p < 0.0001$).

Calculated the tacrolimus C/D ratio, which corresponded to the tacrolimus target concentration range from 6 to 10 ng/mL, was 130.98 ± 97.11 ng/mL/mg/kg. In the patients with TTC

over therapeutic range (> 10 ng/mL) calculated tacrolimus C/D ratio was 174.36 ± 118.57 and in the patients with subtherapeutic concentration range (< 6 ng/mL) the tacrolimus C/D ratio was 104.46 ± 72.23.

Disscusion

Corticosteroid dose, comedication use and patients' gender are known to be among the numerous factors that have been identified as contributors to a large tacrolimus intra- and inter-individual pharmacokinetic variability. Due to this and to the fact that tacrolimus is the NTI drug, the use of TDM, in conjunction with clinical assessment of the patients, is particularly important. The results of this study demonstrate the relevance of TDM in renal transplant recipients who are normally subjected to numerous drugs with a potential to interact with tacrolimus, but in the context of clinical covariates, such as a patient gender. TDD, TDD *per* body weight, TTC and tacrolimus C/D ratio were chosen to be used as TDM tools.

Tacrolimus is well-known to be primarily metabolised in the intestine and liver, by the CYP 3A family, especially CYP 3A4 and CYP 3A5 members, and is a substrate for P-gp efflux pump [2, 4]. Some drugs that are substrates of CYP 3A4, including tacrolimus, show a higher clearance in women than in men, and that the difference persists after correcting some physiologic factors, such as body weight [35, 36]. According to our results, TDD *per* body weight was not significantly different between the genders, but the average TTC and tacrolimus C/D ratio in renal transplant patients were significantly lower in the females comparing with the males. Stratta et al. [5] suggested the tacrolimus C/D ratio as an alternative to the classic methods for evaluating tacrolimus exposure. Therefore, our results indicate a lower tacrolimus exposure in the females than in the males, which is in accordance with the previous findings of significantly lower values of tacrolimus AUC in female, as well as a longer mean $t_{1/2}$ in male patients compared with female ones [16]. The fact that total clearance of some substrates for CYP 3A are faster in females compared with males can be, at least partly, attributed to a higher hepatic CYP 3A4 content in females [37, 38].

According to the KDIGO clinical practice guidelines, the first-line agents for patients subjected to renal transplantation should include basiliximab induction, for low-risk patients, and an anti-thymocyte globulin for high-risk patients, in conjunction with maintenance immunosuppression, including tacrolimus, mycophenolate and steroids [39]. Since CYP 3A is responsible for > 90% of tacrolimus metabolic elimination, the inhibition or induction of CYP 3A4 will lead to clinically important drug interactions [34].

In our study, the patients treated with corticosteroid doses higher than 0.15 mg/kg had higher TTC and TDD *per* body weight, while their tacrolimus C/D ratio was lower in comparison to patients treated with doses lower than 0.15 mg/kg. Anglicheau et al. [34], similarly to our results, demonstrated that the higher the dose of steroids, the higher the dose of tacrolimus was needed to achieve target blood concentrations. Moreover, the higher the steroid dose was given, the lower tacrolimus C/D ratio was found. Since corticosteroids share metabolic CYP 3A and transporter P-gp pathways with tacrolimus, they are potential sites for pharmacokinetic interactions between these drugs. Generaly, corticosteroids are substrate or inducers of CYP 3A enzymes, but they can also act as their inhibitors [13, 34]. Higher TTC and TDD *per* body weight, as well as a lower tacrolimus C/D ratio in our patients treated with higher corticosteroid doses can be explained by corticosteroid induction of CYP 3A and P-gp pathways. Although tacrolimus interactions with corticosteroids are obviously of significant importance [1, 5, 7, 40], there are no enough data from clinical trials concerning their importance in kidney transplantation. However, the recently performed study indicated that corticosteroid withdrawal protocol profoundly affected tacrolimus levels and dosing [41]. Namely, a mean tacrolimus dose necessary to maintain similar TTC was higher in the group which was receiving corticosteroids during the whole study, compared to the group with an early steroid withdrawal (seven days after transplantation).

Studies investigating gender differences concerning corticosteroid treatment in renal transplant recipients have had conflicting results. Most of the data indicate that females generally have higher metabolism and clearance of drugs than males, owing to the higher activity of CYP 3A4 [42]. Considering corticosteroids, for example, the total clearance of methylprednisolone itself was 55% higher in females than in males [43]. On the other hand, corticosteroid IC_{50}, drug concentration which inhibits 50% of the maximum lymphocyte proliferation, as the indicator of immunosuppressive effects, is lower in females than in males, as far as prednisone and methylprednisolone are concerned [44]. Therefore, in comparison with males, immunosuppressive effect is achieved by lower blood steroid concentrations in females. Our results showed that although the female patients received a lower total daily steroid dose, if it is expressed in miligrams *per* kg of body weight, they were actually treated with significantly higher doses than the males. However, tacrolimus parameters monitored in this study (TTC, TDD *per* body weight and the tacrolimus C/D ratio) indicate that administration of significantly higher corticosteroid doses in the females was associated with faster metabolism of tacrolimus in comparison to the males. According to our results, this was not the case only with the group of patients treated with the highest corticosteroid doses (more than 0.25 mg/kg).

In our study, in the renal transplant recipients who were treated with proton pump inhibitors, TDD *per* body weight and TTC were significantly higher compared to the patients without this treatment, while the tacrolimus C/D ratio was significantly lower in those whose treatment included one of the drugs from this group. Considering gender, our results showed that the males treated with one of these drugs were given lower doses of tacrolimus in comparison to the females, while their tacrolimus C/D ratio was higher in comparison with the females. Therefore, it can be concluded that males are slower metabolisers of tacrolimus when they are comedicated with proton pump inhibitors, in comparison with the females. Proton pump inhibitors are well-known to be metabolised by cytochrome CYP 3A4, as well as by CYP 2C19 [45–47]. Their typical representative, omeprazole is a com-

petitive inhibitor of CYP 3A4-mediated tacrolimus metabolism, especially in poor metabolisers for CYP 2C19 [48]. In the patients with CYP 2C19 gene mutations, proton pump inhibitors tend to be metabolised by CYP 3A4, and, therefore, such patients have a higher risk of interactions between proton pump inhibitors and tacrolimus. Moreover, both tacrolimus and most of the proton pump inhibitors are substrates for P-gp drug transporter, while proton pump inhibitors also act as P-gp inhibitor [49–51]. In the paper concerning our previous study, in patients subjected to kidney transplantation, a significant correlation between the increase of TTC and omeprazole application was shown [29]. However, most of the patients in the present study were treated with pantoprazole, which is only marginally metabolised *via* CYP 2C19 and CYP 3A4 [37, 39] and is a not substrate for P-gp [52]. The fact that the patients treated with pantoprazole in comparison with the ones not treated with this drug also showed increased TTC and a significantly decreased tacrolimus C/D ratio is probably in accordance with the aforementioned findings. On the other hand, Takahashi et al. [52] found that the tacrolimus C/D ratio was markedly higher during transplant recipient treatment with omeprazole in comparison with those treated with ranitidine and rabeprazole. Although we can only speculate on the influence of pantoprazole on the tacrolimus metabolism at the moment, it can be concluded that tacrolimus exposure in these patients was less prominent when compared with the patients not treated with this proton pump inhibitor.

Tacrolimus is known to lead to adverse events in the patients with calcium channel blockers comedication [32, 53, 54]. Drug interactions between calcium channel blockers (diltiazem, verapamil and nifedipine) and tacrolimus, both competitive substrates of CYP 3A4 and CYP 3A5 system, as well as P-gp, can result in the rapid TTC increase [53]. The potential of calcium channel blockers for interactions with tacrolimus is thought to be mediated through their common metabolism by the CYP 3A system, as well as by the P-gp efflux mediated transport. The decrease of the tacrolimus clearance by this partial competitive inhibition of the metabolic pathways can lead to the significantly elevated tacrolimus blood level and the related toxicity [32, 54]. Moreover, diltiazem is a potent mechanism-based inhibitor of CYP 3A, whose metabolite becomes as a result of its N-demethylation by this enzyme. Inactivation of CYP 3A occurs by binding this metabolite tightly and irreversibly [55]. The P-gp pump can be inhibited by blocking drug binding sites with calcium channel blockers. As a result, the efflux of tacrolimus in the intestinal lumen is reduced, and increased TTC appears [56, 57]. Although TTC did not differ between the groups, TDD *per* body weight was significantly lower in the calcium channel blocker treated group, compared to the patients from the non-treated group, while the tacrolimus C/D ratio was significantly higher in the renal transplant recipients whose treatment included one of the drugs from this group (amlodipine and nifedipine). This is in accordance with the suggestion of Stratta et al. [5] who stated that when taking into account targeted tacrolimus concentration, the higher the tacrolimus C/D ratio, the slower the metabolic efficiency (requiring low tacrolimus dose). This reduced metabolic efficacy was obviously caused by its interaction with calcium channel blockers. This was actually shown in healthy subjects evaluated for tacrolimus-amlodipine interactions, in whom amlodipine significantly increased tacrolimus blood exposure in CYP 3A5*1 carriers [58]. On the other hand, amlodipine significantly increased tacrolimus blood levels in CYP 3A4*1 carriers, but decreased it in CYP 3A4*3 homozigote carriers [58]. In renal transplant recipients included in our study, amlodipine exerted this effect, while this was not the case with nifedipine. Namely, the examined tacrolimus parameters in the nifedipine-treated group were not significantly different from the parameters in the group not treated with calcium channel blockers. The differences in the examined tacrolimus parameters concerning genders also indicated faster metabolism of this immunosuppressive drug in females comparing to males in the calcium channel blocker treated group.

Two-way between-groups analysis of variance pointed out that the tacrolimus C/D ratio is more sensitive parameter than TTC, taking into accounts the influence of all the examined variables on tacrolimus exposure. However, since the variability of the tacrolimus C/D ratio was rather large, both parameters should be evaluated in clinical studies in order to define rational tacrolimus dosing approach.

The target concentration intervention (TCI) approach as an alternative conceptual strategy to TDM enables evaluation of pharmacotherapy by comparing the clinical outcomes associated with different target concentrations [59].

Conclusion

According to the results of our study, the renal transplant recipients showed lower tacrolimus exposure in the females than in the males. When gender differences were considered in the context of different comedications, a faster elimination of tacrolimus in the females was also seen, with the exception of the highest corticosteroid doses (> 0.25 mg/kg). As far as the influence of corticosteroid dose on tacrolimus exposure is concerned, if a higher steroid dose was given, the lower tacrolimus C/D ratio was found. It can also be concluded that tacrolimus exposure in the proton pump treated patients, mainly with pantoprazole, was significantly less prominent in comparison with the patients not treated with them. On the other hand, a reduced elimination efficacy of tacrolimus in the patients treated with chalcium channel blockers, predominantly with amlodipine, was probably caused by interactions with these drugs.

According to the findings of this study, together with TTC, the tacrolimus C/D ratio would enable better estimation of the infuence of additional factors, like gender and comedication, on tacrolimus exposure in the patients subjected to renal transplantation. Therefore, further study should be done in order to define the target tacrolimus C/D ratio and associated clinical endpoints for rational dose individualization in the real clinical settings.

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Age-related changes of vitamin C levels in aqueous humour

Promene vrednosti vitamina C u očnoj vodici u vezi sa starenjem

Vladimir Čanadananović*†, Slobodanka Latinović‡, Sava Barišić*, Nikola Babić*†, Sandra Jovanović*†

***Eye Clinic, Clinical Center of Vojvodina, Novi Sad, Serbia; †Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia; ‡Eye Hospital "VIDAR-ORASIS Swiss", Novi Sad, Serbia**

Abstract

Background/Aim. Age-related cataract is a common disease among senior population. Vitamin C is the most effective reducing hydrosoluble antioxidant causing reduction in the levels of free radicals in crystaline lens. The aim of this study was to evaluate the age-related change of vitamin C (L-ascorbic acid) level in the aqueous humor of patients with senile cataract. **Methods.** This prospective study included 74 patients, divided into 3 age groups, with age-related cataract, underwent routine phaco cataract surgery with intraocular lens implantation. Aqueous humor was aspirated from 74 eyes at the beginning of phacoemulsification. The levels of vitamin C (L-ascorbic acid) in aqueous humor were determined with high-performance liquid chromatography (HPLC). **Results.** The average age of patients included in the study was 65 ± 9.85 years (54–87 years). The level of vitamin C in aqueous humor was 152.78 ± 7.0125 μg/mL in the group A (50–59 years), 134.15 ± 5.1569 μg/mL in the group B (60–69 years) and 106.51 ± 5.44 μg/mL in the group C (over 70 years). **Conclusion.** The amount of vitamin C in aqueous humor of patients with age-related cataract is decreasing with age. There was a statistically significant change ($p < 0.001$) of vitamin C aqueous concentration in the patients of different age. This decrease could play a role in susceptibility to cataract formation in older population.

Key words:
cataract; aqueous humor; aging; ascorbic acid; chromatography.

Apstrakt

Uvod/Cilj. Starosna katarakta je uobičajena bolest kod starijih osoba. Vitamin C je veoma efikasan redukujući hidrosolubilni antioksidant koji uzrokuje sniženje nivoa slobodnih radikala u očnom sočivu. Cilj studije bio je da se utvrdi promena količine vitamina C (L-askorbinska kiseline) u očnoj vodici u zavisnosti od starosti bolesnika sa senilnom kataraktom. **Metode.** Ova prospektivna studija uključivala je 74 osobe sa senilnom kataraktom, podeljene u 3 starosne grupe, podvrgnuti rutinskoj hirurgiji katarakte metodom fakoemulzifikacije sa ugradnjom intraokularnog veštačkog sočiva. Očna vodica aspirirana je iz 74 oka na početku operativnog zahvata. Nivo vitamina C (L-askorbinske kiseline) u očnoj vodici određen je metodom tečne hromatografije visokih performansi (HPLC). **Rezultati.** Prosečna starost bolesnika uključenih u studiju iznosila je 65 ± 9,85 godina (54–87 godina). Nivo vitamina C u očnoj vodici iznosio je 152,78 ± 7,0125 μg/mL u grupi A (50–59 godina), 134,15 ± 5,1569 μg/mL u grupi B (60–69 godina) i 106,51 ± 5,44 μg/mL u grupi C (preko 70 godina). **Zaključak.** Količina vitamina C u očnoj vodici bolesnika sa senilnom kataraktom opada sa starenjem. Utvrđena je statistički značajna promena ($p < 0,001$) količine vitamina C kod osoba različite starosti. Ovakav pad može imati ulogu u sklonosti ka nastanku senilne katarakte.

Ključne reči:
katarakta; očna vodica; starenje; askorbinska kiselina; hromatografija.

Introduction

Age-related cataract is a very common disease and continues to be one of the major health problems in developing countries, especially among senior population.

It is estimated that more that 18% of those older than 65 have cataracts, and that the cataract is the leading cause of reversible blindness.

Age-related cataract has a multifactor etiology [1, 2]. Besides biochemical changes in the crystalline lens as a part of aging process, multitude of other factors are involved, as well.

The free radical theory of aging states that organisms age because cells accumulate damage caused by free radical over time [3].

Ageing leads to morphological and biochemical changes in the crystalline lens. Contrary to other organs having epithelium,

Correspondence to: Vladimir Čanadanović, Eye Clinic, Clinical Center of Vojvodina, Hajduk Veljkova 1, 21 000 Novi Sad, Serbia. Phone: +381 21 423 650. E-mail: ocna_klinikans@yahoo.com

the crystalline lens does not shed its old cells. Instead, they are being kept inside lens capsule throughout the entire lifetime.

The lens obtains nutrients necessary for metabolism and maintaining of its transparency, as well as antioxidative elements from aqueous humor. On the other hand, products of metabolism and oxidative processes in lens affect the composition of aqueous humor as well [4]. Production of aqueous humor decreases with ageing, affecting normal metabolism of the lens.

Human cataract in the older age groups seems to be caused by accumulation of various risk factors. There is an evident decrease in the antioxidative mechanism, levels of glutathione and vitamin C, as well as activity of catalase and superoxide dismutase, all of which can lead to cataract formation [5].

Current hypothesis emphasises the role of free radicals and oxidative stress in the genesis of cataract [6, 7].

A certain number of metabolic reactions inside human organism require the presence of free radicals. However, on cellular level they can cause damage of important biomolecules leading to a wide range of pathological changes.

Within physiological conditions, the formation of free radicals is in the state of equilibrium with antioxidative systems in the organism. The disruption of this balance causes the increase of free radicals production or decrease in concentration of antioxidants, and it is termed oxidative stress [8–10].

Antioxidative protection inside human body is based on the action of antioxidative substances that, depending on its concentration in substrate, can prevent or significantly reduce oxidation [11].

Vitamin C (L-ascorbic acid) is the most effective reducing hydrosoluble antioxidant and one of essential nonenzymatic components of antioxidative protection [12].

Ascorbic acid is especially active in oxidoreductive processes causing reduction in levels of free radicals in the crystalline lens [13, 14].

The aim of this study was to evaluate the level of vitamin C (L-ascorbic acid) in the aqueous humor of patients with age-related, senile cataract.

Methods

This prospective study included 74 patients with age-related cataract who were undergoing routine phaco cataract surgery with intraocular lens implantation at the University Eye Clinic, Clinical Center Vojvodina in Novi Sad.

According to age, the patients with cataract were divided into 3 groups: the group A with 24 patients of 50–59 years; the group B with 26 patients of 60–69 years; the group C of 24 patients, 70 and more years.

Complete ophthalmological examination included: distance visual acuity testing using the Snellen method, near visual acuity using Jaeger's tables, intraocular pressure measurement with applanation tonometry, slit lamp examination of anterior and posterior segment of the eye with artificial mydriasis.

Patients with other ophthalmic (glaucoma, uveitis, retinal diseases etc.) and systemic diseases (diabetes, hyperlipemia etc.) that might have influence on oxidative stress and the level of ascorbic acid in patients with cataract were excluded.

Aqueous humor samples were obtained before creation of the scleral tunnel. A small amount (0.2 mL) of aqueous humor was aspirated through *ab externa* (outside- in) limbal paracentesis with a 27 gauge needle on a tuberculin syringe.

The extracted quantity of aqueous humor was replaced with the same amount of isotonic Ringer lactate solution, and the operating procedure was resumed in regular fashion.

This method of aqueous sampling did not disrupt the regular course of cataract surgery in any way.

The levels of vitamin C in aqueous humor were determined with high-performance liquid chromatography (HPLC). Vitamin C produced by J. T. Baker (The Netherlands) was used as a standard, and it was dissolved in 3% solution of metaphosphor acid (Riedel-de Haën, Germany) and 8% acetic acid solution (Zorka, Šabac, Serbia).

Standard vitamin C solution was prepared in the following manner: 0.003 g of the standard was dissolved in 100 mL of 3% metaphosphoric acid. Such a solution contained 30 μg/mL of vitamin C.

All solutions were prepared in bidistilled water meeting HPLC quality guidelines.

All analysis used high-performance liquid chromatography (HPLC system „Agilent 1100", USA), with 20 μL sample injection loop, C-18 column, particle size of 5 μm and UV-DAD detector. The flow of mobile phase was 0.4 mL/min, and column temperature was 37°C. Analysis duration was 5 minutes with three repetitions. Standard vitamin C solution, as well as native samples of aqueous humor filtrated with a membrane filter, were passed through the system.

Twenty microlitres of aqueous humor stabilised with a buffer system in the presence of metaphosphoric acid was injected. The amount of vitamin C was calculated by comparing the area under the peak of vitamin C standard solution, and the area under the peak of vitamin C from aqueous of patients with age-related cataract.

Results

A total of 74 patients, average age of 65 ± 9.85 years (54–87 years), were included in this study, of who 56% were men and 44% women. There was no statisticaly significant difference in gender distribution in the patients with senile cataract ($p = 0.662$).

The level of vitamin C in aqueous humor was 152.78 ± 7.0125 μg/mL in the group A, 134.15 ± 5.1569 μg/mL in the group B and 106.51 ± 5.4416 μg/mL in the group C ($p < 0.01$) (Table 1).

Table 1

Average vitamin C content in aqueous humor

Patients	Vitamin C (μg/mL) $\bar{x}$ ± SD (min-max)
Group A (n = 24) 50–59 years	152.78 ± 7.01 (142–168)
Group B (n = 26) 60–69 years	134.15 ± 5.15 (120–149)
Group C (n = 24) > 70 years	106.51 ± 5.44 (99–115)

The variation interval and average vitamin C content (μg/mL) in aqueous humor in relation to age (n = 74) was statistically significant ($p < 0.01$).

Discussion

The aqueous humor has low protein concentration. It maintains the intraocular pressure, provides nutrition (e.g. glucose and amino acids) for the avascular ocular lens and serves to transport ascorbate in the anterior segment which acts as an antioxidant agent.

Nutrients diffuse in and waste diffuses out through the constant flow of fluid from the anterior/posterior poles of the lens and out of the equatorial regions.

Numerous risk factors for the different types of age related cataracts have been identified. These include environmental factors, sunlight exposure, systemic diseases, cigarette smoking, indices of nutrition, socioeconomic factors etc. [15].

A discovery made by Bietti in 1940, that the concentration of vitamin C in aqueous humour is much higher than the one in plasma, played a major role in our understanding of the important function of this vitamin. It is known to be the most important non-enzymatic antioxidant, taking part in numerous complex biochemical reactions, and protecting the eye from oxidative stress.

The lack of vitamin C causes the multitude of degenerative and pathological disorders [16, 17].

Our research showed that the amount of vitamin C in aqueous humour of the patients with age-related cataract is decreasing with age. There was a statistically significant variation ($p < 0.001$) of vitamin C aqueous concentration in patients of different age and we, therefore, concluded this decrease may be the cause of susceptibility to cataract formation in older population.

In the same time, during the process of cataract maturation, vitamin C aqueous concentration in the patients included in this study decreased. The reason for this is thought to be its oxidation, due to accumulation of free radicals and hydrogen peroxide [18].

The degree of vitamin C aqueous concentration fall in our study is consistent with previous results of other authors [19].

Being hydrosoluble, vitamin C is always present in aqueous fraction of the cell in two of its forms: reduced (ascorbic acid), and oxidized form (dehydroascorbic acid). Vitamins C primary function is electron donor, i.e. reducing agent, preventing oxidation of other compounds.

Vitamin C serum concentration ranges from 0.6 to 2 mg/mL and maximal aqueous humor concentration is up to 20 times higher [20, 21].

Jacques and Chylack [22] have found out that there is a decrease of 75% in cataract formation in people taking large amounts of vitamin C (≥ 490 mg/day). In people with high vitamin C serum concentrations (> 90 μmol/L) incipient cataract is one third rarer than in people with lower serum concentration (< 40 μmol/L). The Roche European American Cataract Trial (REACT) reported small deceleration of cataract progresion in persons with high daily dose supplements (vitamin C 750 mg, vitamin E 268 mg and β-karoten 15 mg) combined for 3 years [23]. Supplemens with low doses of vitamin C (180 mg/day) and vitamin E (40 mg/day) decreased risk of nuclear cataracts among participans aged ≥ 65 years in the Linxian cataract studies [24]. Decreased risk of cataract was also reported in a Beaver Dam Eye Study [25].

Other observational studies found that blood levels of antioxidants are inversely associated with age related cataract in Asian population but not in Western population [26].

However, the hypothesis that high doses vitamin C supplement use may decrease the risk of age-related catarct has not received support in several studies.

Antioxidants in free radical rich environment may function as prooxidants and high doses of vitamin C may contribute to a disturbance in redox homeostasis promoting cataractogenesis [27]. A harmful effect from dehydroascorbate (oxidized vitamin C) has been reported. It contributes glycation of lens proteins, generation of superoxide anions and modification of lens proteins increasing risk of cataract formation [28–30].

Rautiainen et al. [31] and Selin et al. [32] observed statistically significantly increased risk of age related cataract extraction among high doses of vitamin C supplement users but not among multivitamins with recommended daily allowances of vitamin C users. The positive association beetween high doses vitamin C use and risk of age-related ctaract was stronger among patients with higher oxidative stress (reactive oxigen species-generating factors): older patients, corticosteroid users, long-term users.

Jacques et al. [33] and Rock [34] determined that steep increase in serum and aqueous humor vitamin C concentrations appear with daily intake of 150–250 mg of vitamin C, while higher intake (> 250 mg/day) fails to further increase its concentration, neither in serum, nor in aqueous humor. It was also found that increase in serum and aqueous humor concentrations of vitamin C raise its concentration within crystalline lens in linear fashion, while increase in serum concentration of vitamin C induces its increase in aqueous humor, but in non-linear way, indicating the existence of saturating concentration.

Conclusion

The amount of vitamin C in aqueous humor of patients with age-related cataract is decreasing with age. There was a statistically significant change ($p < 0.001$) of vitamin C aqueous concentration in the patients of different age. This decrease could play a role in susceptibility to cataract formation in older population.

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CASE REPORT

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Neurotoxic effects of oxygen in hyperbaric environment: A case report

Neurotoksični efekat kiseonika u hiperbaričnim uslovima

Milorad Rabrenović*, Saša Trešnjić*, Violeta Rabrenović[†‡], Nikola Čikiriz[§], Siniša Mašić[§], Radomir Matunović[‡‖]

***Center of Hyperbaric Medicine, [†]Clinic of Nephrology, [§]Institute of Hygiene, [‖]Clinic of Cardiology, Military Medical Academy, Belgrade, Serbia; [‡]Faculty of Medicine of the Military Medical Academy, University of Defense, Belgrade, Serbia**

Abstract

Introduction. Oxygen is an essential element of life in aerobic organisms. However, if not controlled, inhalation of oxygen under increased pressure in conditions of hyperbaric oxygen therapy can lead to serious damage and even death. **Case report.** We presented a 20-year-old male who had begun exhibiting symptoms of epilepsy during diving test in a hyperbaric chamber while inhaling 100% oxygen. He was immediately taken off oxygen mask and started breathing air and began rapid decompression. He lost consciousness, began foaming at the mouth, and had a series of tonic spasms. The patient was previously completely healthy and not on any medications. He was admitted for emergency treatment in our hospital, where he was treated for epilepsy. On admission, he complained of muscle and joint pain, and had erythematous changes on the forehead, neck and chest. All these changes occurred after leaving the hyperbaric chamber. Bloodwork revealed leukocytosis with neutrophil (Leukocytosis 16.0 × 10^9/L (reference values 4.00–11.00 × 10^9/L), Neutrophili 13 × 10^9/L (reference values 1.9–8.0 × 10^9/L), with elevated enzymes aspartate aminotransferase (AST) 56 U/L (reference values 0–37 U/L), alanin aminotransferase (ALT) 59 U /L, (reference values 25–65 U/L), creatine kinase (CK) 649 U/L, (reference values 32–300 U /L), lactate dehydrogenase (LDH) 398 U/L (reference values 85–227 U/L). Because of pain and his condition we began treatment in a hyperbaric chamber at a pressure of 2.0 ATA for 70 minutes, resulting in a reduction of symptoms and objective recovery of the patient. Within 24 h, repeated laboratory tests showed a reduction of leukocytosis (13 × 10^9/L and neutrophils (7.81 × 10^9/L), and the gradual reduction of the enzymes AST (47 U/L), ALT (50 U/L, CK (409 U/L), LDH (325 U/L). Since head CT and EEG were normal, epilepsy diagnosis was ruled out. This fact, along with medical tests, facilitated the differential diagnosis and confirmed that this was a case of neurotoxic effects of oxygen while the patient was in a hyperbaric chamber, not epileptic seizures. **Conclusion.** This case report suggests that in patients with symptoms of epileptic seizures while undergoing treatment in a hyperbaric chamber, it is always important to think of neurotoxic effects of pure oxygen which occurs at higher pressures and with a longer inhalation of 100% oxygen. In these patients, reexposure to hyperbaric conditions leads to recovery. This effect is important in daily inhalation of 100% oxygen under hyperbaric conditions which is why the use of pure oxygen is controlled and diving is allowed in shallow depths and for a limited time.

Key words:
oxygen; epilepsy; skin manifestations; hyperbaric oxigenation; treatment outcome.

Apstrakt

Uvod. Kiseonik je element od životne važnosti za aerobne organizme. Međutim, udisanje kiseonika pod povišenim pritiskom u uslovima hiperbarične oksigenoterapije može, ukoliko nije kontrolisano, da dovede do ozbiljnih oštećenja, pa i do smrti. **Prikaz bolesnika.** U radu je prikazan 20-godišnji muškarac koji je tokom testa za ronioca u hiperbaričnoj komori, prilikom udisanja 100% kiseonika, dobio simptome epilepsije. Odmah mu je skinuta maska sa kiseonikom, prešao je na disanje vazduha i započeta je brza dekompresija. Kod bolesnika je došlo do gubitka svesti, do pojave pene na ustima i serije toničkih grčeva. Bolesnik je ranije bio potpuno zdrav i nije koristio nikakvu medikamentoznu terapiju. Primljen je na lečenje u našu ustanovu kao hitan slučaj, i lečen kao da je imao epileptični napad. Na prijemu je zapaženo da se žali na bolove u mišićima i zglobovima i da ima eritematozne promene na čelu, vratu i grudima koje su se javile posle izlaska iz hiperbarične komore. Laboratorijski nalazi pokazali su da u krvnoj slici postoji leukocitoza sa neutrofilijom (leukociti 16,0 × 10^9/L (referentne vrednosti 4,00–11,00 × 10^9/L), neutrofili 13,0 × 10^9/L (referentne vrednosti 1,9–8,0 × 10^9/L), uz povišene enzime aspartat aminotransferazu (AST) 56 U/L; (referentne vrednosti 0–37 U/L), alanin aminotransferazu (ALT) 59 U/L, (referentne vrednosti 25–65 U/L,), kreatin kinazu (CK) 649 U/L (referentne vrednosti 32–300 U/L) laktat dehidrogenazu (LDH) 398 U/L (referentne vrednosti 85–227 U/L). Zbog bolova i opšteg stanja bolesnik je podvrgnut tretmanu u hiperbaričnoj komori na pritisku od 2.0 ATA u trajanju od 70 minuta, nakon čega je došlo do nestanka tegoba i objektivnog oporavka. Ponovljene labora-

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

torijske analize posle 24 časa pokazale su sniženje leukocita na 13 × 10^9/L, neutrofila na 7,81 × 10^9/L, kao i na postepeno smanjenje aktivnosti enzima u serumu (AST 47 U/L, ALT 50 U/L, CK 409 U/L, LDH 325 U/L). Budući da su multislajsni skener (MSCT) glave i elektroencelafogram (EEG) bili uredni, isključena je dijagnoza epilepsije. Uz ostale nalaze, to je olakšalo diferencijalnu dijagnozu i potvrdilo da se radilo o neurotoksičnom efektu kiseonika koji je pacijent imao u hiperbaričnoj komori, a ne o epileptičnom napadu. **Zaključak.** Prikazani bolesnik upućuje na zaključak da bi u slučaju sa razvojem stanja epinapada u toku tretmana u hiperbaričnoj komori, trebalo uvek razmišljati o prouzrokovanom neurotoksičnom delovanju kiseonika koje se javlja pri većim pritiscima i kod duže inhalacije 100% kiseonika i da ponovno izlaganje hiperbaričnim uslovima kod takvih bolesnika dovodi do oporavka. Ovaj efekat je važan kod svakodnevnog udisanja 100% kiseonika pod hiperbaričnim uslovima zbog čega se kontroliše njegova upotreba i dozvoljava ronjenje na malim dubinama i na ograničeno vreme.

Ključne reči:
kiseonik; epilepsija; koža, manifestacije; hiperbarička oksigenacija; lečenje, ishod.

Introduction

People's attempts to stay under water while breathing air go back to the distant past. Throughout history there have been many attempts to create machines that would enable people to stay under water with more or less success. There also was always a desire to treat patients with many diseases under these conditions.

In 1662, British doctor Henshaw [1] constructed a ball-shaped chamber into which he pumped air using two organ bellows, and tried to cure a number of diseases. The idea was that breathing compressed air has the therapeutic effect.

A Frenchman, Antoine Lavoisier (1743–1794), even though he was not a doctor, came to the conclusion that gas exchange takes place in the lungs. He was sure that the oxygen in the body converts into carbon dioxide, and that the nitrogen which he discovered is removed from the body unchanged.

Following the discovery of oxygen, Joseph Priestley (1733–1804) was the first to raise the question about the possibility of harmful effects of this gas on the body [2]. Oxygen was called "a vital gas" at that time, and Lavoiser described changes in the lungs caused by its inhalation. Only in 1899, after several experiments, Lorain -Smith found that breathing oxygen at a pressure greater than 0.6 bar after a prolonged exposure causes irreversible pathological changes in the lungs. In his honor, this phenomenon is called the Lorain -Smith effect.

In the excellent book "La pression barométrique", French physiologist Paul Bert in 1878, pointed out that breathing oxygen pressurized above 2 absolute bar causes convulsions similar to epilepsy. In deference to him, such cases are referred to as "Paul Bert effect" or neurotoxic effects of oxygen [2]. Others, starting with Berta, have proposed to replace air with oxygen during treatment process [4]. Behnke and Shaw [5] were the first to try this. Their work resulted in a recommendation of therapy based on the severity of the condition and first application of mixtures of oxygen and nitrogen different than normal air. Paul Bert effect is important in daily inhalation of oxygen, which is why its use is limited and diving is allowed in shallow depths and for a limited time. The most accepted mechanism is that oxygen inactivates ferments necessary for the performance of normal processes in nerve cells [6].

Bert [4] also noted that rapid transition from higher to lower pressure leads to sickness and even fatality, so he suggested the prevention of decompression sickness by gradually decreasing pressure (gradual decompression). This is actually the first introduction of the prophylactic decompression procedure based on the principle of slow and continuous emergence. An important aspect of this early work was the recognition of safe limits of exposure to oxygen in relation to dose and time, and the highest possible oxygen pressure and the longest exposure time with the minimum possible risk of oxygen toxicity to the central nervous system [7, 8]. Later, navies became interested in oxygen and expanded its use, speeding up the process of decompression and improving the efficiency and treatment of divers [9, 10].

Bert's other significant contribution to the practice of hyperbaric medicine was the recognition of oxygen toxicity to the central nervous system with application of oxygen under high partial pressures [6, 7]. Oxygen toxicity on the central nervous system was not significant for many years as it was mainly related to diving until the use of sufficiently high partial pressures of oxygen in the clinical setting [11]. The mechanism of oxygen neurotoxicity explans that hyperbaric oxygen inactivates ferments responsible for bioregulation of vital processes in nerve cells, but there is also a view that dissolved oxygen disrupts a regular transport of oxygen and removal of carbon dioxide [6].

Case Report

A 20-year-old male, a diver candidate became ill during testing in the hyperbaric chamber, and admitted to our hospital as an emergency case. The patient's history and medical records indicated that previously he was a healthy person who did not take any medication. While breathing 100% oxygen at the depth of 18 m for 60 minutes in the chamber, he developed problems that were manifested as the loss of consciousness, foaming at the mouth, and a series of convulsions of the entire body and limbs.

A companion in the chamber reacted by removing the mask and the patient began rapidly to ascent, and from that moment he stopped breathing 100% oxygen and breathed air during the ascent. After surfacing and exiting the hyperbaric chamber the patient was confused, he had nausea and queasiness, and within the next 50 minutes, he developed redness on the forehead, neck and chest, and conjunctival suffusion, with pain in the shoulders and muscles (Figure 1).

Post-admission laboratory analysis and blood work showed leukocytosis with neutrophilia (leukocytes 16.0 × 10^9/L, neutrophils 13.0 × 10^9/L), with elevated aspartate ami-

Fig. 1 – Appearance of the patient before treatment with hyperbaric oxygen therapy: a) Changes on the skin of the face; b) Changes on the skin of the neck; c) Changes on the conjuctive (conjuctival suffusion)

notransferase (AST) 56 U/L, alanine aminotransferase (ALT) 59 U/L, creatine kinase (CK) 649 U/L, lactate dehydrogenase (LDH) 398 U/L. Other laboratory tests were within the normal range. Radiography of the heart and the lungs, and head computed tomography (CT) were also normal (Figure 2), as were cardiological and neurological examinations. Head magnetic resonance imaging (MRI) with contrast was also normal. Despite the demonstrated symptoms that resembled the epileptic seizure, the tests confirmed that it was the neurotoxic effect of oxygen. Despite the manner of onset of the disease and the findings that were suggestive of neurotoxic effect of oxygen, the patient was treated in a hyperbaric chamber for 70 minutes at the pressure of 2.0 ATA, primarily due to the redness of the forehead, neck and chest, conjunctival suffusion, and pain in the shoulders, muscles and joints.

During the treatment in the hyperbaric chamber, within 30 minutes the patient's condition improved, skin redness and muscular and joint pain disappeared, and conjuctival suffusion became less pronounced (Figure 3).

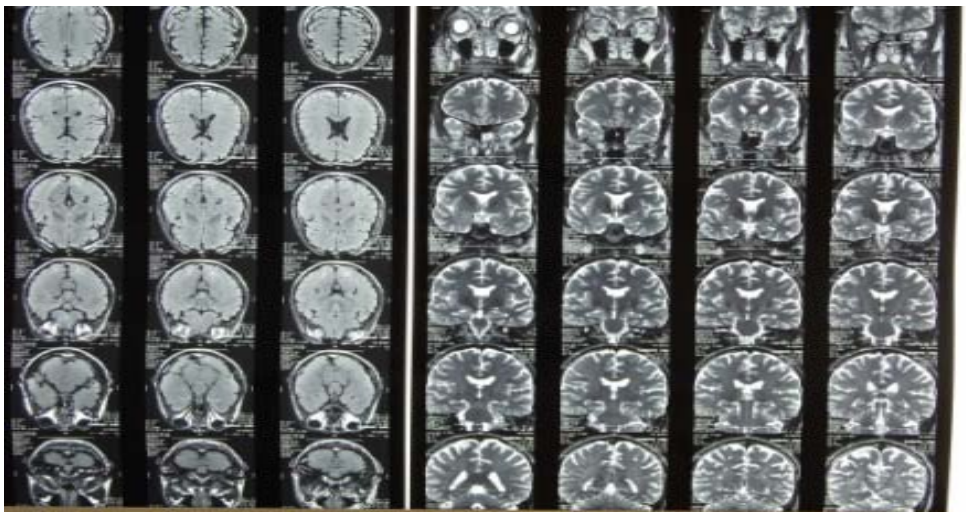

Fig. 2 – Computed tomography (CT) scan of the head – normal findings.

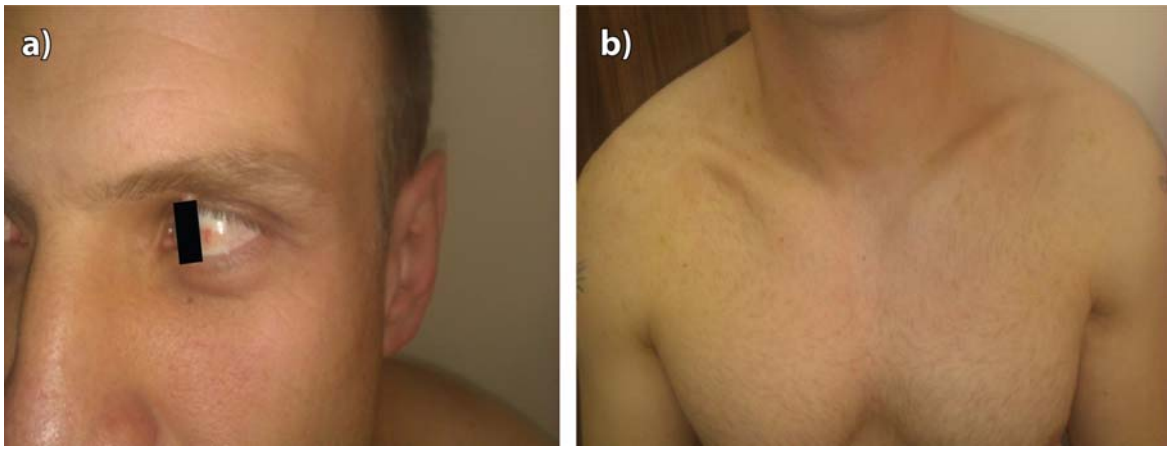

Fig. 3 – The patient after first treatment in the hyperbaric chamber: a) A significant reduction in conjunctival suffusion; b) A significant regression in skin changes.

The next day, laboratory analysis was repeated, revealing a reduction in leukocytes count to 13×10^9/L, neutrophils to $7{,}81 \times 10^9$/L, as well as a gradual reduction in serum enzymes activities (AST 47 U/L, ALT 50 U/L, CK 409 U/L, LDH 325 U/L).

Discussion

Occurrence of neurotoxic effects of oxygen has been described in divers who do not comply with the depth of dive, and time for inhalation of 100% oxygen in a closed apparatus [12]. This effect can occur in a hyperbaric chamber in the event of inhalation of 100% oxygen over 2.0 ATA pressure in people who are particularly sensitive to elevated partial pressure of oxygen.

In this case report, we presented healthy person who had been tested in a hyperbaric chamber. While breathing 100% oxygen at the pressure of 2.8 ATA he exhibited a neurotoxic effect, with the appearance of clinical symptoms that resembled epileptic seizures .

This effect is not unknown in the literature and is also called oxygen epilepsy or Paul Bert effect [2]. The clinical picture is consistent with epileptic seizure and may represent a major differential diagnostic problem to clinicians who are not familiar with diving diseases. Only later, as in the case of our patient, after neurological examination (EEG, CT and MRI of the head) neurotoxic effect of oxygen that the patient breathed at high pressure, and not epileptic seizure, was confirmed.

Also, with pressure reduction in the chamber and cessation of breathing 100% oxygen, symptoms disappear spontaneously and there is no need for further treatment in most cases.

However, immediately upon admission and after examination and the diagnosis, we placed our patient in a hyperbaric chamber where he was treated for pain in the muscles, joints, and erythema which appeared at the head, neck and chest and conjunctival suffusion. We did that at the pressure of 2.0 ATA for 70 minutes. Already in the course of treatment in the hyperbaric chamber, the patient's condition objectively and subjectively improved which was confirmed by laboratory findings.

Conclusion

We presented a case with neurotoxic effects of oxygen that occurred in the hyperbaric chamber. Such cases occur frequently when diving in a closed type apparatus. In particular, in such cases we emphasize the need to carefully and quickly perform a differential diagnosis to severe neurological diseases, but taking into account inhalation of 100% oxygen, which precedes the condition. The diagnosis is easy to establish following normal EEG, CT and MRI of the head findings, unlike a true epileptic attacks.

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CASE REPORT

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Unrecognised adrenergic symptoms and the delayed diagnosis of urinary bladder paraganglioma

Kasna dijagnoza paraganglioma mokraćne bešike zbog neubedljivih adrenergičkih simptoma

Tamara Dragović*†, Dejan Marinković*, Snežana Kuzmić-Janković*, Rade Prelević‡, Novak Milović†‡, Božidar Kovačević§, Zoran Hajduković*†

***Clinic for Endocrinology, ‡Clinic for Urology, §Institute of Pathology, Military Medical Academy, Belgrade, Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

Abstract

Introduction. Paraganglioma is a rare neuroendocrine neoplasm that may arise from the extra-adrenal autonomic paraganglia. Urinary bladder paraganglioma is typically presented as repeated episodes of palpitations, headache or blood pressure rise immediately after micturition. Management of these tumors includes radical surgical treatment with preoperative antihypertensive preparation, and a life-long follow-up. **Case report.** We presented a middle-age female patient with functional urinary bladder paraganglioma, with a 3-year history of repeated episodes of abdominal pain, dysuria and hematuria. After obtaining more precise anamnestic data, the patient reported occasional simultaneous presence of mild adrenergic symptoms, that did not cause any particular attention at first. Morphological and biohumoral examinations suggested paraganglioma of the urinary bladder. Open partial cystectomy was performed, detecting a submucosal mass, while immunohistochemical analysis confirmed the presence of chromaffin tissue. Clinical manifestations, diagnostic approach, management and histopathological findings of urinary bladder paraganglioma are discussed. **Conclusion.** Since the prognosis with localized paraganglioma is good, we underlined the importance of a well-timed, accurate and detailed medical history in all the patients with even mild, inexplicable micturition-provoked adrenergic symptomatology.

Key words:
urinary bladder neoplasms; paraganglioma; diagnosis; urologic surgical procedures; histological techniques.

Apstrakt

Uvod. Paragangliom je redak neuroendokrini tumor koji vodi poreklo od ekstraadrenalnih autonomnih paraganglija. Paragangliom mokraćne bešike u tipičnom slučaju manifestuje se rekurentnim epizodama palpitacija, glavobolje i skokova krvnog pritiska, nastalih neposredno nakon mokrenja. Lečenje ovog tumora podrazumeva radikalni hirurški zahvat sa karakterističnom preoperativnom pripremom, antihipertenzivnom terapijom i dugotrajnim postoperativnim praćenjem. **Prikaz bolesnika.** Prikazali smo bolesnicu, staru 54 godine, sa funkcionalnim paragangliomom mokraćne besike i trogodišnjom evolucijom ponavljanih epizoda suprapubičnih bolova, praćenih hematurijom i dizuričnim smetnjama. Tokom prikupljanja detaljnijih anamnestičkih podataka, bolesnica je navela istovremeno prisustvo blagih adrenergičkih simptoma koji su nastupali neposredno nakon mokrenja, na koje inače ranije nije obraćala naročitu pažnju. Nakon sprovođenja dopunskih biohumoralnih i morfoloških ispitivanja dijagnostikovan je paragangliom mokraćne bešike. Tumorska masa je odstranjena parcijalnom cistektomijom, a imunohistohemijskom analizom potvrđeno je postojanje hromafinog tkiva. U radu su analizirane moguće kliničke prezentacije paraganglioma mokraćne besike, dijagnostički pristup i patohistološka analiza. **Zaključak.** S obzirom na to da bolesnici sa lokalizovanim paragangliomom imaju povoljnu prognozu, naglasili smo značaj pravovremeno uzete, tačne i detaljne anamneze kod bolesnika sa blagom, nerazjašnjenom, adrenergičkom simptomatologijom, koja je provocirana mokrenjem.

Ključne reči:
mokraćna bešika, neoplazme; paragangliom; dijagnoza; hirurgija, urološka, procedure; histološke tehnike.

Correspondence to: Dejan Marinković, Clinic for Endocrinology, Military Medical Academy, Crnotravska 17, Belgrade, Serbia.
E-mail: dejmarinkovic@gmail.com

Introduction

A paraganglioma is a rare neoplasm that originate from chromaffin tissue of the extra-adrenal autonomic paraganglia, and have the ability to secrete catecholamines. The tumor is closely related to pheochromocytoma, and undistinguished at the cellular level [1]. Sympathetic paraganglioma may be localized in different parts of the sympathetic paravertebral ganglia of thorax, abdomen or pelvis. Paraganglioma of the urinary bladder accounts for less than 0,06% of all bladder neoplasms, and less than 1% of all pheochromocytomas [2].

Clinical manifestation of these tumors may be various and nonspecific, since they can be functional or nonfunctional. In most of the cases, signs and symptoms are due to excessive catecholamine secretion, or may be related to the mechanical impact on the surrounding tissues. Typical symptoms are repeated episodes of dizziness, palpitations, headache or hypertensive crises provoked by micturition. In rare cases, the clinical picture is dramatic with general symptomatology or sudden cardiac death [2, 3].

We presented a patient with functional urinary bladder paraganglioma, expressed with a dominant dysuric disabilities and mild inconclusive signs of catecholamine excess, provoked by micturition.

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Case report

A 54-year-old female was admitted to our department, after urological follow-up and suspicion of functional tumor of the urinary bladder. The patient had a history of repeated episodes of suprapubical abdominal pain, dysuria with occasional signs of urinary infection and hematuria over a 3-year period. These symptoms were usually treated with anti-infection drugs.

Her ambulatory urological examination started 6 months prior to admission, when suprapubical pain became more intense, with pelvic ultrasonography showing heterogeneous mass located on the posterior wall of the bladder. Cystoscopic examination revealed solitary submucosal mass of 50 mm in diameter, with normal mucosal covering. Transurethral tissue biopsy of the submucosal mass was taken. Unfortunately, it did not get sufficient amount of tissue material for histopathological examination. During the intervention, no hypertension or other signs of catecholamine excess occurred.

Because of the symptoms persistence and newly registered episodes of high blood pressure, the patient required further examination by the endocrinologist. Only at his insistence, the patient reported the occasional presence of weakness, dizziness, palpitations, headaches and sweating with episodes of sudden blood pressure rise, immediately following micturition. Nevertheless, the patient did not find it necessary to mention at the first time.

On admission, physical examination of the patient was unremarkable. Except for discrete suprapubical palpatory sensitivity, we did not observe any unusual findings. The patient was normotensive, with heart rate of 80 beats *per* minute, and with no organomegaly. The patient had no orthostatic hypotension. Electrocardiogram showed sinus rhythm, with rare supraventricular extrasystoles and no other abnormalities.

The patient had no medical history of cardiovascular illness or hypertension, and she did not take any lasting medications.

Due to the previous anamnestic data, additional endocrine test were performed. Laboratory blood test revealed the elevated serum metanephrine level of 271 pg/mL (normal range < 90 pg/mL), normetanephrine level of 728 pg/mL (normal range < 180 pg/mL) and chromogranin A level of 114 mcg/L (normal range < 100 mcg/L).

Abdominal and pelvic computed tomography (CT) scan demonstrated highly vascularized mass 53 mm × 35 mm × 50 mm in diameter on the upper-posterior wall of the urinary bladder (Figure 1). No signs of any metastatic disease were found. A ^{131}I-metaiodobenzylguanidine (^{131}I-MIBG) scanning showed a single focus of increased radionuclide activity in urinary bladder (before and after micturition) suggesting residual urine, but also the presence of chromaffin tissue (Figure 2).

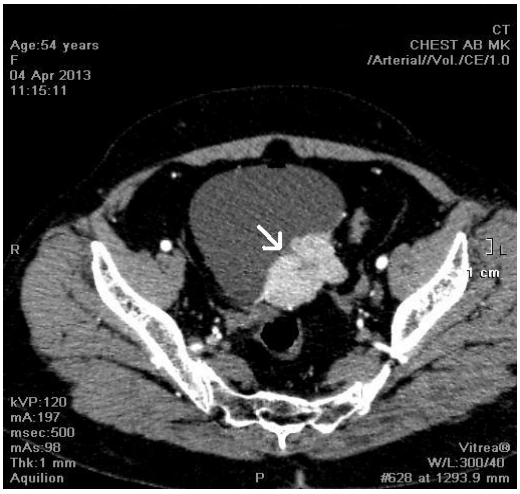

Fig. 1 – Multislice computed tomography (MSCT) demostrated a mass of 53 mm × 35 mm × 50 mm, located on the upper-posterior wall of the urinary bladder (white arrow).

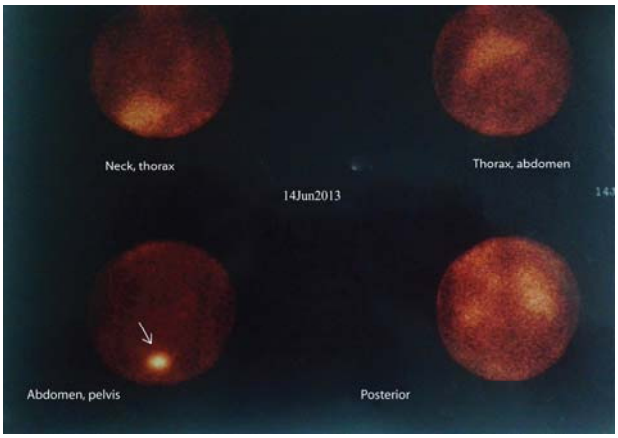

Fig. 2 – Metaiodobenzylguanidin (MIBG) scan 48 h after administration of ^{131}I-MIBG-sequential images of neck, thorax, abdomen and pelvis (anterior and posterior projections). Radionuclide uptake is seen in the urinary bladder (white arrow).

The diagnosis of paraganglioma of the urinary bladder was made.

During the three weeks of preoperative medical treatment with dual adrenoreceptor blocking agents (doxazocine followed by propranolol), our patient exhibited 4 episodes of acute blood pressure rise, up to 240/120 mmHg with the heart rate of 100 beats *per* minute, provoked by micturition. These episodes resolved spontaneously, or by sublingual calcium channel blockers.

The patient was operated and open partial cystectomy was performed revealing a submucosal mass of 50 mm in diameter, with a suspect vesical wall infiltration (Figure 3). No other tumor or lymphnode enlargement were found.

Fig. 3 – Macroscopic appearance of paraganglioma of the urinary bladder.

Despite preoperative medical preparation, blood pressure of the patient increased rapidly during the surgery. Postoperative period proceeded with mild hypotension and no complications.

The histopathology result described infiltrative tumor growth with focal prominent cellular polymorphism and poor, well-vascularized tumor stroma. Mitotic figures were rare, and the proliferative activity was very low (Ki67 about 1%), with no tumor necrosis and lymphovascular invasion. Immunohistochemical examination showed strongly positive staining for chromogranin A, synaptophysin and vimentin; thus the diagnosis of paraganglioma was confirmed (Figure 4).

One month after the surgery, the patient was normotensive and micturition-related symptoms disappeared. Plasma levels of metanephrine and normetanephrine normalised. Six months after the surgery, repeated biohumoral measurement and pelvic ultrasonography showed no local recurrence of the tumor.

Discussion

Urinary bladder paraganglioma is a rare neoplasm, accounting for only 0.06% of all bladder tumors and 6% of all extra-adrenal pheochromocytoma [4]. The tumor usually develops in young adult women. The majority of paragangliomas appear to be sporadic; still, approximately one-third to one-half are hereditary associated with familiar paraganglioma, von Hippel-Lindau disease, neurofibromatosis type 1 or multiple endocrine neoplasia type 2 [5].

Since the first report in 1953, about 270 cases of bladder paraganglioma have been reported worldwide [2, 6–32]. The most common presentation of bladder paraganglioma is a characteristic clinical picture resulting from a hypertensive crisis accompanied by headache, palpitations, flushes, dizziness or sweating. These symptoms are typically provoked by micturition or overdistension of the bladder, and appear in 90% of the patients [6]. Post-micturition syncope is presented in 65%, while painless hematuria appears in 60% of the patients [7]. Someti-

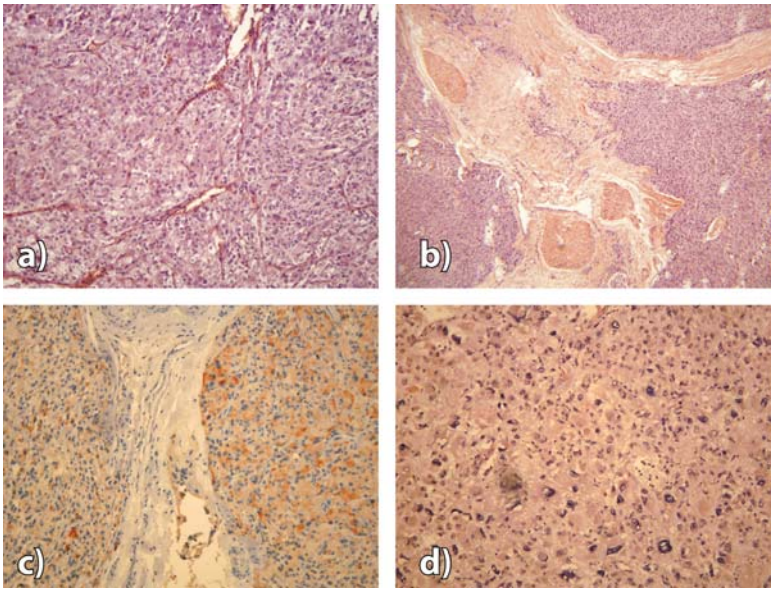

Fig. 4 – Pathological findings (histological and immunocytochemical staining).
a) Typical arrangement of alveolar tumor cells ("Zellballen" growth pattern) (hematoxylin and eosin, × 100);
b) Stromal septa with a beach of tumor cells (hematoxylin and eosin, × 40); The chief cells of the tumor have immunoreactivity to neuroendocrine markers; c) Chromogranin A is strongly positive (intense and diffuse cytoplasmic positive reaction in a large number of tumor cells) (3-amino-9-ethylcarbazole, 100 ×); d) Synaptophysin is positive (reaction is intensively detected in a large number of tumor cells) (Papanicolau, × 100).

mes patients present with long term complications of unregulated hypertension such as intracranial bleeding, retinopathy, cardiac failure or sudden cardiac death. Other symptoms include orthostatic hypotension, abdominal pain, weight loss, nausea, blurred vision, weakness, malaise and paresthesia, presented in 20% of the patients [2–6].

In the presented patient, the medical history was atypical. From the very beginning she had dysuria with abdominal pain and the occasional presence of hematuria. Subsequently, she got mild, short duration, post-micturition symptoms related to excessive catecholamine secretion. These symptoms did not cause particular attention by the patient, so she did not mention any of them at the beginning of examination. Interestingly, cystoscopic examination and transurethral manipulation during biopsy did not provoke any typical signs and symptoms.

There are several similar presentations of bladder paraganglioma described in the literature. Li et al. [7] reported a case of unsuspected functional paraganglioma of the urinary bladder with a 2-year history of mild painless hematuria, with no hypertension attacks prior to surgery. Chen et al. [8] observed a male patient with gross hematuria and abdominal pain which radiated to the inguinal region suggesting ureteral stone. Only after the diagnosis of urinary bladder paraganglioma was conducted, he revealed previous presence of postural hypotension and palpitations. In the case of nonfunctional paraganglioma of the urinary bladder, lower abdominal pain or dysuria, could be the single present symptom [9]. There is also an unusual case of postponed detection of bladder paraganglioma, misdiagnosed with basilar-type migraine due to the permanent headache lasting for 8 years [10]. Rarely, the presence of dyspnea provoced by micturition could be the only sign of unrecognized bladder paraganglioma [11].

In patients who are thought to have paraganglioma, biochemical test should be done to measure the levels of metanephrine and normetanephrine, since they are more sensitive than catecholamines [33]. These levels should be measured either in plasma or in a 24-h urine collection. Urinary levels, if determined shortly after or during the hypertensive crisis, have greater sensitivity. It is found that plasma-free metanephrines and urinary fractionated metanephrines offered higher sensitivity (99% and 97%, respectively) than plasma catecholamines, urinary catecholamines, total urinary metanephrines, and urinary vanillylmandelic acid [33].

The levels of chromogranin A, acidic monomeric protein that is stored and secreted with catecholamines, are increased in more than 80% of patients. Its specificity for paraganglioma is low, and the level of chromogranin A can be increased in other neuroendocrine tumors, too [34]. The presented patient had more than a three-fold increase in normetanephrine plasma value, with slightly elevated chromogranin A plasma value, that is typical for paragangliomas.

Further diagnostic procedures include exact localization and morphological description. Using cystoscopy, paraganglioma are presented as a submucosal globular mass protruding into bladder, with smooth surface and intact mucosa. Although biopsy is usually not recommended, for provoking catecholamine excess by manipulation, we performed biopsy of the submucosal mass for the first time. As we mentioned before, despite the endocrine activity of bladder tumor, our patient showed no adrenergic symptomatology or acute attack of hypertension during biopsy. CT scanning of the abdomen and pelvis is usually one of the first study performed. CT and magnetic resonance imaging (MRI) scans have similar sensitivity of 98–100%, but their specificity is only 70% [35]. For that reason it is recommended to perform functional imaging. The most commonly used functional study for detecting paragangliomas is scintigraphy with ^{131}I-MIBG, with the sensitivity ranging from 77% to 90% and a specificity of 95% to 100% [36]. Scintigraphy using 123I-MIBG is reported to obtain superior images, with the sensitivity ranging from 83% to 100% and a high specificity from 95% to 100%, but the cost and shorter half-life of this isotope limits its use [5].

During the last two decades, several novel functional imaging techniques have been developed and recommended. These involve somatostatin receptor scintigraphy with ^{11}In-pentetreotide and positron emission tomography (PET) using ^{18}F-fluorodeoxyglucose, ^{18}F-fluorodopa, and ^{18}F-fluorodopamine, particularly recommended in cases in which other functional imaging tests are negative [18, 36, 37].

The diagnosis of bladder paraganglioma in the presented patient was confirmed by CT scan of highly vascularized mass in the posterior wall of the urinary bladder associated with a single focus of increased uptake of ^{131}I- MIBG in that region.

As we previously mentioned, extra adrenal paragangliomas can rarely be inherited as an isolated autosomal dominant trait, or as a part of the multiple endocrine neoplasia type II syndrome, as well as with neurofibromatosis and von Hippel-Lindau disease. Since the presented patient had no symptoms, clinical or biochemical manifestations suggesting any of these syndromes, no geneting testing was conducted.

After localization of the tumor, surgical removal should be performed.

For all the patients with paraganglioma, it is essential to conduct adequate preoperative medical treatment based on antihypertensive control. An alpha-blocker, phenoxybenzamine, gradually increased up to 1–2 mg/kg body weight daily, is the first choice therapy. Beta-blockers should be taken only after alpha-blockers have been started, mostly if tachycardia is present [2, 4]. We used doxazosin followed by propranolol for a 3-week period, since the presented patient had got 4 episodes of catecholamine excess after micturition in a preoperative period.

Surgical treatment modalities include transurethral resection, or open laparotomy with partial or total cystectomy, combined with pelvic lymph node dissection. Transurethral resection is not recommended in case of suspect invasion of the muscular layer. Total cystectomy is often performed in case of pelvic lymphadenopathy [3, 16]. Some authors consider laparoscopic resection as the first choice modality in treatment of bladder paraganglioma [15, 38]. Nevertheless, since paraganglioma is easy to recur it is recommended that the tumor and its peri-tissue should be resected completely. Since patients with localized tumors have the favorable prognosis, they could be treated with less radical modalities [4, 5, 32]. Our patient underwent open laparotomy and partial resection of the urinary bladder with *ex tempore* verification of no microinvasion into the bladder wall.

The final diagnosis is based upon histological and immunohistochemical analysis. Paragangliomas show histological features similar to adrenal pheochromocytoma. The chief cells of the tumor show immunoreactivity to neuroendocrine markers such as chromogranin A, synaptophysin and neuron-specific enolase [31, 39]. It is difficult to histopathologically diagnose paraganglioma as malignant or benign. There is no certain way to predict which tumor will progress to malignancy. In clinical practice, the only reliable criterion of malignancy is the presence of distant metastases.

Due to the unpredictable course, late endocrinal manifestations and metastasis, long-term follow up is advised [24]. Although there are no official guidelines for postoperative follow-up, first measurement of plasma metanephrine and normetanephrine values are recommended a month after surgery, and later every 6 months for a period of 2 years. In the suspect cases, abdominal and pelvic CT imaging is recommended every 3 months for a year; then every 6 months for the period of one year, and later yearly for the period of 3 years subsequently [4, 5].

Conclusion

Paraganglioma of the urinary bladder is a rare neoplasm with varied clinical presentation, sometimes very subtle and easily overlooked. Since patients with localized tumor have the good prognosis, it is important to recognize possible symptoms in time, proceeding an accurate and detailed medical history. A combination of specific symptoms, biohumoral testing and image investigation is crucial in diagnosis, while the partial and total cystectomy is the treatment of choice. Since paraganglioma is more likely to recur or metastasize, it is essential to make a long term follow-up of these patients.

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CASE REPORT

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Typical chest pain and precordial leads ST-elevation in patients with pacemakers – are we always looking at an acute myocardial infarction?

Tipičan bol u grudima i prekordijalna ST-elevacija kod bolesnika sa pejsmerkerima – Vidimo li uvek akutni infarkt miokarda?

Marina M Ostojić*, Tatjana S Potpara*†, Marija M Polovina*, Mladen M Ostojić*

***Clinic of Cardiology, Clinical Center of Serbia, Belgrade, Serbia; †Faculty of Medicine, University of Belgrade, Serbia**

Abstract

Introduction. Electrocardiographic (ECG) diagnosis of acute myocardial infarction (AMI) in patients with paced rhythm is difficult. Sgarbossa's criteria represent helpful diagnostic ECG tool. **Case report**. A 57-year-old female patient with paroxysmal atrial fibrillation and a permanent pacemaker presented in the Emergency Department with prolonged typical chest pain and ECG recording suggestive for AMI. Documented ECG changes correspond to the first Sgarbossa's criterion for AMI in patients with dual pacemakers (ST-segment elevation of ≥ 5 mm in the presence of the negative QRS complex). The patient was sent to catheterization lab where coronary angiogram reveled normal findings. ECG changes occurred due to pericardial reaction following two interventions: pacemaker implantation a month before and radiofrequency catheter ablation of AV junction two weeks before presentation in Emergency Department. **Conclusion.** This case report points out to the limitations of proposed criteria that aid in the recognition of AMI in patients with underlying paced rhythm and possible cause(s) of transient electrocardiographic abnormalities.

Key words:
pacemaker, artificial; myocardial infarction; pericarditis; heart conduction system; catheter ablation; diagnosis, differential.

Apstrakt

Uvod. Elektrokardiografska (EKG) dijagnostika akutnog infarkta miokarda (AIM) kod bolesnika sa ugrađenim pejsmejkerom je otežana. Za postavljanje adekvatne dijagnoze od pomoći su Sgarbossa EKG kriterijumi. **Prikaz bolesnika.** Bolesnica, stara 57 godina, primljena je u Urgentni centar sa tipičnim bolom u grudima i EKG promenama koje odgovaraju slici anteriornog AIM. EKG promene ispunjavale su prvi Sgarbossa kriterijum za AIM kod bolesnika sa pejsmejkerom (ST elevacija ≥ 5 mm u odvodima sa negativnim QRS kompleksom). Bolesnica je upućena na hitnu koronarografiju kojom nisu nađene opstruktivne promene u koronarnim arterijama. Registrovane EKG promene nastale su kao posledica perikarditisa izazvanog prethodnim interventnim procedurama: implantacijom trajnog pejsmejkera i radiofrekventnom kateter ablacijom AV čvora izvršenim mesec dana i dve nedelje pred prezentaciju u Urgentnom centru. **Zaključak.** Ovaj prikaz bolesnika ukazuje na ograničenja Sgarbossa EKG kriterijuma za postavljanje dijagnoze AIM kod bolesnika sa pejsmejkerom kao i na moguće uzročnike prolaznih EKG abnormalnosti.

Ključne reči:
srce, veštačko usklađivanje ritma; infarkt miokarda; perikarditis; srce, provodni sistem; ablacija preko katetera; dijagnoza, diferencijalna.

Introduction

The number of patients with pacemakers is constantly growing, and the standard ECG criteria for acute myocardial infarction (AMI) are of limited value in these patients, as acute myocardial injury can be masked by the presence of paced QRS complexes. The Sgarbossa's criteria (e.g. GUSTO criteria) usually allow recognizing AMI in patients with pacemakers but localization of MI is still difficult or even impossible [1]. The undetermined type of AMI is recognized in 6.5% patients and intrahospital mortality is reported to be 11.8% [2]. There are three Sgarbossa's criteria: ST-

Correspondence to: Marina Ostojić, Clinic of Cardiology, Clinical Center of Serbia, Koste Todorovića 8, 11 000 Belgrade, Serbia. Phone: +381 11 3616319, Fax: +381 11 3616318. E-mail: marina.licina1@gmail.com

segment elevation of ≥ 5 mm in the presence of the negative QRS complex, ST-segment elevation of ≥ 1 mm in the presence of the positive QRS complex, and ST-segment depression of ≥ 1 mm in lead V1, V2 or V3 [1].

Case report

We presented a 57-year-old female patient with paroxysmal atrial fibrillation (AF) and a permanent pacemaker admitted to the Coronary Care Unit due to prolonged typical chest pain and ECG recording suggestive for AMI.

The patient had the 7-year history of frequent, highly symptomatic paroxysms of AF despite active treatment with beta blocker, propafenone, flecainide, sotalol and, finally, amiodarone (in combination with beta blocker or verapamil). During propafenone treatment, several paroxysms of typical atrial flutter with fast ventricular response had occurred, and radiofrequency catheter ablation of atrial flutter was successfully performed. However, highly symptomatic frequent paroxysms of AF reoccurred, amiodarone was discontinued due to thyroid dysfunction (hyperthyreosis), and the patient refused radiofrequency catheter ablation (RFCA) of pulmonary veins. At that point, transthoracic echocardiography (TTE) confirmed previous normal findings. Yet, AF symptoms were poorly controlled and the patient's quality of life was significantly impaired. Finally, we performed RFCA of atrioventricular (AV) junction, with permanent pacemaker (DDDR) implantation. A month following pacemaker implantation, RFCA of AV junction was performed without periprocedural complications. At discharge (4 days later), the patient was asymptomatic with ECG showing artificial pacemaker rhythm (Figure 1).

Two weeks later, the patient referred to emergency room with typical chest pain, fatigue and ECG abnormalities suggestive for AMI (ST elevation 1–5 mm with negative T waves in precordial leads V2–V6 and ST elevation in the inferior leads) (Figure 2).

Coronary angiography revealed normal findings (Figure 3), and over the next 10 days biochemical markers of myocardial necrosis remained within referent values: troponin I 0.029 μg/L–0.018 μg/L (normal < 0.04 μg/L) and creatine kinase 125 U/L–

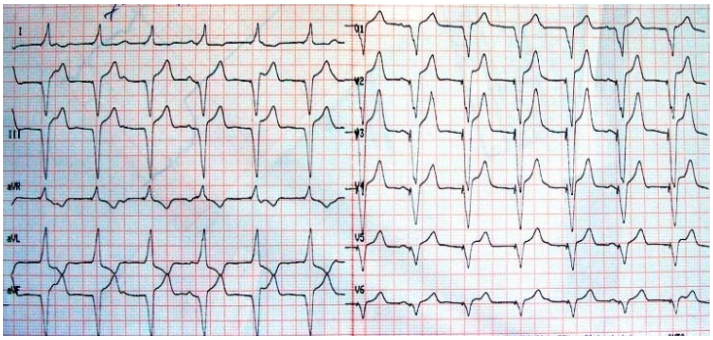

Fig. 1 – Electrocardiogram following atrioventricular node catheter ablation.

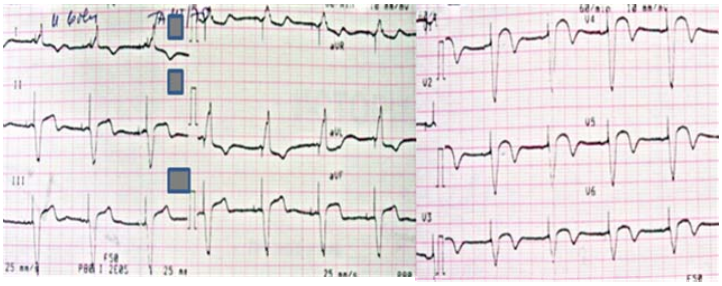

Fig. 2 – Electrocardiogram at admission to the Coronary Care Unit.

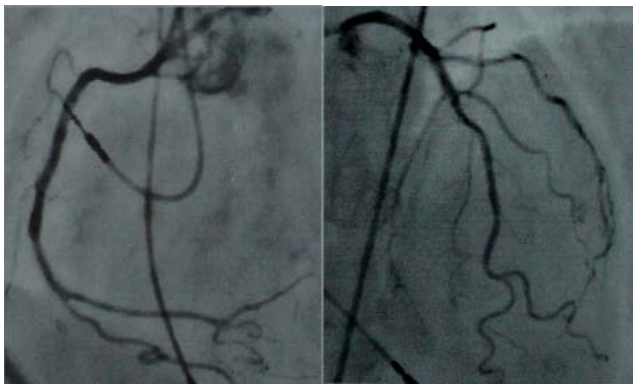

Fig. 3 – Coronary angiogram.

130 U/L (normal < 150U/L); TTE showed a localized non-homogeneous pericardial effusion of 4–10 mm along the right ventricular free wall (suggesting effusion in regression).

Other findings were normal, including chest radiography with pacemaker electrodes in the correct position (Figure 4).

At discharge, ECG abnormalities resolved (Figure 5), and TTE revealed only minimal pericardial effusion of 1–2 mm along the right ventricular free wall.

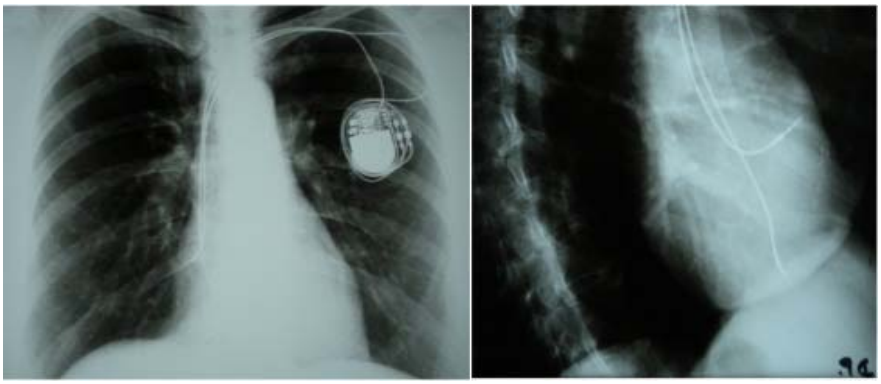

Fig. 4 – Chest radiography.

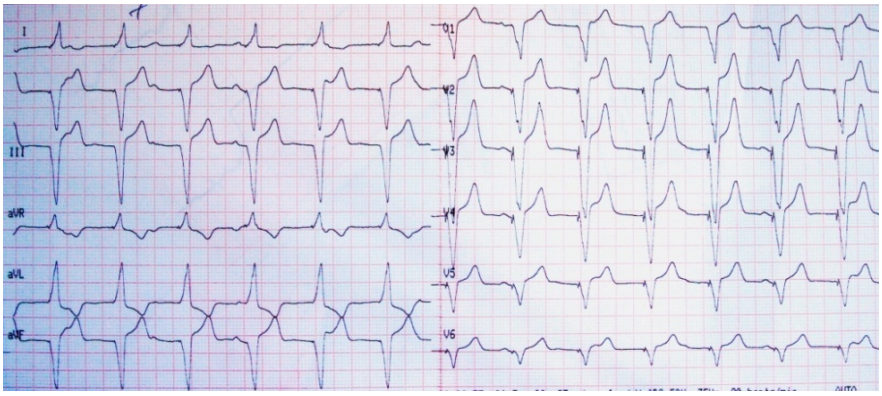

Fig. 5 – Electrocardiogram recording at discharge.

Discussion

Electrocardiographic (ECG) evaluation of patients with permanent pacemakers and typical chest pain might be difficult.

This case report presented a female patient with typical chest pain and ECG suggestive for AMI, ultimately diagnosed with transient pericardial effusion and normal coronary angiogram.

In GUSTO trial with 26,003 AMI patients, ECGs of 17 patients with a pacemaker were analyzed and only one of Sgarbossa's criteria was relatively highly sensitive: ST-segment elevation of ≥ 5 mm in the presence of the negative QRS complex [1]. However, it should be underlined that these criteria, which compose the guidelines for ECG diagnosis of AMI in patients with ventricular pacing, were based on the analysis of only 17 patients selected from the trial.

In the presented patient ST-segment elevation of ≥ 5 mm in the presence of the negative QRS complex was documented, but she did not have AMI. In the literature, ST elevation was also interpreted as ventricular fusion due to the presence of a ventricular pacing artifact and the QRS complex being a hybrid of spontaneous and totally paced beats [3]. However, symptoms of the presented patient and ECG changes occurred a month following pacemaker implantation, and two weeks post RFCA of AV junction. Pericardial reaction is a potential complication of invasive procedures such as pacemaker implantation or RFCA. Moreover, a recurrent chronic pericarditis may occur eventually leading to the development of large pericardial effusion or cardiac tamponade [4]. Myocardial microperforation with spontaneous resolution of a small pericardial effusion has also been described [4]. The incidence of acute myocardial perforation with dual chamber pacemaker implantation is < 1%, while late perforation following device implantation is less well recognized and deemed to be rare [5, 6].

On the other hand, major complications of RFCA occur in approximately 3% of patients, and the overall incidence of myocardial perforation and pericardial effusion is unknown. A multicenter retrospective study of the European Radiofrequency Survey on 2,222 patients reported cardiac perforation in 0.72% of patients, and a clinically relevant pericardial effusion in only 12 patients [7]. The incidence of cardiac complications varied according to the experience of the center and the type of ablation. Indeed, there is a very low risk of serious complications with RFCA of AV junction [8]. Regarding the presented patient, pacemaker electrode placement had most probably caused a localized myocardial damage (and possible myocardial microperforation) which led to the pronounced late pericardial reaction with pericardial effusion (and possible hematopericardium) and ST-segment elevation in precordial leads.

Conclusion

The interpretation of ECG abnormalities in patients with permanent pacemakers should be carefully undertaken, especially with the detailed insight into their history and recent invasive procedures, if any. Application of Sgarbossa's criteria may be helpful, but further prospective studies are needed on a larger number of patients with permanent pacemakers to elucidate more reliable criteria for more accurate interpretation of ECG abnormalities.

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CASE REPORT

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Surgical treatment of orbital floor blowout fracture in children: A case report

Hirurško lečenje *blowout* preloma poda očne duplje kod dece

Saša Z. Tabaković*, Ivana Ilić Dimitrijević†

***Clinic of Maxillofacial Surgery, Department of Dentistry, Faculty of Medicine, University of Prishtina with temporary seat in Kosovska Mitrovica, Kosovska Mitrovica, Serbia; †Department of Maxillofacial Surgery, Faculty of Dentistry, University of Belgrade, Belgrade, Serbia**

Abstract

Introduction. Orbital floor blowout fracture is a common traumatic lesion of the craniofacial complex, but rarely in children population, consequently representing challenge in surgical treatment. Timely diagnosis and surgical treatment prevent the probability of the occurrence of the functional complications. **Case report.** We presented surgical treatment of on 8-year-old girl with a blowout orbital floor fracture one month after the injury. The predominant symptoms were: ocular bulb motility disorder with consecutive strabismus and double vision. Orbital floor reconstruction was made by an autogenous mandibular symphyseal graft. A year after the orbital floor reconstruction additional correction of strabismus was performed due to functional disorder of the bulbomotor muscles. **Conclusion.** Delayed surgical treatment of blowout orbital floor fracture in children leads to unsatisfactory functional results in the majority of cases. In such a situation surgical correction of strabismus is necessary in order to obtain functionally quality vision and satisfactory aesthetic appearance.

Key words:
orbital fractures; diagnosis; child, ophtalmologic surgical procedures; treatment outcome.

Apstrakt

Uvod. *Blowout* prelom poda očne duplje česta je traumatska lezija kraniofacijalnog kompleksa, međutim, retka u dečijoj populaciji, tako da predstvalja terapijski izazov. Blagovremenom dijagnozom i hirurškim lečenjem, sprečava se mogućnost nastanka funkcionalnih komplikacija. **Prikaz bolesnika.** U radu prikazano je lečenje osmogodišnje devojčice sa *blowout* prelomom poda očne duplje mesec dana posle povrede. Dominantni simptomi bili su: poremećaj motiliteta očnog bulbusa sa prisutnim strabizmom i diplopijom. Rekonstrukcija defekta poda orbite urađena je autolognim koštanim transplantatom simfizne regije mandibule. Godinu dana posle rekonstrukcije poda orbite urađena je dodatna korekcija strabizma zbog funkcionalnog poremećaja bulbomotornih mišića. **Zaključak.** Kasno hirurško lečenje *blowout* preloma poda orbite kod dece najčešće ne daje zadovoljavajuće funkcionalne rezultate. Kod najvećeg broja bolesnika, neophodna je hirurška korekcija strabizma kako bi se omogućio funkcionalno kvalitetan vid i estetski zadovoljavajući izgled.

Ključne reči:
orbita, prelomi; dijagnoza; deca; hirurgija, oftalmološka, procedure; lečenje, ishod.

Introduction

Injuries of the craniofacial region in children are not frequently found [1]. Blowout orbital fractures create less than 10% of injuries of facial skeleton in the pediatric population [2]. Fractures of the floor and the inner orbital wall are the most frequent fractures sites [1, 3].

Bulbar conjunctiva ecchymosis, periorbital hematoma, diplopia, enophthalmos and paresthesia of the infraorbital nerve distribution are the commonest symptoms of blowout orbital floor fracture [4]. The defect results in herniation of the periorbital fatty tissue and bulbomotor muscles to the maxillary sinus. Incarceration (entrapment) of the periorbital tissue leads to ischemia, atrophy and scarring on the bulbomotor muscles, resulting in motility disorder of ocular bulb as well as esthetic problems.

Due to the craniofacial disproportion of the medial facial massif, underdevelopedness of the maxilla and paranasal cavities as well as protrusion of the frontal region, blowout fractures of the orbital floor are very rare in children below 8 years of age [5].

The young bone is rich in osteoblasts making it elastic and less fragile. Due to the greater elasticity of the bones in

Correspondence to: Saša Z. Tabaković, Clinic of Maxillofacial Surgery, Medical Faculty-Dentistry, University of Prishtina with temporary seat in Kosovska Mitrovica, Anri Dinana b.b, Kosovska Mitrovica, Serbia. Phone: +381 60 150 0161. E-mail: sasataba@yahoo.com

children, the orbital floor fracture appears more in the form of a linear fracture – "trapdoor" and less in the form of "blowout" fracture with defect. If a fracture is not treated on time, serious complications may occur such as bulb motility with the presence of diplopia and enophthalmos. Contrary to children, in adults, where the bone is more fragile, fractures with comminution are present more frequently [6].

Adequate diagnosis and successful treatment require, in addition to clinical examination, pre-operative computed tomography (CT) with 3D-CT reconstruction of the orbit and ophthalmological Hess-Lancaster test.

Opinions differ as to the indications and time of surgical treatment, operative approach and material used for reconstructive defects in blowout fractures [7]. Good clinical results, in the sense of corrected ocular motility disturbance, diplopia and enophthalmos are achieved if the surgical treatment is carried out within maximum 7 to 10 days after the injury. This standpoint pertains to blowout orbital floor fractures in adults. If the treatment is not carried out within 24 to 72 hours after injury in children, the risk of functional and cosmetic complications is much higher [8, 9].

Case report

An 8-year-old girl underwent treatment of orbital floor blowout fracture one month after the injury. Medical records were obtained heteroamnestically, provided by parents. Two weeks after the injury, motility restriction of the left ocular bulb was observed. The patient was referred for further treatment based on suspicion of a posttraumatic dysfunction of the bulbomotor muscles within blowout fracture.

On clinical examination convergent strabismus was present with disturbance of the left ocular bulb motility (Figure 1).

Fig. 1 – Globes possition before surgical treatment.

Double vision (diplopia) was present on upward and left gaze. Enophthalmos was evident in addition to infraorbital nerve paresthesia. Fracture with an orbital floor defect on the left side was diagnosed by CT with 3D reconstruction (Figure 2).

Fig. 2 – Computed tomography (CT) of the orbit showing left orbital floor facuture.

Ophthalmological examination (Hess-Lancaster test) also confirmed the dysfunction of the bulbomotor muscles with a limited elevation which was pronounced in the medial position and adduction.

After the preoperative preparation, exploration of the orbital wall was performed under general endotracheal anesthesia by subciliary approach. Prolapse-herniation of the periorbital fatty tissue and muscles (*m. rectus* and *m. obliqus inferior)* into the maxillary sinus area was established (Figure 3).

Fig. 3 – Prolapse of the orbital tissue.

The periorbital fatty tissue, muscles and fibrous adhesions were released by blunt preparation taking care of the infraorbital nerve. The defect in the medial orbital floor area was about 0.5 × 1cm. The reconstruction was done by an autogenous mandibular symphyseal graft. The size of the bone graft was 1 × 1.5 cm, 2–3 mm thick, which represented a safety margin in the zone of the permanent teeth germs (Figure 4).

Fig. 4 – Donor site after symphyseal graft harvesting.

The bone graft was placed with the peripheral edges positioned minimum 3 mm from the edge of the orbital floor defect on a firm bone (Figure 5).

Fig. 5 – Insertion of the autogenous mandibular symphyseal graft.

One month following the operation the patient did not feel paresthesia of infraorbital nerve. Diplopia was present even after six months with a manifest alternating strabismus. The patient was referred to the ophthalmologist for continuation of the treatment. Surgical correction of strabismus was performed and bulb motility established (Figures 6 and 7).

Discussion

Similar to adults, orbital floor fracture in children causes the ocular bulb motility disturbance accompanied by diplopia and enophthalmos. The disturbance is a result of incarceration of the orbital contents and bulbomotor muscles. Surgical treatment includes releasing of orbital tissue and muscle entrapment and reconstruction of the orbital floor.

The retrospective study of Gerber et al. [10] including 24 patients with orbital floor fractures (mean age of 13.5 years) show that isolated injury occurred in 14 (58%) cases while the other 10 (42%) had a compound fracture. Also, 11 (46%) patients had a trapdoor fracture and 9 (38%) a blowout fracture. Almost all the patients (n = 22) were treated surgically with the mean time of 4 days after the injury. A better postoperative result was observed in those operated on the first day following the trauma. As a consequence of the injury, diplopia remained in 6 out of 11 patients with trapdoor fracture, as well as limited bulb motility in 3 patients. Out of 9 patients with blowout fracture, secondary enophthalmos was registered in one and paresthesia in three. Regardless of the type of fracture the authors conclude that surgical treatment within the fourth day from the injury gives better postoperative results in children [10].

The other authors present similar results, i.e. surgical treatment of blowout fracture of the orbital floor in children give best results if treatment takes place before the seventh day after the injury [9].

Entrapment of the periorbital fatty tissue and bulbomotor muscles is present in orbital floor fracture. As a result of poor development of the periorbital fatty tissue in children, lesions of muscles (*m. rectus* and *m. obliqus inferior)* are more frequent in children than in adults. Considering that entrapment causes shortening of the muscle which leads to bulb motility disturbance and diplopia, the release of the entrapped muscles is vital as this prevents their dysfunction caused by ischemia and scarring [11]. Reconstruction of the orbital floor also prevents the possibility of secondary enophthalmos which can cause functional as well as cosmetic problems. The bulb motility disturbance after surgery can also be improved conservatively – by functional orthoptic exercises. When orthoptic exercises do not give satisfactory results, surgical correction of bulbomotor muscles is required [12].

Cases describing orbital floor reconstruction in children with an autogenous mandibular symphyseal graft are very

Fig. 6 – Globes possition after retroposition of bulbomotor muscles.

Fig. 7 – Globes possition on upward gaze.

rare although they gave excellent results. Because of the corticocancellous nature of the graft which insures stability and less susceptibility to resorption, the absence of immune reaction, the possibility of occurrence of secondary enophthalmos is minimized. Also, no deformities in the donor region remained and continuity of the mandible is preserved. However, disturbance of sensitivity of the mental nerve (*n. mentalis*) is possible. To prevent that complication, it is extremely important to avoid strong retraction of the mucoperiosteal flap and the soft tissues during surgery [13].

The presented reconstruction of the orbital floor with an autogenous mandibular symphyseal graft, prevented the appearance of secondary enophthalmos, and paresthesia of the infraorbital nerve disappeared a month after the surgical treatment. The vitality of the teeth in the donor region was completely preserved. However, bulb motility disturbance and diplopia remained as the consequence of fracture. Due to the limited elevation of the ocular bulb, orthoptic exercises were conducted, which did not yield results in improved motility and elimination of diplopia. The Hess-Lancaster test was conducted six months after the surgical treatment and has showed a dysfunction of the bulbomotor muscles which resulted in alternating strabismus. Because of that, the patient underwent surgical correction of strabismus by the ophthalmologist. Bulb motility was established by bilateral retropositioning of the internal rectus muscle (*m. rectus internus*). Functional correction also achieved a satisfactory cosmetic result.

Conclusion

Timely diagnosis and surgical treatment of blowout fracture of the orbital floor in children can prevent complications such as disturbance of binocular vision leading to diplopia. Releasing of the periorbital tissue with reconstruction of the orbital floor in children, should be done as soon as possible, within the first 3 days after the injury. Surgery done later may not give satisfactory results in most of the cases. In the presented case, surgical correction of bulbomotor muscles was necessary in order to improve functional quality vision, as well as to provide satisfactory aesthetic appearance.

The orbital floor can be reconstructed successfully with autogenous mandibular symphyseal graft. Enough bone could be collected avoiding injury of teeth roots or teeth buds in the symphyseal region without deformities in the donor region. Complications like mental nerve paresthesia are rare if careful retraction of the mucoperiosteal flap was used.

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CASE REPORT

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Multiple lateral sinus pericranii – A case report

Multipli lateralni perikranijalni sinus

Dražen Ivetić*, Goran Pavlićević*†, Branislav Antić*†, Dejan Kostić‡

***Department of Neurosurgery, ‡Institute of Radiology, Military Medical Academy, Belgrade, Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

Abstract

Introduction. Sinus pericranii is a rare vascular anomaly. It is characterized by abnormal communication between the extracranial and intracranial venous system, usually involving the superior sagittal sinus and occasionally the transverse sinus. Off the midline lesions are extremely rare. Multiplicity, associated venous lakes, venous angioma and lateral location are unusual and unique presentation of sinus pericranii. **Case report.** A case of multiple congenital off-midline sinus pericranii in the left frontotemporal and parietal region is presented. Magnetic resonance imaging showed an extracranial vascular anomaly connected with the intracranial venous system through abnormal diploic or emissary veins. The lesions were removed completely by surgery. **Conclusion.** Sinus pericranii is a rare vascular malformation with unique clinical and radiological features. Sinus pericranii may cause fatal complications, and it must be treated by surgical or endovascular procedures.

Key words:
sinus pericranii; neurosurgical procedures; diagnosis; treatment outcome.

Apstrakt

Uvod. Perikranijalni sinus predstavlja veoma retku vaskularnu anomaliju. Karakteriše se patološkom komunikacijom između ekstrakranijalnog i intrakranijalnog venskog sistema, obično komunicira direktno sa gornjim sagitalnim sinusom, a retko sa transverzalnim sinusom. Lateralne pozicije perikranijalnog sinusa su izrazito retke. Multiple promene, prisustvo venskih jezeraca, venski angiomi i lateralna pozicija predstavljaju neobičnu i jedinstvenu prezentaciju perikranijalnog sinusa. **Prikaz bolesnika.** U radu je prikazan bolesnik sa multiplim kongenitalnim lateralnim perikranijalnim sinusima u levoj čeonoslepoočnoj i parijetalnoj regiji. Magnetna rezonanca ukazala je na ekstrakranijalnu vaskularnu anomaliju koja komunicira sa intrakranijalnim venskim sistemom preko diploične ili emisarne vene. Anomalija je u potpunosti odstranjena hirurškom intervencijom. **Zaključak.** Perikranijalni sinus je veoma retka vaskularna malformacija sa karakterističnom kliničkom i radiološkom prezentacijom. Može prouzrokovati veoma ozbiljne komplikacije, zbog čega mora biti rešen hirurškim ili endovaskularnim procedurama.

Ključne reči:
perikranijalni sinus; neurohirurške procedure; dijagnoza; lečenje, ishod.

Introduction

Sinus pericranii (SP) is a rare vascular anomaly. It is characterized by an abnormal communication between the extracranial and intracranial venous system, usually involving the superior sagittal sinus and occasionally the transverse sinus [1]. Lateral sinus pericranii or sinus pericranii of the transverse sinus is exceedingly rare [1].

The venous collections receive blood from and drain into the intracranial venous sinuses [2]. Usually, this lesion presents as a soft, fluctuant, painless scalp mass that is easily compressed and more prominent with Valsalva maneuvers. In general, sinus pericranii is considered to be a congenital anomaly, but trauma is believed to be a possible causative factor in acquired sinus pericranii [3]. An extremely rare case of multiple congenital and off-midline located sinus pericranii is presented. Multiplicity, associated venous lakes, venous angioma and lateral location are unusual and unique presentation of sinus pericranii [4].

Case report

A 22-year-old man presented with a round soft swelling, nontender scalp masses in the left frontotemporal and another mass in the left parietal region of the cranium (Figure 1). The masses presented in early childhood and in-

Correspondence to: Dražen Ivetić, Department of Neurosurgery, Military Medical Academy, Crnotravska 17, 11040 Belgrade, Serbia. Phone: +381 63 7777770. E-mail: iveticd@yahoo.com

creased in size in time. On physical examination, there was a large soft scalp mass in the left frontotemporal region, and another mass in the left parietal region. The swelling in the frontotemporal region was larger, dimensions 48 × 40 × 6 mm, whereas another mass dimensions were 30 × 18 × 3 mm. Either mass was easily compressible and increased in size on coughing, and other kind of Valsalva maneuvers. The skin over the masses was normal. According to history he had some minor trauma of the cranium during his sport activities, and he registered increasing in size of masses in time. The computed tomography (CT) scan with angiography revealed a soft tissue isodense lesions in frontotemporal and parietal left region of the skull with a focal bony defect and a small transosseus vascular structure were also detected (Figures 2 and 3). The contrast enhanced magnetic resonance (MR) imaging revealed multiple formations with laminar morphology; they had mixed signal intensity on T1-weighted images and appeared hyperintense on T2-weighted and FLAIR images. MR images also detected small transosseus vascular structures which appear like emissary/diploic vein and connect extracranial mass with intracranial venous structures, usually with venous sinuses, in our cases with sphenoparietal sinus in frontotemporal located lesion and with intracranial cortical vein and superior sagittal sinus in parietal region (Figures 4 and 5). MR angiography did not detect any other intracranial vascular anomaly. According to the history, physical examination and MR images, we concluded that it was the epicranial vascular venous anomaly of the sinus pericranii type. Due to the risk of future complications the patient elected to undergo surgery. The operation was performed under general anestesia. The anomaly was resected by soft tissue and emissary vein dissection, without craniectomy, and bone venous channels, including emissary vein, was closed with bon wax (Figures 6 and 7). The same procedure was performed in both anomalies. Histopathology examination was made and cavernous vascular channels with thin vascular endothelium were detected, which is typical for congenital sinus pericranii. Two months after the operation, control MRIs showed a total extirpation of anomalies and no signs for rest and/or recidive of the illness (Figure 8).

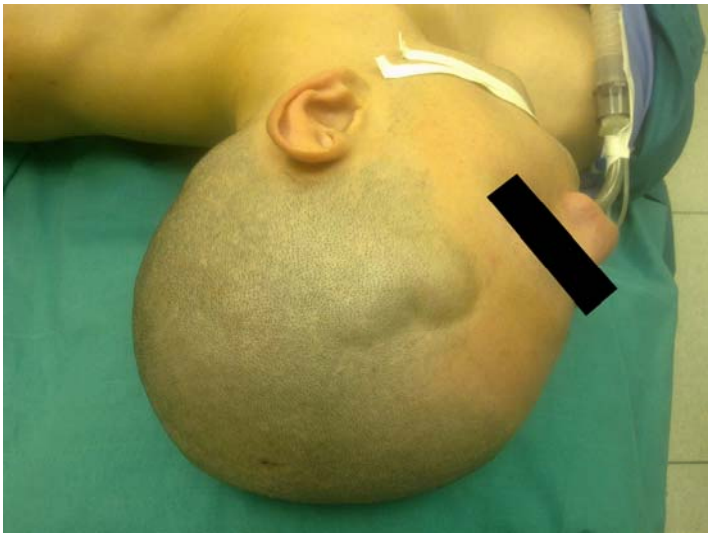

Fig. 1 – Sinus pericranii – round soft swelling scalp mass.

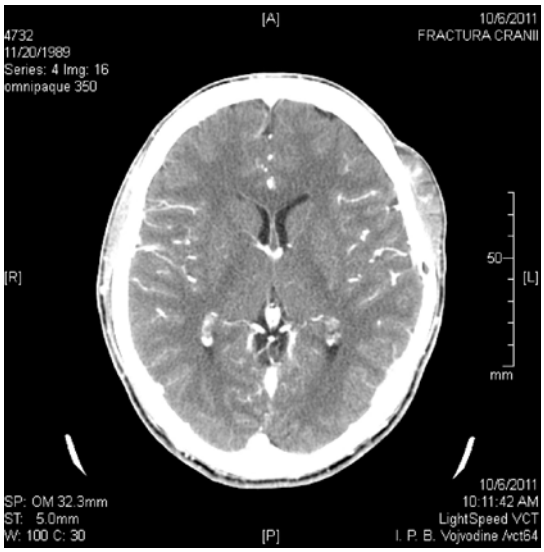

Fig. 2 – Computed tomography showing in isointense soft tissue mass.

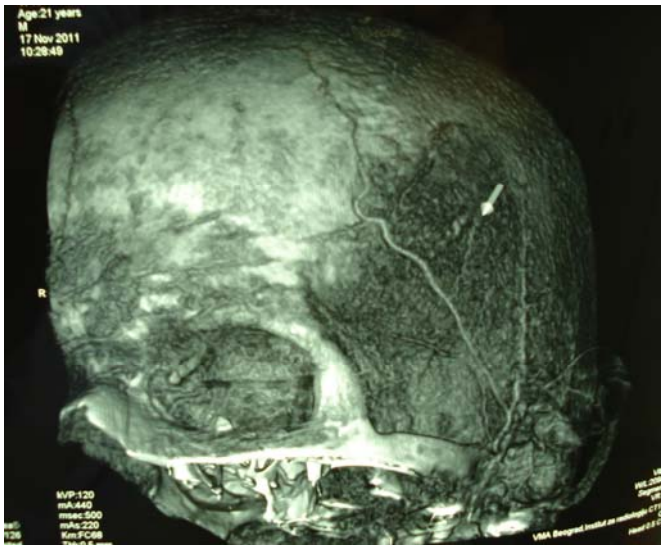

Fig. 3 – Multislice computed tomography (MSCT) angiography with reconstruction revealed a transosseus vessel.

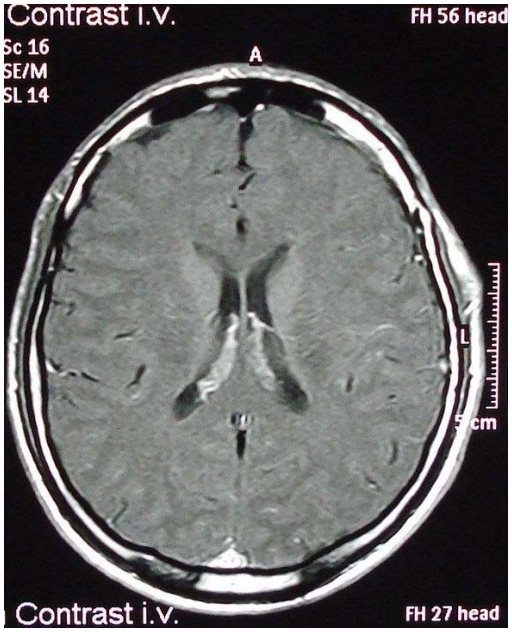

Fig. 4 – Magnetic resonance (MR) image showing the transosseus vessel (emissary or diploic vein) which connects intracranial venous system with extracranial lesion.

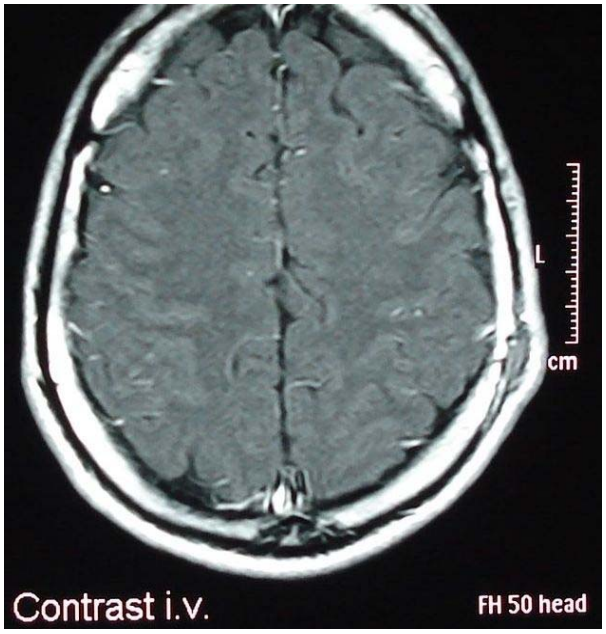

Fig. 5 – Magnetic resonance (MR) image showing sinus pericranii and the transosseus vessel in parietal region.

Fig. 6 – Intraoperative picture of large sinus pericranii.

Fig. 7 – Intraoperative picture after removal of sinus pericranii – the bone channel with the emissary vein closed with bone wax.

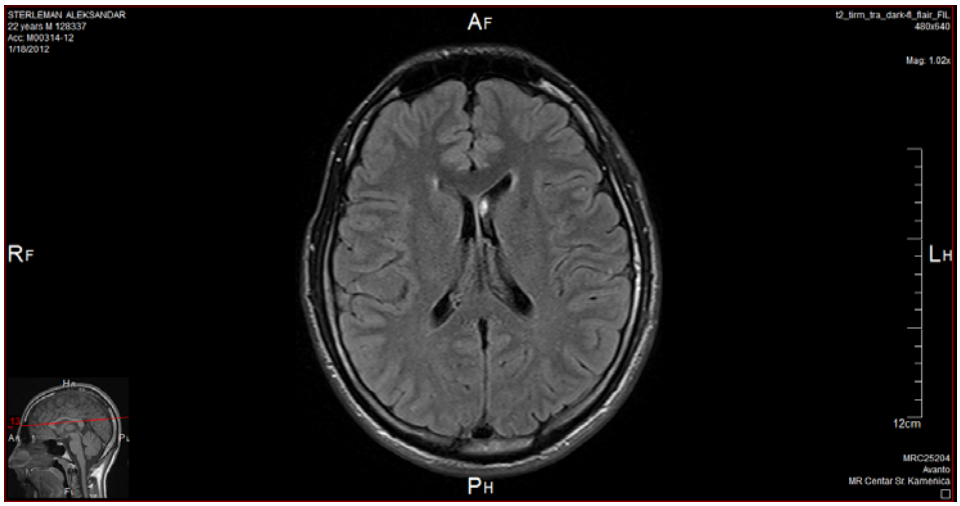

Fig. 8 – Postoperative magnetic resonance (MR) image – total removal of the lesion.

Discussion

Sinus pericranii was first described by Hecker in 1845 as “varix spurious circumscriptus venae diploicae frontalis” [1]. In 1850, Stromeyer referred this anomaly as a "subperiosteal blood-filled cyst of the skull communicating with an intracranial sinus" and proposed the term "sinus pericranii" [5]. Sinus pericranii is a vascular lesion, consistent with an abnormal transdiploic connection between intracranial and extracranial venous circulation [6]. They usually increase in size on Valsalva manoeuvre and reduce on nondependent position; they disappear with compression [1]. According to Ota et al. [2], sinus pericranii is a collection of venous blood vessels without tunica muscularis or "venous hemangioma". Sinus pericranii can appear at any age, usually under 30 years, common in males and although usually asymptomatic, may present with nausea, vomiting and vertigo [7, 8]. It is most commonly located on the frontal region close to the midline connected to the superior sagittal sinus through a skull defect (Table 1) [6]. Off-midline and multiple lesions are extremely rare. Its multiplicity, association with dural venous lakes, and developmental venous anomaly such as a venous angioma, and its peritorcular and lateral location are unique presentation [4]. The pathogenesis and etiology of this peculiar disorder is not definitively known. Mainly congenital, traumatic and spontaneous causes are described for the development of sinus pericranii [8]. Some authors suggest an acquired pathophysiology because of the development of sinus pericranii after head trauma, skull fracture, emissary vein tear, or birth trauma [9, 10]. The main difference between spontaneous and traumatic lesions is the presence of endothelial lining. The presence of connective tissue suggests a traumatic origin, whereas the presence of vascular endothelium indicates a congenital or spontaneous origin [11]. Sinus pericranii is usually asymptomatic and the problems are cosmetics. Rarely there may be headache, vertigo and fatal complications such as hemorrhage, thrombosis or air embolism [1, 2, 11]. The diagnosis of sinus pericranii can be made by the appearance of soft, fluctuant mass located near the intracranial sinus which can vary in size with change in the intracranial pressure. It can be investigated by X-ray, CT scan, MR imaging and/or digital subtraction angiography. MR imaging study with contrast medium administration will demonstrate a soft-tissue mass of mixed signal intensity with the areas of flow void, and usually demonstrate the diploic vein which connects intracranial sinus with the abnormal extracranial vessels. Today, according to excellent opportunity of MR imaging and MR angiography, digital subtraction angiography is rarely necessary, except in cases when sinus pericranii is associated with brain vascular anomaly. The differential diagnosis include leptomeningeal cyst, meningocele, encephalocele, epidermoid tumor, cavernous haemangioma, scalp areteriovenous malformation. The diagnosis of sinus pericranii and its differentiation from scalp arteriovenous malformation can be difficult. Strictly speaking, sinus pericranii is a collection of nonmuscular venous blood vessels tightly adhering to the outer surface of the cranium and communicating directly with an intracranial venous sinus [1, 12]. Treatment for sinus pericranii has mainly been recommended for cosmetic reasons, prompt treatment after diagnosis is required for prophylactic purposes to prevent complications such as thrombosis, traumatic air embolism or massive hemorrhage [1, 11]. Surgery is the usual method of treatment. In surgical treatment, the goal is to resect the extracranial venous package and ligate the emissary communicating vein [11]. This can be done by craniectomy or by dissection of soft tissue and vein without craniectomy, like in our case. Endovascular treatment using transvenous route and percutaneous direct puncture has also been described [13, 14]. Although spontaneous regression of sinus pericranii has been reported, most patients require removal of the sinus and blocking the communicating veins [15–17].

Table 1

The commonest location of the sinus pericranii lesions [6]

Location	Number of cases	Percentage (%)
Frontal	46	43
Parietal	39	36
Occipital	8	7.4
Frontoparietal	5	4.6
Parieto-occipital	4	3.7
Occipito-temporal	4	3.7
Temporal	2	1.9

Sinus pericranii is a rare vascular malformation with unique clinical features. Sinus pericranii may cause fatal complications, and it must be treated by surgical or endovascular procedures. Exceptionally, sinus pericranii may have a prominent role in the venous drainage of the intracranial compartment, and represents the main venous drainage of the underlying brain in case of bilateral occlusion of the jugular veins, and no surgical nor endovascular treatment are recommended [18, 19].

Conclusion

Sinus pericranii is a rare vascular malformation with unique clinical and radiological features. Sinus pericranii may cause fatal complications, and it must be treated by surgical or endovascular procedures.

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LETTER TO THE EDITOR

Legionnaires' disease: Is it really that rare in Serbia?

Da li je legionarska bolest zaista retka u Srbiji?

To the Editor:

These hot summer days, when mass use of air conditioning systems is increased, we aimed to put into attention the problem of the disease, which escalates in importance as a cause of severe morbidity and mortality in many parts of the world. Is Legionnaires' disease, which is an important public health problem in many countries, really that rare in Serbia? The illness usually presents as a severe pneumonia and may be accompanied by systemic symptoms. It results primarily from exposure to aerosolized water contaminated with Legionella. Known risk factors for the disease are increasing age, male sex, chronic underlying lung disease, tobacco smoking, diabetes and a variety of conditions associated with immunodeficiency [1, 2]. Its milder form is also known as Pontiac fever resembling an influenza. An American research showed that residents of long-term care facilities are at higher risk for Legionnaires' disease than the general population [3].

Legionella spp. are Gram-negative bacteria found worldwide in fresh water environments and tend to contaminate and thrive in man-made water systems [4]. Historically, the name of *Legionella* spp. and related disease are derived from an event – a large outbreak of pneumonia caused by a previously known organism. It happened 40 years ago in a hotel in Philadelphia (PA, USA), affecting 182 persons (29/182 died). All of them were participants of a convention of the American Legion – a United States wartime veteran service organization [5].

In the United States of America, the number of reported cases of legionellosis more than tripled between 2001 and 2012 [6]. The disease deserved a higher public health priority and guidance across public health agencies for its primary prevention has been strengthened. A formal and comprehensive review of national public health guidelines for prevention of legionellosis is recommended [6].

In December 2013 issue of the European Respiratory Journal, Editorial referred to Legionnaires' disease in Europe [7]. A map was provided to show a known distribution of legionellosis (Figure 1). While the incidence rate of the disease largely varies in western European countries, the authors ask if all is quiet on the eastern front. While Slovenia, one of the countries with presented highest incidence rates (more than 15 cases *per* million), and Croatia, reported outbreaks of the disease, no such event has been reported to happen in our settings. Performed diagnostic methods have differed among countries as well with the reference method (culture) used in only 12% of all European cases reported in 2011 [8].

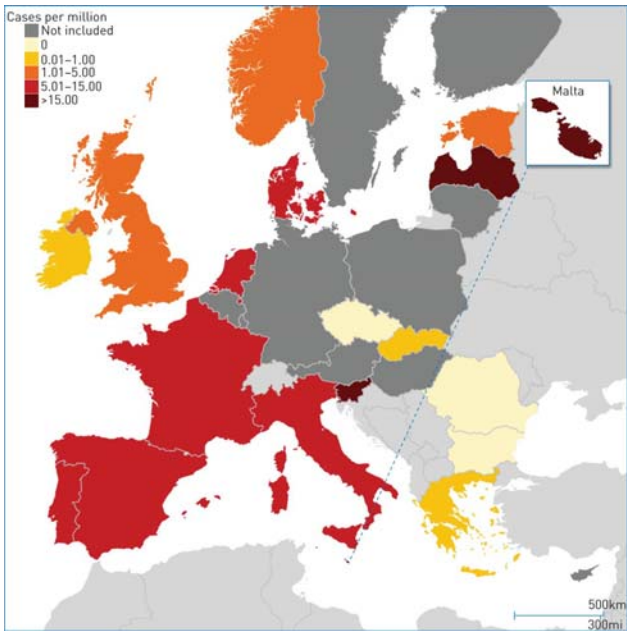

Fig. 1 – Large differences in Legionnaires' disease notification rates among European Union countries.
(Reproduced with permission of the European Respiratory Society. Eur Respir J December 2013; 42:1454-1458; doi: 10.1183/09031936.00089113).

Not only general practitioners and pulmonologist should be aware of the disease. All the other clinicians could be interested in the latest case report, which showed that Legionnaires' disease can masquerade acute pyelonephritis, with the complete absence of respiratory symptoms on the very beginning 9. A 45-year-old man was admitted to hospital with dysuria and the right loin pain and diagnosed with Legionnaires' disease two days after presenting to hospital. He then became critically ill with headache, fever, breathlessness, and respiratory failure with decreasing oxygen saturations. Fortunately, treatment with ciprofloxacin and rifampicin led to a full recovery.

When it comes to treatment, antibiotics with good intracellular action are most effective against any form of legionel-

losis. Azithromycin and levofloxacin are usually considered as the best first-line option [10, 11]. Having in mind doctrinal approach to treatment of community acquired pneumonia, many cases, especially of mild disease, have a chance to be cured even without proof of *Legionella* as a causative agent. Our 55-year-old patient, servicer of cooling towers in Belgrade, who might suffer from a typical Legionnaires' pneumonia, cured and never tested for *Legionella pneumophila*, could be a good example for that. On the other hand, mortality from Legionnaires' disease remains substantial (<10%), even in patients who receive appropriate treatment [12]. This appoints to the importance of primary prevention measures of the disease.

The results of a recent review reveal that the most guidelines emphasize adequate design and maintenance of water systems and water temperatures, but guidance regarding routine preventative environmental testing for Legionella is not uniform if it exists at all [3]. The World Health Organization recommends routine environmental testing and some European countries such as Ireland, France and the Netherlands perform it. The testing is also performed in the Austrian capital Vienna, then South Africa and Queensland, Australia [7]. Recommendations of two different health protection agencies in the same American country happen to vary and the variations in frequency of testing for *Legionella* in water systems are also found.

Knowing all these, one could not but ask how frequently legionellosis might appear in Serbia. The answers to some other questions related to domestic settings would be interesting to know, as well. The first one arisen from the 16-year distance: Were the small outbreaks of "influenza" that happened in many underground collective shelters in Belgrade in early spring 1999 – outbreaks of Legionnaires' disease, or at least its milder form – Pontiac fever? Could the ventilation systems, out of use for years, be properly prepared for sudden use, and were *Legionella* spp. the agents coming from the systems? Finally, how effective is the maintenance of currently used (especially central) ventilation systems and cooling towers in terms of *Legionella* spp?

The actual degree of *Legionella* spp. presentation in water systems in Serbia is not known. Research to inform recommendations on the usefulness of routine environmental testing and other measures for the primary prevention of legionellosis are needed. According to experience of other countries,[7] cooling towers, hot and cold water systems and recreational pools and spas should be given priority in the control. In the meantime, the accepted guidance for antibiotic treatment of community acquired pneumonia is expected to cure some of sporadic cases of Legionnaires' disease. The fact that the cases mainly stay without diagnostic confirmation for many reasons, may strengthen the idea of "quiet" epidemiologic situation with legionellosis in our settings.

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Dragica P. Pešut, Jelena V. Vasić
Faculty of Medicine, University of Belgrade, Belgrade, Serbia; Teaching Hospital of Pulmonology, Clinical Center of Serbia, Belgrade, Serbia,

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