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Personnel of the State Hospital in Skopje in 1936. In the photo, Dr. Viktor Šošić, the head of the Hospital in the period 1937–1941, is sitting in the centre of the first row (see the article by Verica Josimovska, pp. 997–1003).

Osoblje Državne bolnice u Skoplju 1936. godine. Dr Viktor Šošić, upravnik bolnice u periodu 1937–1941. sedi u prvom redu u sredini (vidi članak Verice Josimovske, str. 997–1003).

ORIGINAL ARTICLES

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The role of three-phase ^{99m}Tc-MDP bone scintigraphy in the diagnosis of periprosthetic joint infection of the hip and knee

Uloga trofazne scintigrafije kostiju sa ^{99m}Tc-MDP u dijagnozi periprotetske infekcije kuka i kolena

Dragan Pucar*, Zoran Janković*†, Zoran Baščarević‡, Srdjan Starčević†§, Milica Čizmić†‖, Marija Radulović*

Military Medical Academy, *Institute of Nuclear Medicine, §Clinic for Orthopedic Surgery and Traumatology, ‖Clinic for Endocrinology, Belgrade, Serbia; University of Defence, †Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; University of Belgrade, Faculty of Medicine, ‡Institute for Orthopedic Surgery "Banjica", Belgrade, Serbia

Abstract

Background/Aim. In the last five decades primary hip and knee arthroplasty is the most common and effective surgical intervention worldwide. Infection, although unfrequented, is the most serious complication. Nuclear medicine imaging, not affected by metallic hardware, is the current imaging modality of choice for the evaluation of suspected joint replacement infection. The aim of this study was to estimate the diagnostic accuracy of three phase 99m technetium methylene diphosphonate (^{99m}Tc-MDP) bone scintigraphy in periprosthetic hip and knee joint infection. **Methods.** Inclusion criteria of patients in the study were suspected knee or hip periprosthetic joint infections. In this study, we examined 45 patients (14 men and 31 women) with 39 hip and 24 knee prosthesis (total 63). In all patients, three-phase bone scintigraphy was performed after intravenous application of 555 MBq of ^{99m}Tc-MDP. The final confirmation of infection was microbiological or pathohistology finding. **Results.** Infection was confirmed in 29 prosthetic joints, in 13 (44.8%) knee and 16 (55.2%) hip joints while there was no infection in 34 prosthetic joints. The connection of different modalities of negative and positive findings ^{99m}Tc-MDP three-phase bone scintigraphy with the final confirmation of infection showed a high statistical significance ($p < 0.001$). Three phase bone scintigraphy showed a high sensitivity of 90% but a modest specificity of 69.7% in the detection of periprosthetic infection with the diagnostic accuracy of 79%. The calculated positive predictive value was 73% but the negative predictive value was high 89%. Our results of three-phase bone scintigraphy with calculated sensitivity, specificity and diagnostic accuracy of 79% are in consent with the majority of published studies, or even slightly better. **Conclusion.** Bone scintigraphy is sensitive in the diagnosis of periprosthetic infection but insufficiently specific. In the detection of periprosthetic infections three-phase bone scan can be used as a diagnostic method of the first line only aimed at its exclusion. The only reasonable use of bone scintigraphy is in combination with other radionuclide methods with high specificity.

Key words:
hip prosthesis; knee prosthesis; infection; diagnosis; sensitivity and specificity; technetium tc99m medronate.

Apstrakt

Uvod/Cilj. U poslednjih pet decenija primarna artroplastika kuka i kolena predstavlja jednu od najčešćih i najefikasnijih hirurških intervencija širom sveta. Infekcija, iako retka, jeste najozbiljnija komplikacija. Nuklearno medicinsko snimanje, koje nije ometeno metalnim hardverom proteskog zgloba, je trenutno modalitet izbora za procenu sumnje na periprotetsku infekciju zgloba. Cilj ovog istraživanja bio je da se proceni dijagnostička tačnost trofazne 99m *technetium methylene diphosphonate* (^{99m}Tc-MDP) scintigrafije kostiju kod periprotetske infekcije kuka i kolena. **Metode.** Kriterijumi za uključivanje bolesnika u studiju bili su postojanje sumnje na periprotetsku infekciju zgloba kuka i kolena. U ovoj studiji smo ispitivali 45 bolesnika (14 muškaraca i 31 žene) sa 39 implanta kuka i 24 proteze kolena (ukupno 63). Kod svih bolesnika bila je urađena trofazna scintigrafija skeleta nakon intravenske aplikacije 555 MBq ^{99m}Tc-MDP. Konačna potvrda infekcije ustanovljena je bio mikrobiološkim ili patohistološkim nalazom. **Rezultati.** Infekcija je bila potvrđena kod 29 protetskih zglobova, 13 (44,8%) kolena i 16 (55,2%) kuka, a isključena kod 34 protetska zgloba. Povezanost raz-

Correspondence to: Dragan Pucar, Military Medical Academy, Institute of Nuclear Medicine, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: draganpucar@yahoo.com

ličitih modaliteta negativnih i pozitivnih nalaza ^{99m}Tc-MDP trofazne scintigrafije kostiju sa konačnom potvrdom infekcije pokazuje visoku statističku značajnost ($p < 0,001$). Trofazna scintigrafija skeleta pokazala je visoku osetljivost od 90%, ali skromnu specifičnost od 69,7% u otkrivanju periprotetske infekcije, dok je dijagnostička tačnost bila 79%. Izračunata pozitivna prediktivna vrednost za metodu iznosila je 73%, ali negativna prediktivna vrednost bila je visoka (89%). Naši rezultati trofazne scintigrafije skeleta sa vrednostima osetljivosti od 90%, specifičnosti od 69,7% i dijagnostičke tačnosti od 79% u saglasnosti su sa većinom objavljenih studija, pa i diskretno bolji. **Zaključak.** Scintigrafija skeleta je osetljiva metoda u dijagnostici periprotetske infekcije, ali je nedovoljno specifična. U otkrivanju periprotetske infekcije, trofazna scintigrafija kostiju može se koristiti kao dijagnostička metoda prve linije samo u cilju njenog isključivanja. Jedino razumno korišćenje scintigrafije kostiju je u kombinaciji sa drugim radionuklidnim metodama visoke specifičnosti.

Ključne reči:
kuk, proteza; koleno, proteza; infekcija; dijagnoza; senzitivnost i specifičnost; tehnecijum tc99^{m} medronat.

Introduction

Over the last five decades primary hip and knee arthroplasty procedures have been among the most common and effective surgical intervention worldwide [1]. The number of performed joint replacements has significantly increased in response to demographic change. The number of total hip arthroplasties (THA) carried out in the USA increased 2.5 times from 200,216 in 1993 to 497,419 in 2005. In the same period, the amount of primary total knee arthroplasties (TKA) grew 1.7-fold [2]. The most implanted artificial joints are hip and knee prostheses (more than 95%). Patients who failed conservative and comprehensive care may be considered for joint replacement surgery. Intractable pain, severe osteoarthritis (OA), and limitation of motion are the main reasons to consider joint replacement surgery. Inability to flex the knee more than 90 degrees considerably limits the functional capacity of rising from a chair. Life expectancy is also an important consideration regarding the longevity of joint replacements and the risk of future loosening or implant failure. However, some patients have prosthesis more than 20 years. The most important contraindications are an acute infection and severe obesity, while relative contraindications include: poor health and high anesthetic risks, poor bone stock, significant deformities, severe neuropathy. Although hip or knee joint replacement is highly successful surgical intervention, long-term outcomes continue to suffer from aseptic loosening and periprosthetic joint infection (PJI). More than 25% of all prostheses will demonstrate evidence of loosening, often after a revision arthroplasty [3]. Most authors agree that many cases understood as aseptic failure may be due to an unrecognized infection.

Infection, although unfrequented, is the most serious complication. In patients with primary joint replacement, the infection rate in the first 2 years usually ranges from 0.5 to 2%. Commonly isolated microorganisms are Gram-positive cocci: coagulase-negative *staphylococci, Staphilococcus aureus*, and *enterococci* (65%) [4]. Infection rates after a surgical revision are usually higher (25% to 40%) than after a primary replacement [5]. Important risk factors for arthroplasty are a postoperative surgical site infection, previous arthroplasty, older age, malnutrition, joint disease, obesity, diabetes mellitus, remote infection [6, 7].

Increased erythrocyte sedimentation rate (ESR) and high C-reactive protein (CRP) levels are sensitive for non-specific inflammation but not specific for PJI. Findings such as radiolucency, osteolysis, and migration obtained by plain radiography are not enough sensitive or specific and may be present in both infection and aseptic loosening [8]. Cross-sectional imaging techniques, such as computed tomography and magnetic resonance imaging, are of limited value in the presence of metallic prosthetic implants owing to beam hardening and dephasing artefacts [9].

Nuclear medicine imaging, which is, in general, not affected by metallic hardware, is the current imaging modality of choice for the evaluation of suspected joint replacement infection [10]. One of the oldest radionuclide imaging modalities used for this purpose is bone scintigraphy (BS) [11]. BS is widely available, relatively inexpensive, easily performed, and quickly completed. Uptake of bone-seeking tracer such as 99m technetium methylene diphosphonate (^{99m}Tc-MDP), which accumulates on the surface of the bone mineral matrix, depends on blood flow and especially on the rate of new bone formation [12]. The cause of accelerated new bone formation may be seen as increased periprosthetic activity on BS in infection, but also in the postoperative physiological bone remodeling, as well as pathological conditions such as fracture, heterotopic ossification and aseptic loosening. Persistent uptake more than 12 months after arthroplasty is usually abnormal. In general, BS is highly sensitive but not very specific for PJI. The literature on the diagnostic efficiency of bone scintigraphy shows a considerable variability due to several factors, such as the use of different scan interpretation criteria (quantitative *vs* qualitative approach) and performing a three-phase study instead of only a delayed bone scan.

The aim of this study was to estimate the diagnostic accuracy of three phase ^{99m}Tc-MDP bone scintigraphy in periprosthetic hip and knee joint infection.

Methods

A total of 45 patients (14 men and 31 women) with 39 implanted hip and 24 knee prosthesis (total 63) were included in this study. The mean age of patients was 68.6 (range 43–82) years. The study was performed during a 5-months period from August 2015 to January 2016. Criteria for including patients in this study were suspected PJI based on: painful prosthetic joint (especially in locomotion but also in peace), restricted joint movements and increased value of erythrocyte sedimentation rate and/or high levels of C-reactive protein. All patients underwent plain radiography.

In all patients, after intravenous injection of 555 MBq ^{99m}Tc-MDP, three-phase bone scintigraphy was performed on ADAC vertex gamma camera using a large field of view dual detectors filtered with low energy all-purpose collimator. Imaging was performed at various times in supine position: 0–5 minute (15 frames in the first minute and 6 frames in further 4 minutes), 6–10 minute (one frame with 300 seconds duration) and late static imaging after 3 hours. Scintigrams were analyzed visually without any quantification.

The bone scintigraphy findings were semiquantitative: value 1 – normal, 2 – borderline normal, 3 – borderline abnormal, and 4 – clearly abnormal. The final diagnosis of infection was confirmed by microbiological or histopathological results.

For the purpose of an initial analysis and description, usual descriptive statistics were used (mean age, standard deviation, number and percentage of frequency characteristics within a given set). To test the statistical significance of differences the results of bone scintigraphy were compared with the gold standard. The χ^2 test was used and statistically significant differences were evaluated at a level of at least $p < 0.05$.

A software package SPSS version 18.0 (USA) was used. Sensitivity, specificity and predictive values of bone scintigraphy were calculated using the standard formulas.

Results

Descriptive parameters of patients are shown in Table 1.

The relationship between the results of three-phase ^{99m}Tc-MDP bone scintigraphy and confirmed infection is shown in Table 2.

Infection was confirmed in 29 prosthetic joints – 13 (44.8%) knee and 16 (55.2%) hip joints, while not found in 34 patients.

The association between different modalities of negative and positive findings of ^{99m}Tc-MDP three phase bone scintigraphy crossed with the final confirmation of infection showed high statistical significance ($p < 0.001$).

Table 1

Descriptive parameters of the patients

Parameters	Values
Age (years), mean ± SD	68.64 ± 9.56
Gender, n (%)	
male	14 (31.1)
female	31 (68.9)
Confirmation of infection (the gold standard), n (%)	
confirmed	16 (35.6)
unconfirmed	29 (64.4)
Total	45 (100.0)

SD – standard deviation

Table 2

The relationship between the results of ^{99m}Tc-MDP and confirmed infection

Results of ^{99m}Tc-MDP scyntigraphy	Infection		Total
	No	Yes	
Negative finding			
n	9	0	9
MDP finding (%)	100.0	0.0	100.0
Infection (%)	26.5	0.0	14.3
Borderline negative finding			
n	14	3	17
MDP finding (%)	82.4	17.6	100.0
Infection (%)	41.2	10.3	27.0
Borderline positive finding			
n	5	6	11
MDP finding (%)	45.5	54.5	100.0
Infection (%)	14.7	20.7	17.5
Positive finding			
n	6	20	26
MDP finding (%)	23.1	76.9	100.0
Infection (%)	17.6	69.0	41.3
Total			
n	34	29	63
MDP finding (%)	54.0	46.0	100.0
Infection (%)	100.0	100.0	100.0
Significance	$\chi^2 = 23.498$; $p < 0.001$		

^{99m}Tc-MDP – technetium methylene diphosphonate.

Three phase bone scintigraphy had a high sensitivity of 90% but a modest specificity of 69.7% in detecting periprosthetic joint infection, with a diagnostic accuracy of 79%. Calculated positive predictive value (PPV) was 73%, but the negative predictive value was high – 89%.

Clearly positive findings of ^{99m}Tc-MDP three-phase bone scintigraphy in detecting periprosthetic joint infection of the knee are shown in Figure 1.

The high negative predictive value of 89% resulted in a frequent number of true negative findings (Figure 2).

Modest specificity of 69.7% in detecting periprosthetic joint infection resulted in false positive findings (Figure 3).

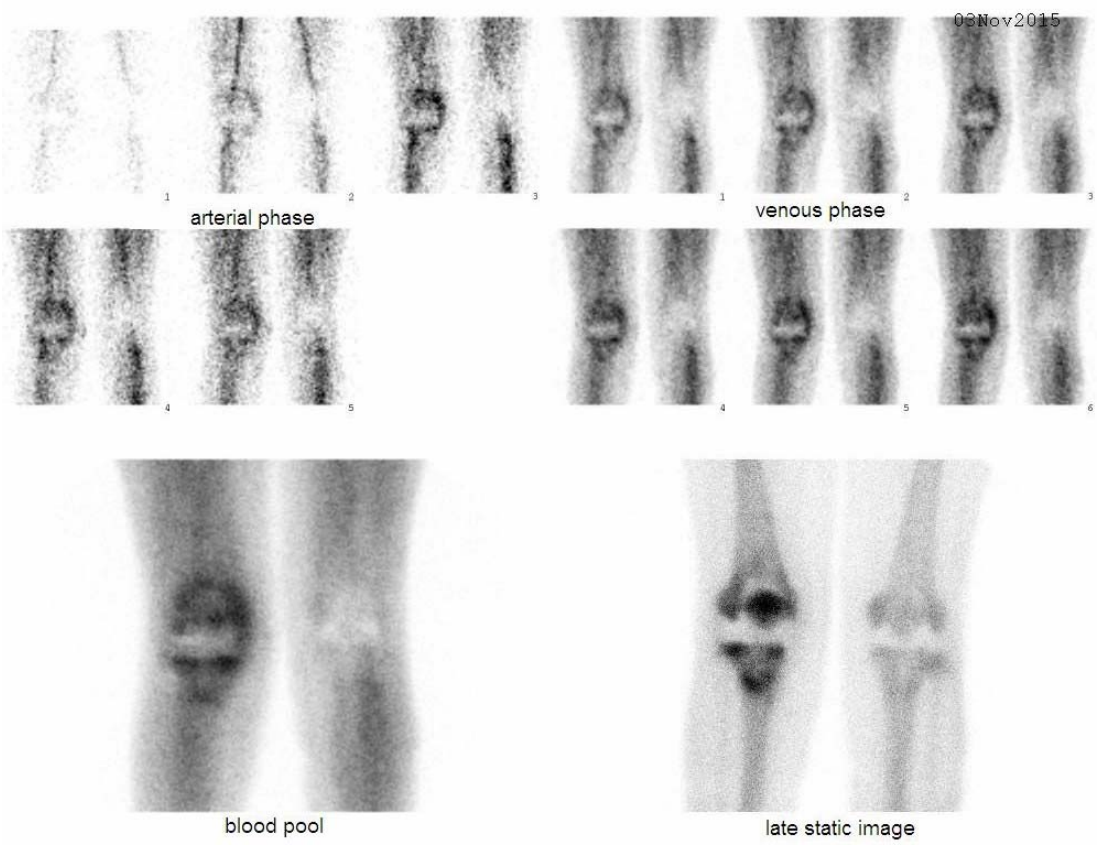

Fig. 1 – A 72-year-old female patient with bilateral knee joint replacement. In the region of right knee replacement extensive increased activity is observed in all three phase of bone scintigraphy indicating infection.

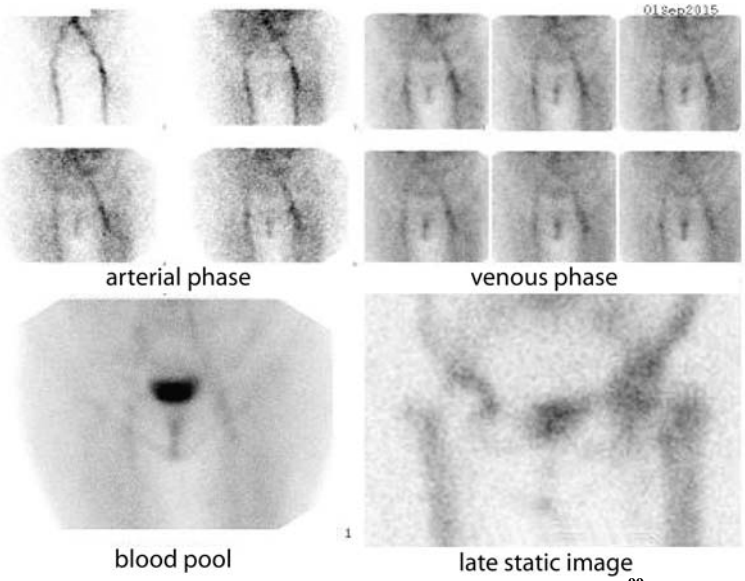

Fig. 2 –The results of three-phase 99m technetium methylene diphosphonate (^{99m}Tc-MDP) bone scintigraphy are negative at all stages of the imaging. Definite result: inadequate joint biomechanics due to adductor muscle weakness – aseptic.

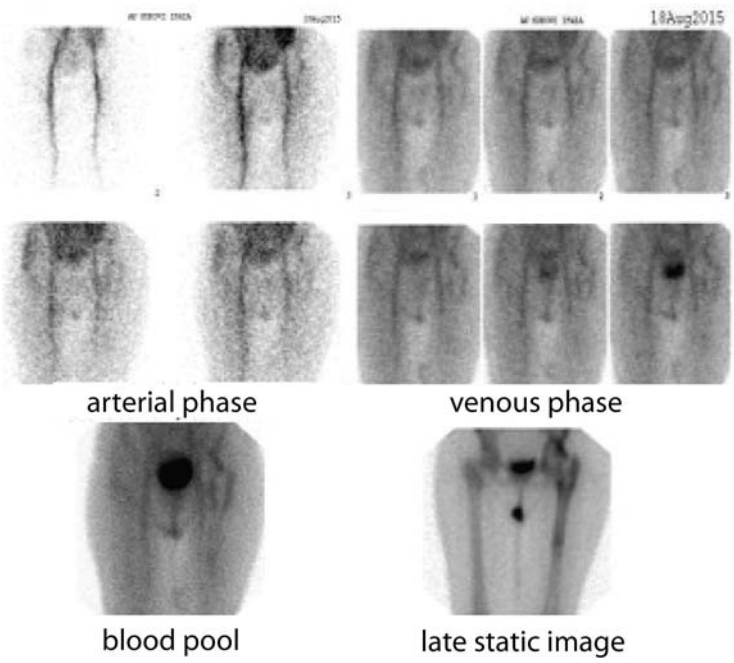

Fig. 3 – Positive findings at all stages of the imaging (increased uptake in the region of the greater trochanter and discreetly increased along the femoral stem). Definitive result: aseptic loosening.

Discussion

Assessment of the presence of periprosthetic joint infection using bone scan is considered problematic by many authors. Persistently increased binding of radiopharmaceuticals in the first year after application of arthroplasty, suggesting accelerated bone remodeling, occurs in approximately 60% of cases around the femoral component of the prosthesis and in about 90% around the tibial component [13–15].

Most authors agree that the accuracy of bone scintigraphy in the evaluation of possible causes of painful prosthesis varies between 50 and 70% and that it is too low value for its clinical use unless combined with some other radionuclide imaging method of higher specificity [16].

However, according to the recent study of Zajonz et al. [2], which included 320 prosthetic hip and knee joints, the obtained accuracy of three-phase bone scintigraphy was as much as 90%. Probably the greatest advantage of using this method alone is its high negative predictive value of 95%, making it a screening method with a high probability of periprosthetic joint infection of the hip and knee [17]. In that case should not be forgotten that the negative predictive value is lower in the first year after arthroplasty and that two-thirds of infections occur precisely in that period [8].

Early efforts made by some authors in differentiating aseptic loosening from periprosthetic joint infection by analyzing the increased uptake were very interesting. Williamson et al. [18] found in 1979 that the focal zone of increased radiopharmaceutical uptake indicated aseptic loosening, while diffused increased radiopharmaceutical binding around the femoral or acetabular endoprosthesis indicated infection. Two years later, Williams et al. [19] reported that diffusely increased radiopharmaceutical binding around the implant might be an indication of infection and aseptic loosening. Aliabadi et al. [20] published in 1989 that bone scan could accurately detect loosening, but not separate aseptic loosening from the loosening caused by the presence of infections. Palestro et al. [21] have long argued that bone scintigraphy is neither enough sensitive nor specific method in the diagnosis of a periprosthetic knee infection. This statement was supported by some recent studies, except when it comes to its sensitivity. The accuracy of this method is slightly increased if carried out as a three-phase scintigraphy. In 2008 Love et al. [16], considering 150 prosthetic lower limb joints (96 joints of the hip and 54 joints of the knee), obtained a sensitivity of 76% and specificity of 51% in the diagnosis of infection. Diagnostic accuracy of 50% was obtained if only delayed static scintigraphy was done and increased to 62% when performed as a three-phase bone scintigraphy.

Our results of three-phase bone scintigraphy (diagnostic sensitivity 90%, specificity 69.7% and accuracy 79%) are in consent with the majority of published studies and even slightly better.

Conclusion

Bone scintigraphy is sensitive in the diagnosis of periprosthetic joint infection, but due to its unacceptable specificity, it is not a method that could work independently and can not be used alone for detecting a periprosthetic joint infection. For that purpose, the three-phase bone scan can be used as the first line diagnostic method just for the definitive exclusion of infection. The only realistic possibility to use bone scintigraphy to detect a periprosthetic joint infection is its combination with other radionuclide methods of high specificity.

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Risk factors for levodopa-induced dyskinesia in Parkinson's disease patients

Faktori rizika od diskinezija uzrokovanih levodopom kod obolelih od Parkinsonove bolesti

Gordana Djurić*, Vladana Marković†, Tatjana Pekmezović‡, Aleksandra Tomić†, Nikola Kresojević†, Vladimir Kostić†, Marina Svetel†

***Railway Health Care Institute, Belgrade, Serbia; Clinical Center of Serbia, †Neurology Clinic, Belgrade, Serbia; University of Belgrade, Faculty of Medicine, ‡Institute of Epidemiology, Belgrade, Serbia**

Abstract

Background/Aim. Levodopa, the precursor of dopamine, is a substitute therapy for Parkinson's disease. Long-term application of levodopa causes fluctuation in motor response and the occurrence of involuntary movements or dyskinesia. The aim of this study was to assess the risk factors for dyskinesia in Parkinson's disease (PD) patients undergoing treatment with levodopa. **Methods.** We included 177 consecutive outpatients with PD, who had been undergoing treatment for at least six months. A semi-structured interview was used to collect demographic and clinical data as well as a number of clinical scales. **Results.** Patients with dyskinesia (n = 90) were younger at disease onset and had longer disease duration. They had higher Unified Parkinson's Disease Rating Scale (UPDRS) scores and more frequently had other motor complications, such as wearing-off and freezing of gait, as well as non-motor ones, such as psychosis. They took higher levodopa doses and levodopa equivalent doses and were on levodopa therapy for a longer period of time. Multivariate analysis yielded that independent risk factors for dyskinesia were: disease duration of longer than 10 years [relative risk (RR = 2.90), 95% confidence interval (CI) 1.19–7.10; $p = 0.019$), dopaminergic treatment duration of longer than 94 months (RR = 3.21, 95% confidence interval (CI) 1.05–9.87; $p = 0.041$) and levodopa dosage of higher than 537 mg (RR = 3.62, 95%IP 1.57–8.35; $p = 0.002$). **Conclusion.** We highlight the importance of known risk factors for dyskinesia and their occurrence in the context of advanced, complicated disease.

Key words:
parkinson disease; disease progression; levodopa; dyskinesia, drug-induced; risk factors; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Levodopa, prekursor dopamine pretstavlja supstitucionu terapiju Parkinsonove bolesti. Dugotrajna upotreba levodopa uzrokuje fluktacije motornog odgovora i pojavu neželjenih pokreta ili diskineziju. Cilj rada bio je procena značaja faktora rizika od razvoja diskinezija uzrokovanih levodopom kod obolelih od Parkinskonove bolesti (PB). **Metode.** Istraživanje je obuhvatilo 177 bolesnika sa PB, regrutovanih na Neurološkoj klinici Kliničkog centra Srbije (Beograd), čije lečenje je trajalo duže od šest meseci. Za ispitivanje obolelih korišćene su standardizovane skale za kvantifikovanje PB, ali i detaljni posebno konstruisani upitnik za ovu studiju sa demografskim i kliničkim podatcima. **Rezultati.** Bolesnici sa diskinezijama (n = 50) bili su mlađi na početku bolesti i imali su duže trajanje bolesti. Takođe, oboleli sa diskinezijama imali su veće skorove na Unified Parkinson's Disease Rating Scale (UPDRS), učestalije motorne komplikacije – fluktuacije terapijskog odgovora (bilo u formi skraćenja trajanja pojedinačnih doza ("wearing off") ili motornih blokova hoda ("freezing") i češće su ispoljavali medikamentoznu psihozu. Oni su bili i na statistički višim dozama levodope, ali i višoj ekvivalentnoj dozi levodope, pri čemu je njihovo lečenje trajalo značajno duže. Multivarijantna analiza pokazala je da su nezavisni prediktori pojave diskinezija u grupi bolesnika sa PB bili: dužina bolesti preko 10 godina [relativni rizik (RR = 2.90, 95% interval poverenja (IP) 1.19–7.10; $p = 0.019$), dužina terapije više od 94 meseci (RR = 3.21, 95%IP 1.05–9.87; $p = 0.041$), dnevna doza levodope u trenutku ispitivanja veća od 537 mg (RR = 3.62, 95%IP 1.57–8.35; $p = 0.002$). **Zaključak.** Potvrđen je značaj poznatih faktora rizika od razvoja diskinezija u odmakloj fazi Parkinsonove bolesti.

Ključne reči:
parkinsonova bolest; bolest, progresija; levodopa; diskinezija izazvana lekovima; faktori rizika; ankete i upitnici.

Correspondence to: Marina Svetel, Railway Health Care Institute, Dr Subotica 6, 11 000 Belgrade, Serbia.
Phone: +381 11 268 5596. E-mail: marinasvetel@gmail.com

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder whose key motor manifestations (e.g., bradykinesia, rigidity, tremor at rest) mainly result from the degeneration and death of dopaminergic neurons in *substantia nigra pars compacta*, with effects of a hypodopaminergic state and disturbed dopaminergic neurotransmission in the striatum [1]. Levodopa, the precursor of dopamine, is a substitute therapy, which, following more than half of a century since its introduction, continues to be the basis of PD treatment [2] because it enables control of the basic motor symptoms which have occurred due to lack of dopamine. In idiopathic PD a good response to levodopa is the rule, which has also become a part of diagnostic criteria for this disease [3]. However, the long-term application of levodopa has been followed with, amongst others, the occurrence of motor complications which generally involve the fluctuation of motor response and the occurrence of involuntary movements or dyskinesia [4, 5].

The first description of dyskinesia was given by Cotzias et al. [6] in affected patients who were initially successfully treated with levodopa. It has been shown that the dyskinesia additionally disables the affected individuals, sometimes even more so than the basic disease, and moreover, it limits the ability for treatment with dopaminomimetic drugs [7]. Even though it is considered that dyskinesias are unavoidable at some point of disease progression, not all affected individuals will develop them at the same time, nor will their intensity be uniform [8]. As a risk factor for the development of dyskinesias, notables are: an earlier onset of PD, a longer duration of disease, longer exposure to levodopa, higher total dose of levodopa, female sex, and likely, different genetic factors [9, 10]. Their recognition is especially important for the planning of individual therapies [11].

The aim of our study was to identify demographic and clinical factors of risk which most significantly impact the appearance of dyskinesia during the chronic use of levodopa in our population of affected individuals with PD.

Methods

Our research included 177 patients with PD, who were recruited at the Neurology Clinic of the Clinical Center of Serbia from 2009 to 2011. The PD diagnosis was made according to the afore-published criteria of Ward and Gibb [3]. Demographic and clinical data were obtained through the use of a detailed, specially designed (for this study) questionnaire which, among other facts, included the age of the patient at onset of PD, the initial dose of levodopa, the latency period between the first PD symptoms and the beginning of levodopa therapy, the time of the occurrence of dyskinesia, and the therapy that the patient underwent at that time, use of other antiparkinsonian drugs and levodopa-equivalent doses (calculated per the method described by Tomlinson et al. [12]).

The PD severity was assessed with reference to the Unified PD Rating Scale (UPDRS) [13], and in order to determine the stage of disease, the Hoehn and Yahr [14] (HY) system was applied. The functionality and performance of daily activities were assessed using the modified Activities of Daily Living Scale [15]. Dyskinesia was quantified per the modified Abnormal Involuntary Movement Scale (AIMS) [16] and Goetz's scale for the Quantification of Dyskinesia [17]. For the assessment of depression and anxiety, the Hamilton Depression Rating Scale (HDRS) [18] and the Hamilton Anxiety Rating Scale (HARS) [19] were used, respectively, and for patient cognitive function, the Mini Mental State Examination (MMSE) [20] was applied. Testing was performed during the "on" phase (the phase in which the optimal drug therapy and motor improvement were achieved in the patient).

Statistical analysis of the acquired data involved the following methods: descriptive statistics, parametric and non-parametric tests, univariate and multivariate logistic regression analysis. For the comparison of continuous variables, we used the analysis of variance, and for the categorized variables, the χ^2 test. The criteria for the multivariate model were defined by the statistical significance of 0.05, obtained from the univariate analysis. As a measure of the effects, relative risk (RR) was used with a 95% confidence interval (95% CI).

Results

In our study 177 PD patients were involved, 118 (65.5%) men and 61 (34.5%) women, of an average age of 58.9 ± 10.9 years (mean value ± standard deviation), with disease onset at the age of 49.0 ± 11.2 years, and a duration of 9.7 ± 6.2 years.

Of the 177 patients in the study group, 90 developed, whereas 87 did not develop dyskinesia (Table 1). Patients in these two groups did not have any differences based on sex, dominant hand, age, education, heredity, and form of PD, or presence of depression symptoms. The statistically significant difference was noted for age at onset of PD, duration of disease and MMSE score (Table 1).

Patients with dyskinesia were at a statistically significant more advanced PD stage, as well as higher scores on the UPDRS, and more problems in daily functioning as measured per the Schwab and England scale (Table 2). This group of affected patients had statistically significant more frequent fluctuations in therapeutic response (either in the form of a shorter duration of individual doses (referred to as wearing off), or the on-off phenomenon. They developed motor blocks while walking (referred to as freezing of gait) more frequently and with a shorter latency from the both disease onset and the moment in which treatment began, in comparison to the patients without dyskinesia (Table 2). Finally, patients with dyskinesia in a statistically significant manner, more often demonstrated medication psychosis.

PD patients with dyskinesia, as compared to those without dyskinesia, in a statistically significant manner, differed with respect to the duration of treatment, application of therapy (more often were on levodopa, amantadine and clozapine, and less often on monoamine oxidase (MAO) B inhibitors) (Table 3). In addition, these patients were on larger

Table 1

Demographic and clinical characteristics of Parkinson's disease (PD) patients (n = 177), with and without dyskinesia

Parameters	PD-Dys+	PD-Dys-	*p*
Patients, n (%)	90 (50.8)	87 (49.2)	0.214
Male, n (%)	57 (63)	59 (68)	0.530
Right-hand dominant, n (%)	88 (98)	84 (97)	0.623
Age (years), $\bar{x}$ ± SD (range)	60.3 ± 8.9 (38–79)	57.3 ± 12.7 (28–82)	0.067
Education (years), $\bar{x}$ ± SD (range)	11.4 ± 3.7 (3–17)	12.4 ± 3.3 (4–17)	0.136
Positive family history, n (%)	20 (22)	13 (15)	0.214
Age at PD onset (years), $\bar{x}$ ± SD (range)	47.3 ± 9.6 (20–66)	50.7 ± 12.4 (27–72)	0.045
Duration of disease (years), $\bar{x}$ ± SD (range)	12.5 ± 6.7 (3–37)	6.7 ± 5.1 (0–25)	0.001
Form of disease, n (%)			0.241
tremor dominant	44 (49)	43 (49)	
akinetic-rigid	24 (27)	20 (23)	
postural instability	12 (13)	11 (13)	
MMSE score, n (%)	27.6 ± 3.1 (14–30)	28.5 ± 2.2 (16–30)	0.033
HDRS score, n (%)	11.7 ± 8.6 (0–39)	10.3 ± 7.2 (0–26)	0.220
HARS score, n (%)	7.7 ± 6.1 (0–21)	6.9 ± 5.8 (0–22)	0.506

PD-Dys+ – PD patients with dyskinesia; PD-Dys- – PD patients without dyskinesia; MMSE – Mini Mental State Examination; HARS – HDRS – Hamilton's Depression Rating Scale; Hamilton's Anxiety Rating Scale; $\bar{x}$ – mean; SD – standard deviation.

Table2

Severity of motor and non-motor symptoms in Parkinson's disease (PD) patients with and without dyskinesia

Parameters	PD-Dys+	PD-Dys-	*p*
H-Y state, $\bar{x}$ ± SD (range)	2.5 ± 0.7 (1.5–4)	2.1 ± 0.5 (1–3)	0.001
UPDRS total score, $\bar{x}$ ± SD (range)	68.9 ± 22.3 (15–125)	48.8 ± 22.2 (7–101)	0.001
SE score, $\bar{x}$ ± SD (range)	72.6 ± 16.1 (3–100)	80.6 ± 13.2 (50–100)	0.001
Fluctuations, n (%)	72 (80)	26 (30)	0.001
latency from PD onset (months), $\bar{x}$ ± SD (range)	77.2 ± 47.3 (0–252)	61.8 ± 50.9 (0–180)	0.179
latency from introduction of therapy (months), $\bar{x}$ ± SD (range)	73.7 ± 46.7 (12–294)	55.2 ± 30.5 (5–144)	0.073
Wearing off, n (%)	60 (67)	21 (24)	0.001
On-off, n (%)	25 (28)	7 (8)	0.001
Motor blockade (freezing), n (%)	50 (56)	22 (25)	0.001
Latency from PD onset (months), $\bar{x}$ ± SD (range)	97.2 ± 59.6 (6–240)	62.8 ± 67.3 (0–288)	0.037
Latency from introduction of therapy (months), $\bar{x}$ ± SD (range)	102.5 ± 53.6 (20–294)	62.7 ± 39.6 (0–168)	0.004
Medication psychosis, n (%)	31 (34)	10 (12)	0.001

PD-Dys+ – PD patients with dyskinesias; PD-Dys- – PD patients without dyskinesia; H-Y – Hoehn-Yahr; UPDRS – Unified Parkinson's Disease Rating Scale; SE – Schwab and England.
$\bar{x}$ – mean; SD – standard deviation.

Table 3

Therapy applied in Parkinson's disease (PD) patients with and without dyskinesia

Parameters	PD-Dys+	PD-Dys-	*p*
Latency (symptoms onset until therapy introduction) (months), $\bar{x}$ ± SD	22.5 ± 25.6	19.2 ± 17.9	0.320
Duration of therapy (months), $\bar{x}$ ± SD	129.5 ± 62.7	58.1 ± 52.8	0.001
Levodopa, n (%)	90 (100)	70 (81)	0.001
Pramipexole, n (%)	36 (40)	28 (32)	0.279
Ropinirole, n (%)	23 (26)	16 (18)	0.250
Bromocriptine, n (%)	15 (17)	5 (6)	0.022
Amantadine, n (%)	64 (71)	25 (29)	0.001
MAO B inhibitors, n (%)	2 (2)	9 (11)	0.024
COMT inhibitors, n (%)	4 (4)	5 (6)	0.693
Clozapine, n (%)	27 (31)	10 (12)	0.003
Anticholinergics, n (%)	13 (14)	6 (7)	0.117

PD-Dys+ – PD patients with dyskinesia; PD-Dys- – PD patients without dyskinesia; MAO-B – monoamine oxidaze B; COMT – catechol-*O*-methyltransferase.
$\bar{x}$ – mean; SD – standard deviation.

doses of levodopa (605 ± 212 mg *vs* 450.7 ± 200.8 mg), and also on higher levodopa equivalent doses (808.3 ± 256.9 mg *vs* 497.3 ± 286.5; $p = 0.000$).

Multivariate analysis demonstrated that independent predictors of the onset of dyskinesia in the group of patients with PD were: duration of disease of more than 10 years (RR = 2.90, 95% CI 1.19–7.10; $p = 0.019$), duration of levodopa therapy of more than 94 months (RR = 3.21, 95% CI 1.05–9.87; $p = 0.041$), daily dose of levodopa at the date of testing of more than 537 mg (RR = 3.62, 95% CI 1.57–8.35; $p = 0.002$), and amantadine therapy (RR = 2.99, 95% CI 1.29–6.89; $p = 0.010$). The results of univariate analysis are not shown.

In regard to certain types of involuntary movements (dyskinesias) that were noted, the most frequent among them were choreatic ones (82.2%), followed by dystonic movements (77.8%), whereas ballistic dyskinesias (8.9%) were least frequent. Dyskinesias were most often presented at the moment when the highest concentration of levodopa was attained after each individual dose ("peak of dose" dyskinesia, 77.8%), in approximately one-half of patients they appeared at the end of the effectiveness of the individual dose of levodopa ("end of dose" dyskinesia, 45.6%), whereas diphasic dyskinesia (at the beginning and at the end of the effectiveness of the individual dose of levodopa) were identified in only 4.4% of the test subjects. Dyskinesias in 73% of the patients worsened their functional abilities, and such disability was heavy in 15.4%, moderate in 46.2% and light in 38.5% of the PD patients. Dyskinesia appeared with a latency of 74.6 ± 48.8 months from the initiation of dopaminergic therapy.

Discussion

The main findings of our research indicate that PD patients who developed dyskinesia, differed from those who did not, according to the following: age at the onset of disease, length of disease, results on the MMSE scale, and had a more difficult form of Parkinson's disease, higher scores on the UPDRS, greater difficulty in daily functioning, more frequent motor fluctuations and medication-induced psychosis, in the duration of treatment and in larger daily doses of levodopa, or of dopaminometics. As the strongest predictors of dyskinesia induced by levodopa, the following were identified: the length of disease of more than 10 years, length of treatment of more than 94 months and an actual daily dose of levodopa of more than 537 mg.

Dyskinesias developed in approximately 50% of patients involved in our study, which is in accordance with the average findings of other researches (30%–80%). This range is explained by the variety of methodological approaches used in identifying dyskinesia, by the different age groups that were analyzed, and by the difference in the length of monitoring of affected patients. In individuals who have had PD for 4 to 7 years, the presence of dyskinesia has been 20%–40% [21–23]. Schrag and Quinn [24] found dyskinesia in 32% of patients treated with levodopa for 6 to 9 years, and in 89% of patients treated for more than 10 years. In the DATATOP study [23] after 20.5 months of being treated with Levodopa, dyskinesias presented in a third of the PD patients. In short, it is assumed that after a sufficiently long treatment period with levodopa, all patients will, in the end, develop dyskinesia [25].

Similarly to the other authors [26], we identified chorea and dystonia as the most frequent types of dyskinesia that arise from levodopa use. They presented most frequently as the peak of dose dyskinesias, but in almost half of the patients, they manifested at the end of the levodopa dose effectiveness ("end of dose" dyskinesia). Dyskinesia heavily disabled only 15% of our patients. This result is similar to the finding that dyskinesia whose intensity requires therapeutic intervention, or otherwise the adjustment of levodopa therapy after a treatment period of 10 years, is found in 12% of patients [8]. This finding is identical to the Sidney study [25] that revealed that only 12% of patients developed a difficult (or heavy) form of dyskinesia.

The development of dyskinesia is associated with different risk factors such as an earlier onset of disease, a longer duration and difficulty of PD, a longer duration of treatment with levodopa, a larger cumulative dose of levodopa, a higher daily dose of levodopa at the time of testing, and also its initial daily dose, female sex and possible genetic factors [9, 10, 27]. Our study confirmed the significance of these factors in the development of dyskinesia arising from levodopa.

Of particular significance in the planning of individual therapy is the finding that in individuals who had an earlier onset of PD, fluctuations and dyskinesia more frequently presented, and thus such affected patients had a more difficult disease progression. After five years of treatment with levodopa, more than 50% of patients in which PD was presented during the ages of 40 through 59, had dyskinesia, while such frequency decreased in the group with PD was arising during the ages of from 60 to 69, to 26% or even 16% in so far as the PD was presented after the age of 70 [9, 28]. Kostic et al. [28] indicate that after five years of levodopa treatment, they identified dyskinesia in 90% of patients in which PD had been presented before the age of 40. Hence, the same authors suggest initial therapy with dopamine agonists instead of levodopa for patients with PD for whom disease onset is presented at earlier age [11]. Dyskinesia was more prevalent in patients treated with higher daily doses of levodopa. This is in agreement with the findings of the ELLDOPA study [29] which demonstrated that the prevalence of dyskinesia increased with the increase in a daily dose of levodopa.

In our research, the length of the duration and difficulty of PD were significant factors. Van Gerpen et al. [8] showed that in a group of 126 PD patients, the likelihood that dyskinesia will develop during the first five years of illness was 30%, whereas it was 59% during the first ten years of illness. Advanced forms of PD with greater pathological findings upon the introduction of levodopa were followed by the faster onset of heavier forms of dyskinesia in comparison to the lighter forms of the illness [30], which also was confirmed in the animal model of PD [31].

As a key factor in the development of dyskinesia, intermittent ("pulse"), non-physiologic stimulation of dopamine receptors in the basal ganglia utilizing standard oral forms of dopamine mimetics was noted, as in all of our patients [32, 33]. Hence a large segment of pharmacotherapeutic interventions is focused on establishing forms of treatment by which continuous dopaminergic stimulation is achieved [34]. The finding that dyskinesia is more rarely presents in patients being treated with dopamine agonists in relation to levodopa [35] is explained by their longer half-life in blood plasma. The finding of our study that PD patients with dyskinesias, in comparison to those without them, were significantly more frequently treated with amantadine, can be explained by the fact that at this moment, it is the only drug that specifically lessens their intensity [36]. This can in part be an explanation for the more frequent use of clozapine in this

group of patients because it possesses anti-dyskinetic effects too [36], however, it is much more likely that a more frequent presentation of medication psychosis occurred in this group. It is interesting to note that affected patients who did not develop dyskinesia, in a statistically relevant manner more frequently received selegiline therapy (MAO B inhibitor), however the scope and methodology of our study are inadequate so as to be able to present the assumption that this drug has a protective effect involved in the onset of dyskinesia. The application of MAO B inhibitors in the early phase of PD was connected with the statistically relevant, less frequent fluctuations in motor response, whereas such a difference was not noted with respect to dyskinesias [37].

Conclusion

In our research, on average, slightly more than half of our PD patients developed dyskinesia. In comparison to the patients without dyskinesia, they were significantly younger at the onset of disease, the PD had a longer duration, and PD patients were on levodopa therapy for a longer period, and on the statistically significant higher daily dose.

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Depressive, anxious and somatization symptoms and quality of life in stress-related disorders

Depresivni, anksiozni i somatizacioni simptomi i kvalitet života u poremećajima povezanim sa stresom

Olga Čolović*†, Dušica Lečić-Toševski*‡§, Vanja Mandić-Maravić*, Oliver Tošković‖

***Institute of Mental Health, Belgrade, Serbia; Singidunum University, †Department of Psychology, Belgrade, Serbia; University of Belgrade, ‡Faculty of Medicine, ‖Faculty of Philosophy, Laboratory for Experimental Psychology, Belgrade, Serbia; §Serbian Academy of Sciences and Arts, Belgrade, Serbia**

Abstract

Background/Aim. Recent studies have shown a significant relation of the post-traumatic stress disorder and impairment of quality of life. The research on the relations of other stress-related disorders and quality of life is scarce. The aim of this research was to determine which symptoms within the stress-related disorders (depressive, anxious and somatization) have the strongest effect on the quality of life decrease. **Methods**. The study group comprised 80 subjects who have developed a certain stress-related disorder. The diagnosis was made based on the International Classification of Diseases (ICD-10) criteria. Manchester Short Assessment Quality of Life Scale (MANSA) and Symptom Check List-90 Revised (SCL-90-R) were administered. **Results.** The presence of all three types of symptoms (depressive, anxious or somatization) was in negative correlation with the quality of life, contributing to the variation of quality of life with 40%. Depressive symptoms had the greatest impact on the quality of life impairment. **Conclusion.** When it comes to stress-related disorders, the quality of life is mostly impaired by depressive symptoms. Target therapeutic interventions aimed at depressive symptoms might have a significant effect on the quality of life improvement in the person who developed stress-related disorders.

Key words:
stress, psychological; quality of life; surveys and questionnaires; stress disorders, post-traumatic; depression; neurotic disorders; somatoform disorders.

Apstrakt

Uvod/Cilj. Skorašnje studije pokazale su značajnu povezanost posttraumatskog stresnog poremećaja i smanjenja kvaliteta života. Istraživanja o korelaciji drugih poremećaja povezanih sa stresom i kvaliteta života izuzetno su retka. Cilj ovog istraživanja bio je da se utvrdi koji simptomi u okviru poremećaja povezanih sa stresom (depresivni, anksiozni i somatizacioni) najviše utiču na smanjenje kvaliteta života. **Metode.** Studijska grupa sastojala se od 80 ispitanika koji su razvili neki od poremećaja povezanih sa stresom. Dijagnoza je postavljena na osnovu kriterijuma Međunarodne klasifikacije bolesti (MKB-10). Primenjeni su sledeći instrumenti: Mančesterska kratka skala za procenu kvaliteta života (*Manchester Short Assessment Quality of Life Scale* – MANSA) i revidirana lista simptoma (*Symptom Check List-90 Revised* – SCL-90-R) **Rezultati.** Prisustvo sve tri grupe simptoma (depresivni, anksiozni i somatizacioni) bilo je u negativnoj korelaciji sa kvalitetom života, doprinoseći varijaciji kvaliteta života sa 40%. Depresivni simptomi imali su najveći uticaj na smanjenje kvaliteta života. **Zaključak.** Depresivni simptomi kao deo poremećaja povezanih sa stresom najviše utiču na smanjenje kvaliteta života. Ciljane terapijske intervencije usmerene na depresivne simptome mogle bi imati značajan uticaj na poboljšanje kvaliteta života kod osoba koje su razvile neki od poremećaja povezanih sa stresom.

Ključne reči:
stres, psihički; kvalitet života; ankete i upitnici; stresni poremećaji, posttraumatski; depresija; neurotski poremećaji; psihofiziološki poremećaji.

Introduction

Stress-related disorders always appear as a direct consequence of an acute severe stress or of continuous trauma, i.e. they represent a maladaptive response to a severe or continuous stress. It is necessary to emphasize that the stress or continuous trauma are primary etiological factors, i.e. that the disorder would not develop in their absence [1]. According

Correspondence to: Vanja Mandić Maravić, Institute of Mental Health, Palmotićeva 37, 11 000 Belgrade, Serbia. Fax: +381 11 3231 333. E-mail: vanjamandic81@gmail.com

to the Tenth Revision of the International Classification of Diseases and Related Health Problems (ICD-10) [1], stress-related disorders are the disorders that are identified not only by their symptoms and course but also based on one of two causing effects – extremely stressful life events or significant life changes.

The above-mentioned criteria are met by the diagnostic category F 43 (Reaction to severe stress and adjustment disorders) which includes: F 43.0 (Acute stress reaction), F 43.1 (Post-traumatic stress disorder – PTSD), and F 43.2 (Adjustment disorders). Apart from these diagnoses, one more diagnostic category meets the above-mentioned criteria – F 62.1 (Enduring personality changes after catastrophic experience) [1].

During the past two decades, the prevalence of the stress-related disorders significantly increased in our country due to numerous stressful factors [2–5]. Some recent studies have shown a significant relationship between PTSD and the decrease in quality of life (QOL) [6], which we have also shown in our previous research [7]. Studies on the relationship between other stress-related disorders and QOL are rather scarce. Studies on adjustment disorders are mostly done in populations of somatic patients, and their results show that the QOL is significantly lower in those who develop these disorders rather than those who suffer from the somatic disease alone [8].

The QOL as a concept became significant with the emergence of an idea that the impact of the disease was not limited only to symptoms and signs, but also to the global subjective impression of one's health. Therefore, the QOL may be considered as an operational measure of the overall health and welfare [9]. According to the definition of the World Health Organization, the QOL is defined as "individuals' perception of their position in life in relation to their goals and in the context of value systems, incorporated in their decision making" [10]. This definition primarily emphasizes the significance of the individual's readiness and capacity to communicate and participate in the personal QOL assessment.

Mental disorders have a significant impact on the life of an individual. Apart from the symptoms of a disorder, the following are present: changes in functionality and in access to the resources and possibilities, the subjective sense of welfare, burden on the family, and sometimes endangered safety of the society. Due to a wide range of the relevant consequences, and the prevailing opinion that the assessment should include patient's perspective as well, the increased attention has been directed to the development of the measures and procedures for the assessment of their QOL [11, 12].

Clinical experience suggests that specific forms of disorders from the stress-related group can have various impacts on patients' QOL [13, 14], affecting physical as well as mental health [15]. Patients may have difficulties in work or in relationships with others, as well as problems in leisure activities due to cognitive symptoms of fear, worry, and obsessions, they may be upset due to symptoms of increased irritability that are present in PTSD, or can be limited by avoidance symptoms that are inherent to this group of disorders. The researchers have only recently started to examine this topic in a more comprehensive and sophisticated way, using various approaches on different samples [16].

The studies which were not focused on stress-related disorders have shown the influence of anxious and depressive symptoms on the reduction of QOL [17–19], with the notion that depressive symptoms lead to a decrease in quality of life significantly more compared to the anxious symptoms [20]. Furthermore, it has been shown that in PTSD patients who had depressive comorbidity, the QOL decreased to the most significant extent [21]. Studies focusing on QOL in somatic illnesses showed that it decreases significantly if patients develop depression [22, 23].

In our previous study [7], we showed that persons in whom some stress related disorders were diagnosed had a significantly lower QOL compared to persons who experienced stress but did not develop a disorder.

The aim of our study was to determine which type of symptoms within the group of stress-related disorders (depressive, anxious, and somatization symptoms) has the strongest impact on the decrease of the QOL. By defining the type of symptoms that are the most important for the decrease of the QOL, a more directed treatment and prevention of disorders from this group could be achieved, thus improving the QOL of patients.

Methods

Sample

The sample comprised 80 subjects who were recruited during the period from 2002 to 2005. It included patients from a University Psychiatric Clinic who developed some of the stress-related disorders after a stressful life event. This group comprised 31 men (38.75%) and 49 women (61.25%), with average age 42.16 years [standard deviation (SD) = 11.56], ranging from 18 to 68 years. This sample of 80 subjects was the study group described in our aforementioned research [7], for which we have shown significantly greater impairment of quality of life for these 80 subjects, in comparison to the control group (80 subjects who experienced a stressful life event but did not develop a stress-related disorder). The total score of the QoL (measured by Manchester Short Assessment Quality of Life Scale – MANSA) [24] in the study group was 42.99 ± 9.5, while the same score in the control group was 53.01 ± 8.23 ($p < 0.01$) [7].

The diagnosis was based on clinical psychiatric interview and was made according to ICD-10 criteria [1]. The sample did not include patients with the accompanying psychiatric comorbidity. All subjects experienced a traumatic or stressful life event that led to the development of the disorder (acute stress reaction, PTSD, adjustment disorder and enduring personality change after a catastrophic experience); none of them had received any psychiatric treatment. All subjects were given the explanation about the aims of the study and have signed the Informed Consent Form. The confidentiality of obtained results was preserved. The study was approved by the Ethical Committee and was carried out according to the good research practice of the Faculty of Medicine in Belgrade.

Instruments

The following questionnaires were administered to all the subjects: MANSA [24] and Symptom Check List-90 Revised (SCL- 90-R) [25].

MANSA [24] is a short scale which assesses the general level of the QOL often used for evaluation of mental health, and it consists of three parts. The first part includes general data (date of birth, gender, ethnic background, and the diagnosis of disorder). The second part includes nine questions related to education, employment, finances, state support, dwelling conditions, the number of children and number of individuals within the family community person lives in. The third part (the satisfaction scale) measures a subjective satisfaction with the quality of different aspects of life, as well as the QOL as a whole. It consists of sixteen items, four of which are considered as "objective" while the remaining twelve are considered as "subjective" assessment of satisfaction with specific life aspects and with life as a whole. This instrument has a seven-degree scale, where 1 represents unfavorable, while 7 represents favorable pole of the scale.

SCL-90-R [25] consists of ninety items related to the symptoms of different disorders. They range from 0 to 4, for the population aged 13 to 70. The factor analysis of the symptom list distinguishes nine factors measured by this instrument: 1. somatization; 2. obsessions; 3. interpersonal sensitivity and vulnerability; 4. depression; 5. anxiety and phobias; 6. hostility; 7. paranoia; 8. psychoticism; and 9. various symptoms. The discrimination value refers to only three factors: somatization, depression, and anxiety with phobias. By scoring them, three more general indexes of the disorder are obtained: the severity of the disorder, the variability of symptoms, and the level of the subjective feeling about the disease.

Statistical analysis

The following statistical measures were used: arithmetic mean (AM) and standard deviation (SD) for quantitative parameters (subject age).

Pearson's correlation coefficient was used to determine the relationship between the QOL and the presence of somatization, depressive and anxious symptoms in our subjects, while multiple regression analysis has been used to determine to which extent the QOL was determined by the presence of somatization, depressive and anxious symptoms. Student *t*-test has been used to analyze the differences within the sample itself (in different diagnostic categories) – by presence of somatization, depressive and anxious symptoms.

Results

The presence of specific diagnostic categories from the group of stress-related disorders was the following: acute reaction to stress (F 43.0) was diagnosed in one (1.25%) subject, PTSD (F 43.1) in twenty (25%) subjects and adjustment disorder (F 43.2) in fifty seven (71.25%) subjects, while the diagnosis of the enduring personality change after a catastrophic experience (F 62.0) was made in two (2.5%) subjects.

The evaluation of the impact of depressive, anxious, and somatization symptoms on QOL suggested that the presence of all the three groups of symptoms was in negative correlation with the QOL. The multiple regression analysis showed that the presence of three mentioned types of symptoms explains as much as 40% of the variation in QOL ($R^2 = 0.4$; $F_{3,156} = 34.9$; $p < 0.01$).

After further examining which of the three above mentioned groups of symptoms had the greatest effect on the decrease of quality of life, the findings pointed at depressive symptoms, suggesting that the β ponder of this group was the highest, as shown in Table 1.

By using partial correlation, we tried to exclude mutual interlacing effects of somatization, depressive and anxious symptoms. Our findings demonstrated that somatization and depressive symptoms were in negative correlation with the QOL, explaining 2.89% (squared semipartial correlation) and 14.44% of variance, respectively, while anxious simptoms were in positive correlation with QOL, explaining 1.69% of variance, as shown in Table 1.

The comparison of the presence of the somatization, depressive and anxious symptoms among subjects with a different diagnosis of stress-related disorders, is shown in Table 2.

Our findings showed that there was a significant difference in the scores for somatization and anxious symptoms between subjects with adjustment disorders and subjects with PTSD – the subjects with PTSD had higher scores for both somatization and anxious symptoms. For depressive symptoms, there was no significant difference between the two groups of subjects ($p = 0.24$).

Discussion

Our findings have shown that all the three groups of symptoms (somatization, depressive and anxious) were in negative correlation with the QOL (somatization – 0.50; depressive – 0.61; and anxious – 0.43) and that they account for as much as 40% of the variation in QOL. We have found that depressive symptoms (compared to anxious and somati-

Table 1

The significance of contribution of somatization, depressive and anxious symptoms (SCL-90-R) to prediction of quality of life (MANSA)

Symptoms type	β	*t*	*p*	Correlation	Partial correlation	Semipartial correlation
Somatization	-0.267	-2.77	0.01	-0.50	-0.22	-0.17
Depressive	-0.610	-6.16	0.00	-0.61	-0.44	-0.38
Anxious	0.240	2.18	0.03	-0.43	0.17	0.13

SCL-90-R – Symptom Check List-90 Revised; MANSA – Manchester Short Assessment Quality of Life Scale.

Table 2

Average scores for somatization, depressive and anxious symptoms (SCL-90-R) in subjects diagnosed with various stress-related disorders

Symptoms	Diagnosis	n	Mean ± SD	*t*	df	*p*
Somatization	F 43.1	20	2.40 ± 1.03	2.88	75	0.01
	F 43.2	57	1.69 ± 0.93			
Depressive	F 43.1	20	2.33 ± 0.70	1.19	75	0.24
	F 43.2	57	2.08 ± 0.84			
Anxious	F 43.1	20	2.50 ± 0.92	2.61	75	0.01
	F 43.2	57	1.84 ± 0.98			

SCL-90-R – Symptom Check List-90 Revised; F43.1 – Posttraumatic stress disorder; F43.2 – Adjustment disorder; SD – standard deviation.

zation) within stress-related disorders had the greatest impact on the decrease of QOL.

In order to apprehend how anxious, depressive and somatization symptoms independently influence the QOL, we attempted to exclude the mutual overlapping effect of these three groups of symptoms (using partial correlation). By doing so, some interesting findings have been obtained. The negative correlation with QOL has been observed in 4.8% of somatization symptoms, and in 19% of depressive symptoms, while 2.9% anxious symptoms were in positive correlation with QOL.

A question arises – which parts of anxiety have a positive effect on QOL? It is possible that it is the "normal" anxiety, having useful, adaptive function, representing a warning signal suggesting that something should be done, i.e. facilitating an adequate perception of danger which gives a possibility of an appropriate protective reaction proportional to the level of threat[26]. On the other hand, this may also be certain kind of a ''positive" tension stimulating an individual, i.e. provoking an action that is a part of the course towards attaining life goals. Future studies on the impact of anxiety on the QOL may confirm or disapprove these hypotheses.

Comparing the group with adjustment disorders and the group with PTSD by presence of the three groups of symptoms, our findings have shown significant differences in scores for somatization and anxious symptoms (the subjects with PTSD diagnosis had higher scores for these two types of symptoms), while there was no significant difference between the two groups in presence of depressive symptoms.

Our findings are in accordance with the results of some other studies demonstrating the effect of anxious and depressive symptoms on the decrease of QOL [6, 16], where it is emphasized that depressive symptoms have a more compromising effect on QOL compared to anxious symptoms [17].

Furthermore, recent studies have shown that the QOL has been reduced the most in individuals with PTSD and with depressive comorbidity [27, 21, 28]. It was shown in a sample of primary care patients with various anxiety disorders [21], in a clinical sample of patients with PTSD [27], as well as in a sample of survivors of the war who developed PTSD [28].

To our knowledge, there are no other studies that explored the individual effect of specific types of symptoms in the *whole stress-related disorders group*. Although, according to ICD-10, the diagnosis of enduring personality change after catastrophic experience (F62.0) is not in the group F43 (Reaction to severe stress, and adjustment disorders) [1], we included it in our sample of stress-related disorders because, by definition, it can develop exclusively and only after a catastrophic stress experience and it is not necessary to explore personal vulnerability in order to explain its occurrence. This disorder does not exist in the Diagnosis and Statistical Normal of Mental Disorders (DSM) V [29], but there was a great debate whether the classification should include "a disorder related to extreme stress, not otherwise specified" [30], to which some authors relate to as "complex PTSD" [31]. It takes into consideration the functioning of an individual with the history of severe or prolonged trauma.

On the other hand, when it comes to specific stress-related disorders, such as PTSD, to our knowledge there are no other studies that explored the individual effect of somatization symptoms.

Apart from all methods of control that have been implemented, both methodological and statistical, our study may have certain limitations. Firstly, the study was retrospective and the data on the stressful event were collected retroactively. Another limitation of this study may also be the unequal proportion of certain diagnostic categories among subjects.

Conclusion

Our study showed that depressive symptoms (compared to anxious and somatization ones) of stress-related disorders have the greatest impact on the decrease of the QOL. For depressive symptoms, no significant difference was shown between individuals with adjustment disorders and those with a diagnosis of PTSD, while the subjects with PTSD had higher scores for somatization and anxious symptoms.

A clear identification and specific treatment of each of the mentioned groups of symptoms is necessary throughout all phases of treatment of stress-related disorders.

Targeted psychotherapeutic and psychopharmacological interventions aimed at depressive symptoms that are part of stress-related disorders could have a major effect on improvement of QOL of these patients and might be the way for an efficient prevention of the relapse of these disorders.

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Biometric features of the eyes of preterm born babies

Biometrijske karakteristike očiju prevremeno rođenih beba

Zorka Grgić*, Ana Oros*, Jelena Karadžić †, Vladimir Čanadanović*, Sandra Jovanović*, Sofija Bolinovska*

Clinical Center of Vojvodina, *Clinic for Eye Diseases, Novi Sad, Serbia; Clinical Center of Serbia, †Clinic for Eye Diseases, Belgrade, Serbia

Abstract

Background/Aim. Preterm birth and retinopathy of prematurity (ROP) could affect optical and biometric features of eyes and cause refractive errors. The aim of this study was to compare the ocular axial length, anterior chamber depth and lens thickness changes during the first year in preterm born babies with and without ROP. **Methods**. This prospective longitudinal study included 87 preterm born babies. The examinations were performed at 3 and 12 months after birth and included fundus examination and measurements of the ocular axial length, anterior chamber depth and lens thickness. Based on the results of fundus examination at 3 months, the subjects were divided into two groups and the measurements of those with and without ROP were compared. **Results**. At 3 months 60.92% of infants had ROP. The mean values in the ROP group were: axial length 16.56 mm and 16.53 mm, chamber depth 2.34 mm and 2.38 mm and lens thickness 4.04 mm and 3.96 mm, in the right and the left eye, respectively. In the no ROP group these values were: axial length 17.06 mm and 17.08 mm, chamber depth 2.31mm and 2.39 mm and lens thickness 4.16 mm and 4.14 mm in the right and the left eye, respectively. At 12 months 28.74% of the children had a change in the ocular fundus as a result of the ROP therapy. In the ROP group the axial length was 19.94 mm in both eyes, chamber depth 3.01 mm and 2.99 mm and lens thickness 4.28 mm and 4.29 mm, in the right and the left eye, respectively. In the no ROP group the axial length was 20.64 mm and 20.29 mm, lens thickness 4.37 mm and 4.36 mm, in the right and the left eye, respectively and chamber depth 3.10 mm in both eyes. **Conclusion**. In the group of children with ROP axial length of the eye at 3 and 12 months was statistically significantly smaller in comparison to the group without ROP. Statistically significant difference was not found between these groups in the anterior chamber depth and lens thickness.

Key words:
infant, premature; retinopathy of prematurity; biometry; refraction, ocular; eye.

Apstrakt

Uvod/Cilj. Prevremeno rođenje i prematurna retinopatija (ROP) mogu dovesti do nastanka refraktivnih mana, uticajem na optičke i biometrijske karakteristike oka. Cilj ovog rada bio je da se uporede promene u aksijalnoj dužini oka, dubini prednje očne komore i debljini sočiva prematurusa sa i bez ROP tokom prve godine života. **Metode**. Ispitivanje je sprovedeno kao prospektivna longitudinalna studija, koja je obuhvatila 87 prevremeno rođenih beba. U uzrastu od 3 i 12 meseci urađen je pregled očnog dna i merenje aksijalne dužine oka, dubine prednje očne komore i debljine očnog sočiva. Prema nalazu na očnom dnu 3 meseca posle rođenja bebe su podeljene u dve grupe, sa i bez ROP i i izmerene vrednosti praćenih parametara su upoređene. **Rezultati**. Prilikom pregleda beba sa 3 meseca kod 60,92% njih je ustanovljen ROP. Prosečne vrednosti merenih parametara na desnom i levom oku u grupi sa ROP bile su: aksijalna dužina 16,56 mm i 16,53 mm, dubina prednje očne komore 2,34 mm i 2,38 mm i debljina sočiva 4,04 mm i 3,96 mm na desnom, odnosno levom oku. U grupi bez ROP ove vrednosti na desnom i levom oku bile su: aksijalna dužina 17,06 mm i 17,08 mm, dubina prednje komore 2,31 mm i 2,39 mm i debljina sočiva 4,16 mm i 4,14 mm. Sa 12 meseci 28,74% dece je imalo promene na retini kao posledice tretmana ROP. U grupi sa ROP pri prvom pregledu, aksijalne dužine bile su 19,94 mm na oba oka, dubine prednje komore 3,01 mm i 2,99 mm, a debljine sočiva 4,28 mm i 4,29 mm na desnom, odnosno levom oku. U grupi bez ROP aksijalne dužine desnog, odnosno levog oka bile su 20,64 mm i 20,29 mm, dubine komore 3,10 mm na oba oka, a debljine sočiva 4,37 mm i 4,36 mm, desno i levo. **Zaključak**. U grupi dece sa ROP aksijalna dužina oka sa 3 i 12 meseci bila je statistički značajno manja u odnosu na grupu bez promena na retini. Statistički značajna razlika nije nađena između grupe sa i bez ROP poređenjem parametara očne komore i sočiva.

Ključne reči:
nedonošče; retinopatija kod prematurusa; biometrija; oko, refrakcija; oko.

Correspondence to: Zorka Grgić, Clinical Center of Vojvodina, Clinic for Eye Diseases, Hajduk Veljkova 1–3, 21 000 Novi Sad, Serbia. E-mail: zorkagrgic@gmail.com

Introduction

The number of infants born prematurely (that is before 37 weeks of gestation), which survive the neonatal and perinatal period is currently increasing. The focus of medical care is therefore shifted to long-term functional results of all organ systems [1]. Vision is a complex and highly functional set of processes at the levels of the retina and nervous system [2]. The impressions that an infant receives through the sense of sight are highly important for its normal psychological and cognitive development [3]. Potential consequences of premature delivery for visual, motor and cognitive functions are numerous [3]. There are many causes for visual impairment, but they mainly occur as a consequence of the immaturity of the central nervous system and not of localized damage to the eye or cerebral cortex [1]. Retinopathy of prematurity (ROP) is a vasoproliferative disorder of the retina in prematurely born infants [4], which is the most common cause of preventable blindness in children. Preterm birth is also characterized by a higher incidence of strabismus, refractive disorders, nystagmus, glaucoma, optic nerve hypoplasia, eye movement disorders and reading disorders [1].

ROP develops in two distinct phases [2, 5, 6] and according to the International Classification, the severity of the disease is described in five stages, the localization of the process with three zones and the circumferential extent of the disease is based on the clock hours (from 1 to 12), where the extent of five hours is regarded as the critical value [7]. Generally, stage 1 is defined by the presence of the so-called demarcation line, stage 2 by the presence of the so-called ridge, stage 3 by extraretinal fibrovascular proliferation. Stages 4 and 5 are severe conditions, the former of which is characterized by sub-total retinal detachment and the latter by total retinal detachment [7]. The zones are defined according to the posterior position of the process, so that the severity of the disease decreases from zone I to zone III [5, 7].

Screening of prematurely born infants is performed in order to identify cases with a high risk of permanent visual loss due to ROP, which in turn could be decreased by adequate and timely intervention [8, 9]. The criteria for screening infants in our country, following the recommendation of the American Academy of Pediatrics, are the birth weight 2,000 grams or less and gestational age 36 weeks or less, with the postnatal application of oxygen. The presence of additional risk factors during the development of the child points to the need of screening regardless of the criteria mentioned [5, 7]. The first examination should be performed 4 to 6 weeks after birth [5, 8] or between gestational weeks 31 and 33 [8, 10]. After that, the second examination is performed after a week or two, depending on the local findings [8]. The screening takes place until blood vessels reach zone III in infants without ROP in the zones I and II [8, 11, 12], or to the completed vascularization of the ora serrata after intravitreal application of anti-vascular endothelial growth factor or until week 50 postmenstrually for children with prior milder ROP forms or until ROP recedes [12]. A repeated ophthalmological examination of prematurely born babies at 12 months is of crucial importance, regardless of the presence of ROP, in order to identify amblyogenic factors such as strabismus or refractive disorders [11, 13].

Premature birth, i.e. gestational age, birth weight, retinopathy and the development of refractive disorders are closely related [14]. These characteristics indirectly affect refraction, by influencing the optical characteristics of the eye [15]. Refractive disorders occur when the ocular axial length does not correspond to the focal plane, created by the cornea, lens and anterior chamber [16]. The process of balancing out the strength of the refractive power of the optical system and the ocular axial length is called emmetropization [17] and it generally occurs between month 3 and 12 after birth. This process is different in full-term and preterm infants and the differences are noticeable depending on the presence of the retina disorder [15]. The changes in the refractive power of the cornea and lens show a negative correlation with the axial growth of the eye [18]. At the age of one, 6.66% of prematurely born children with ROP at the earliest age and 3.75% of those without ROP have a refractive error [19]. The main causes of the high incidence of myopia, in general, are the greater axial length [20, 21], shallower anterior chamber and the greater thickness of the ocular lens [21]. However, prematurity, lower gestational age and birth weight are characterized by a more convex cornea, shallower anterior chamber, thicker lens, but also, a smaller axial length, compared to the values which might be expected from the dioptric value of the eye [14, 19, 22, 23]. The early effect of growth restriction within retinopathy is later followed by irregularities in the growth of the eye posterior segment [23], so that in preterm infants there is no direct correlation between the ocular axial length and its refractive status. Proportions of the eye are not later fully compensated for by the growth of the eye and visual experience [14, 22].

In this study is shown how the ocular axial length, anterior chamber depth and lens thickness were changing during the first year of life in preterm born babies with and without ROP.

Methods

The research was conducted as a prospective, longitudinal study at the Clinic for Eye Diseases of the Clinical Center of Vojvodina during the period of 5 years, from 2005 till 2010. The study was approved by the Clinical Centre of Vojvodina Ethics Committee. The parents of the participating babies provided their informed consent, after being provided written and verbal information about the study. The study enrolled 87 preterm infants (174 eyes), divided into the two groups according to the presence of ROP. Two examinations were performed, at 3 and 12 months of age.

The study included preterm born babies whose birth weight was 2000 grams or lower and gestational age 36 weeks or lower, who received oxygen, as well as infants satisfying these criteria, but with other risk factors that might affect the development of the retinal vasculature, such as: diseases of the respiratory tract, brain hemorrhage, sepsis, anaemia, enterocolitis, blood transfusion, non-physiological values of partial pressure of oxygen and carbon dioxide, acidosis, phototherapy and multiple pregnancies. The preterm in-

fants with risks were selected by neonatologists. Infants that developed severe forms of retinopathy (stages IV and V) were excluded from the study, since the biometrical data and the refractive status that would have been acquired in those cases would bear no relevance for this research.

For the collection of necessary data, an original questionnaire was used. The research was conducted according to the preset methodological plan with precisely defined criteria. It included: the scrutiny of medical documentation, i.e. the discharge note from the Institute for Child and Youth Health Care of Vojvodina, biometric measurements, including the measurements of the ocular axial length, anterior chamber depth and lens thickness of both eyes, as well as the bilateral fundus examination in mydriasis and with indentation at the two respective ages. The values were taken for each eye separately in order to record differences in values for each eye, taking into account the higher incidence of anisometropia in preterm population.

Medical records provided the basic personal data, the data on the values of birth weight, gestation age and the presence of risk factors.

Biometric measurements were performed after the application of the local anesthetic Tetracaine hydrochloride® 0.5% eye drops, three times at 5 minutes intervals. The ocular axial length, anterior chamber depth and the thickness of the lens were measured by the ultrasonographic A-method using the Sonomed Inc, USA, A-2500 ultrasound machine, with the transducer frequency of 10 MHz. The values were taken three times for all the parameters and the mean value was calculated. The measurements were expressed in millimeters.

The examination of the ocular fundus was performed through dilated pupils. The maximal mydriasis was provided by the application of Phenylephrine hydrochloride® 2.5% eye drops or Cyclopentolate® 0.5% eye drops, twice at 10- minute intervals one hour before the examination. The examination was performed by indirect binocular ophthalmoscope, Indirekte Ophthalmoskop Omega 180, Heine Optotechnik, Hersching, Germany and a 20 D lens. For a more detailed view of the peripheral segments of the retina scleral indentation was used. Eyelid separation was obtained by using blepharostat. The results were expressed in accordance with the International Classification of Retinopathy of Prematurity.

Statistical analysis of the data was performed using the Statistical Package for Social Sciences software - SPSS 21. The numerical values were expressed as mean values and variability measurements (range, standard deviation) and the attributive features by frequencies and percentages. The comparison of the numerical values between the groups was done using *t*-test and the differences between the frequencies of attributive features by χ^2 test. The correlation between two features was determined by Pearson's correlation coefficient. Statistical significance was set at level $p < 0.05$.

The results are presented as tables and figures.

Results

The research included 87 preterm born babies (174 eyes): 43 (49.43%) boys and 44 (50.57%) girls. The mean gestational age at birth was 31.45 weeks, ranging from 24 to 36 weeks. Birth weight ranged from 1,190 grams to 3,620 grams, with the mean value 1,643.51 grams.

The results at 3 months

The incidence of ROP at 3 months was present in 60.92% of the infants and was identical in both eyes. In the right eye 41.5% infants with ROP had ROP 1, 9.4% ROP 2, 43.4% ROP 3, 1.9% ROP 4 and 3.8% ROP 5. In the left eye, the occurrence of specific ROP stages was: 41.5% with ROP 1, 9.4% with ROP 2, 45.3% with ROP 3, ROP 4 was found in none of the infants and ROP 5 was found in 3.8% babies (Table 1).

Table 1

The incidence of specific retinopathy of prematurity (ROP) stages in eyes of premature born infant at the age of 3 months

ROP stages	Right eye, n (%)	Left eye, n (%)
1	22 (41,5)	22 (41.5)
2	5 (9,4)	5 (9.4)
3	23 (43,4)	24 (45.3)
4	1 (1,9)	
5	2 (3,8)	2 (3.8)
Total	53 (100)	53 (100)
Mean	2,17	
Median	2,00	
Mode	3	

The mean values of the axial length in patients with ROP were 16.56 mm (15.02 to 17.78 mm; standard deviation (SD) = 0.72 mm) in the right and 16.53 mm (15.10 to 17.78 mm; (SD) = 0.68 mm) in the left eye. Axial length values in the group of infants without ROP were 17.06 mm (15.97 to 18.27 mm; (SD) = 0.55 mm) in the right and 17.08 mm (15.66 mm to 18.27 mm; (SD) = 0.54) in the left eye. The standard deviation was higher in the group of patients with ROP in both eyes, which points to the higher heterogeneity of this group. The results of the differences in the axial values between the two groups were statistically highly significant both in the right ($t = 3,451$, $p = 0,001$) and in the left eye ($t = 3.996$, $p = 0.000$) (Figure 1).

The analysis of the values of the anterior chamber depth of the right ($t = 0.420$, $p = 0.676$) and the left eye ($t = 0.149$, $p = 0.882$) revealed no statistical difference between the groups with and without ROP, while the values of standard deviation, similarly to the values of axial length, were higher in the group of infants with ROP. The mean of the anterior chamber depth was 2.31mm (1.92 to 2.90 mm; SD = 0.24 mm) in the right and 2.39 mm (2.11 to 3.36 mm; SD = 0.27 mm) in the left eye in the group without ROP, while in the group with ROP it was 2.34 mm (1.92 to 4.34 mm; SD = 0.38 mm) in the right and 2.38mm (1.85 to 4.27 mm; SD = 0.36 mm) in the left eye (Figure 2).

The mean values of the lens thickness in infants without ROP were 4.16 mm (3.40 to 4.90 mm; SD = 0.30 mm) and 4.14 mm (2.75 to 4.79 mm; SD = 0.37 mm) and in babies with ROP they were 4.04 mm (2.10 to 4.70 mm; SD = 0.49 mm) and 3.96 mm (2.26 to 4.75 mm; SD = 0.47 mm) in the right and the left eye respectively. The differences obtained

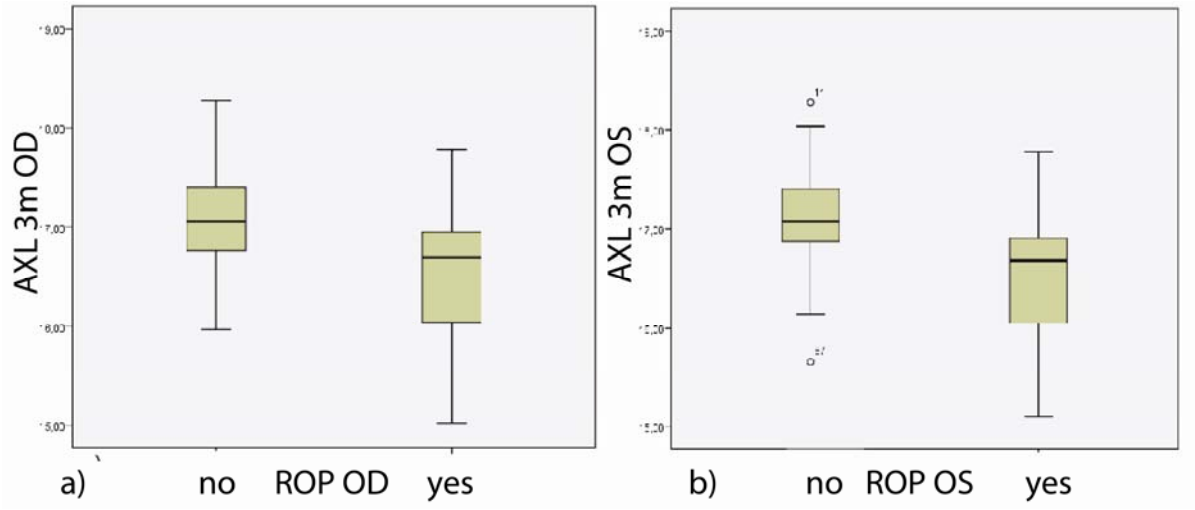

Fig. 1 – Axial length of eyes in premature born infants with and without retinopathy of prematurity (ROP) at the age of 3 months: a) right eye; b) left eye.

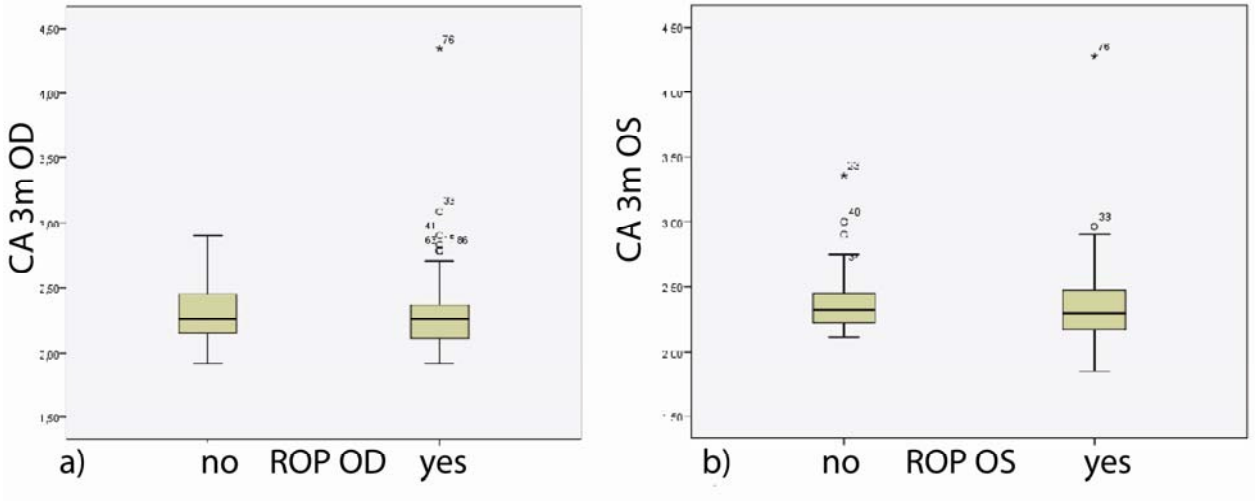

Fig. 2 – Anterior chamber depth of eyes at the age of 3 months in premature born infants with and without retinopathy of prematurity (ROP) at the age of 3 months: a) right eye; b) left eye.

were not statistically significant in either the right (t = 1.360, p = 0.177) or the left eye (t = 1.852, p = 0.067) and the values of higher standard deviation point to the greater heterogeneity of the group with ROP (Figure 3).

The results at 12 months

At 12 months of age 62 (71.26%) babies had a normally developed blood network of the retina. The remaining 25 (28.74%) had a change in the ocular fundus as a result of cryopexy or retinal laser therapy due to ROP. The results were almost identical in both eyes. The axial length of the right (t = 2.329, p = 0.022) and the left eye (t = 2.087, p = 0.040) in the no ROP group was statistically significantly greater than in the group with ROP. The values of standard deviation of this parameter in both eyes were approximately the same in the group with ROP, while the standard deviation in the right eye in the other group was significantly higher, since the values of one child deviated considerably from the others, whose results were homogeneous. In the group without ROP the mean value of the axial length of the right eye was 20.64 mm (19.0 to 30.4 mm; SD =1.89 mm) and of the left one 20.29 mm (19.0 to 22.0 mm; SD = 0.65 mm). In the group with ROP, these values of the right eye ranged from 17.4 to 22.1 mm (SD = 0.87 mm) and of the left eye from 16.7 to 21.8mm (SD = 0.84 mm). The mean value of both eyes was 19.94 mm (Figure 4).

The difference in the mean values of the anterior chamber depth was not statistically significant neither in the right (t = 1.048, p = 0.268), nor in the left eye (t = 1.408, p = 0.163) in both groups, with or without ROP. In the group without ROP the mean value of both eyes was 3.10 mm (right eye: 2.14 to 3.58 mm; SD = 0,34 mm; left eye: 2.26 to 3.81 mm; SD = 0.37 mm) and in the group with ROP the mean values were 3.01mm (2.15 to 3.77 mm; SD = 0.41 mm) and 2.99 mm (2.40 to 3.57 mm; SD = 0.34 mm) in the right and left eye, respectively. The differences in standard deviations were not statistically significant which pointed to close homogeneity of both groups regarding this parameter (Figure 5).

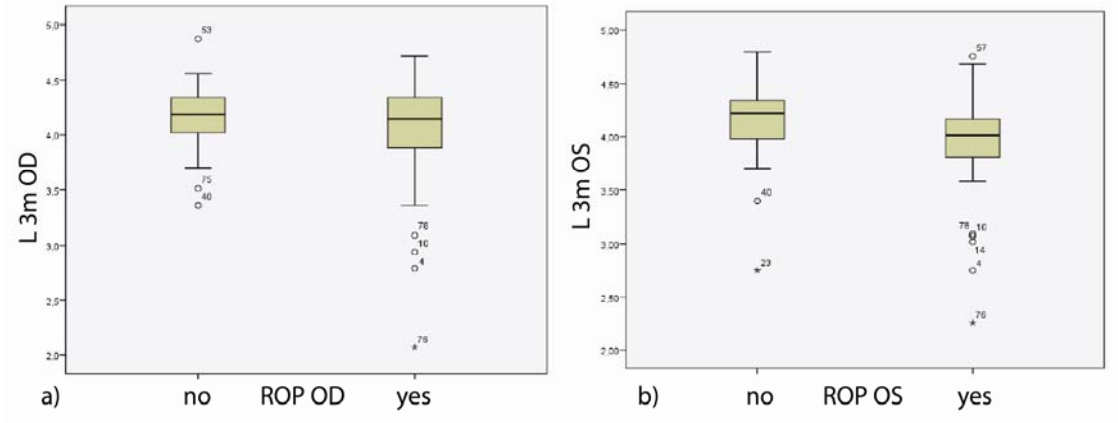

Fig. 3 – Lens thickness of eyes in preterm born infants with and without retinopathy of prematurity (ROP) at the age of 3 months: a) right eye; b) left eye.

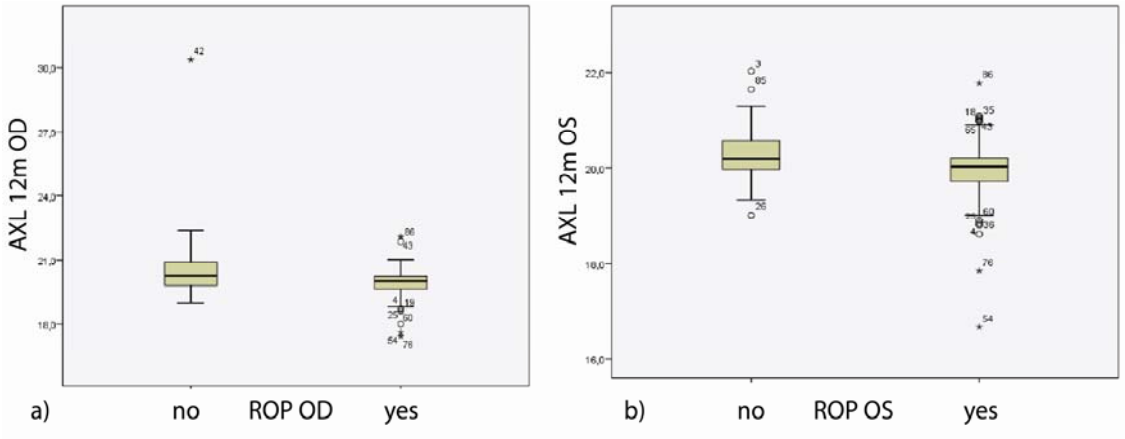

Fig. 4 – Axial length of the eyes in preterm born infants with and without retinopathy of prematurity (ROP) at the age of 12 months: a) right eye; b) left eye.

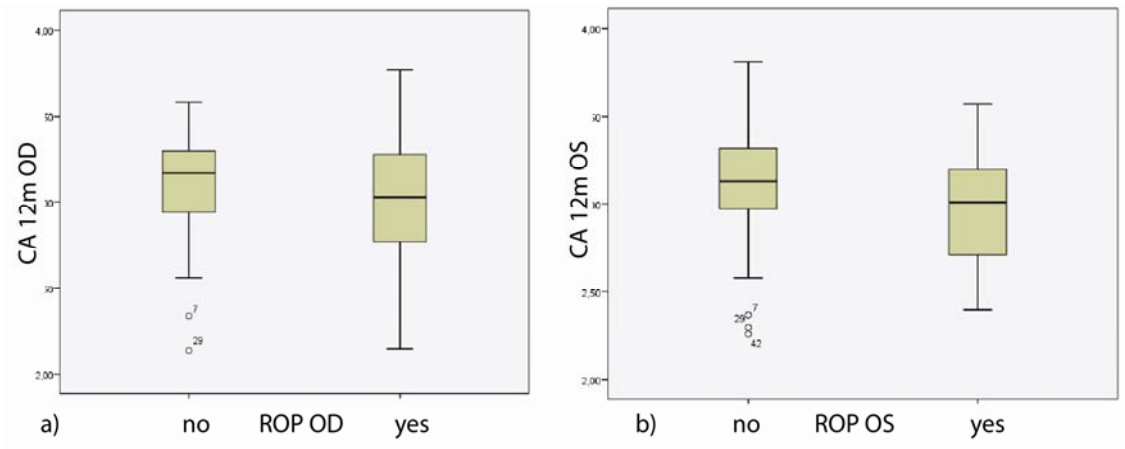

Fig. 5 – Anterior chamber depth of eyes in preterm born infants with and without retinopathy of prematurity (ROP) at the age of 12 months: a) right eye; b) left eye.

No statistical significance was found in the mean values of the lens thickness of both eyes in patients with and without ROP (right: $t = 1.357$, $p = 0.178$; left: $t = 1.092$, $p = 0.278$). Standard deviations of the values obtained in the two groups point to their relative homogeneity, although the ROP group was even somewhat more homogeneous. In the group without ROP the values were 4.37 mm (3.77 to 4.92 mm; SD = 0.30 mm) in the right and 4.36 mm (3.02 to 4.90 mm; SD = 0.36 mm) in the left eye, whereas in the group with ROP they were 4.28 mm (3.69 to 5.13 mm; SD = 0.28 mm) in the right and 4.29 mm (3.81 to 4.96 mm; SD = 0.26 mm) in the left eye (Figure 6).

Discussion

The objective of this study was to examine the effect of retinopathy of prematurity on the growth and development of the eye.

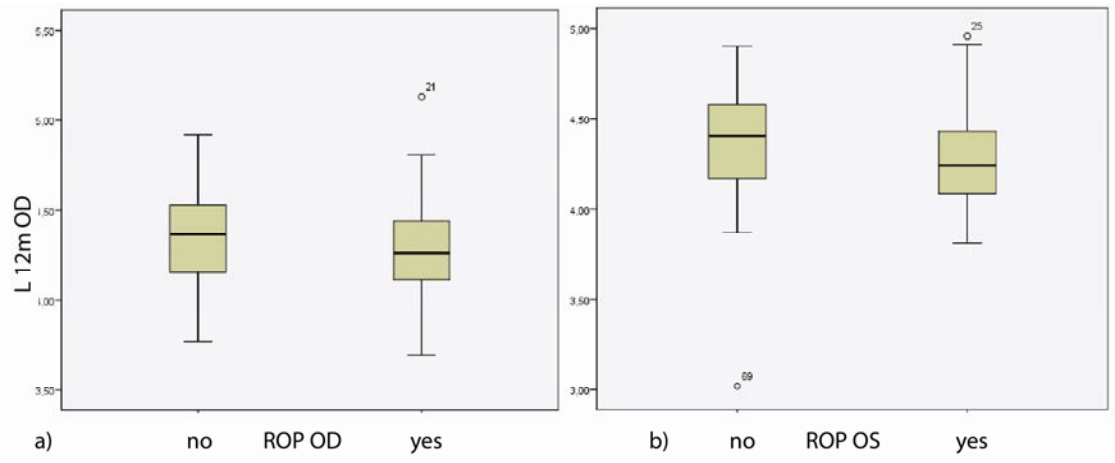

Fig. 6 – Lens thickness of the eyes in preterm born infants with and without retinopathy of prematurity (ROP) at the age of 12 months: a) right eye; b) left eye.

The study enrolled a similar number of boys and girls and therefore, the difference between the genders in the groups was of no statistical significance. The findings reported in the literature regarding the influence of gender on the biometric characteristics of the eye have been inconsistent thus far. Siegwart and Norton [16] claim that gender does affect the development of the eye. According to O'Brien and Clark [24], and Laws et al. [25] the mean axial length of the eye and the rate of its growth are greater in male infants notwithstanding the correction of the birth weight, gestational age and head dimensions. On the other hand, Mutti et al. [18] reported that gender is not a factor affecting the development and change in refraction and eye growth. Similar results were obtained by Chen et al. [22], who also consider the characteristics of the anterior segment of the eye in preterm infants to be independent of the gender.

Gestational age and birth weight are highly important parameters of the development and progression of ROP [23, 26]. Various screening criteria are found in the literature, but they are primarily related to these two characteristics. The differences between the criteria are also conditioned by the differences in the economic prosperity and investments into medical care, including neonatal, one as well as differences in the incidence of more severe forms of ROP [4, 8, 9, 11, 27–29]. The mean gestational age of our subjects was 31.45 weeks, while the mean birth weight was 1,643.51 grams.

The overall incidence of ROP in this sample was 60.92%, which is higher than the data reported in the literature. The incidence of ROP among preterm infants reported in the studies by Chen et al. [22] was 44%. The need for more frequent and long-term follow-up examinations due to the disorders of the retina may explain the obtained differences in incidence. Namely, the infants with normal findings in the first or second examination were not taken to the follow-up examination at 1 year of age, so they were not included in this study. The greatest majority of infants had ROP 1 (41.5% in both the right and the left eye) and ROP 3 (43.4% in the right and 45.3% in the left eye), while the incidence of ROP 2 was lower than ten percent in both eyes (9.4%). ROP 4 was only found in the right eye, in 1.9% cases and the incidence of ROP 5 was the same in both eyes (3.8%). Chen et al. [22] also report the incidence of specific stages of ROP in preterm infants, ROP 1 and 3 being of the highest incidence, then ROP 2 and the lowest incidence is found with ROP 4 and 5.

The mean axial length of the right and the left eye in infants without retinopathy (17.06 mm and 17.08 mm) was statistically significantly higher in comparison to these values in infants with retinopathy (16.56 mm and 16.53 mm). The values of the axial length of the eye in preterm children at birth [30], as well as at 3 months, are lower compared to full-term babies and this difference is particularly marked in the presence of ROP [23]. Fledelius and Fledelius [30] report about the values of this parameter in full-term infants at birth and preterm newborns at the full-term gestational age, pointing that they are similar in the two groups. They also state that visual and biometric parameters in preterm infants with milder forms of ROP and spontaneous regression are similar to those of preterm infants without ROP [30]. Full-term newborns have the mean axial length ranging from 16.6 mm to 17.6 mm, while the mean value in the preterm infants at 40 gestational weeks ranges from 16.6 mm to 17.16 mm [30]. According to the data reported by Pennie et al. [31], at 3 months of age, the axial length in full-term infants is 17.99+/-0.67 mm, whereas Mutti et al. [18] report the mean value of 19.03+/-0.58 mm. Cook et al. [23] report the data on the values of the eye axial length of preterm infants at 3 months, giving a comparison to the findings of the fundus. Thus at 3 months infants without ROP have the approximate mean axial diameter of 18.6 mm and with ROP 18.5 mm. After this period the axial length of the eye increases, so that in full-term children at 9 months it is approximately 20.23+/-0.64 mm [18] but at 12 months approximately 19.71+/-0.87 mm [31]. However, very few studies report on the axial length values of prematurely born children at 1 year of age. Our study found a statistically significant difference in axial length values of both eyes in the groups of infants with and without ROP, such that in the case of normal development of retinal blood vessels the axial length of the eye was greater compared to the group of infants who had ROP at 3 months of age. In addition to this, the group without ROP was significantly more homogeneo-

us. The mean value of the axial length of the right eye in the group of infants with ROP at 12 months was 19.94 mm and without ROP it was 20.64 mm. The corresponding values of the left eye for the two respective groups were 19.94 mm and 20.29 mm.

Since at both ages investigated the axial length in both eyes was statistically significantly smaller in infants with ROP, but also in preterm infants compared to full-term infants, it can be concluded that prematurity, as well as retina disorders related to premature birth, both affect the growth of the eye in total from birth to one year of age. Future research and analyses of older children are also necessary in order to determine the alteration of these values at later ages.

In our population studied, there was no statistically significant difference at 3 months of age between the anterior chamber depth in infants with and without ROP in the right (2.34 mm vs. 2.31 mm) or left eye (2.38 mm vs. 2.39 mm). However, the standard deviation of this parameter points to differences in the homogeneity between the two groups, which disappear at a later age. The depth of the anterior chamber in full-term children is approximately 2.38 mm to 2.90 mm, while in preterm infants at the time of full-term gestation this value ranges from approximately 2.25 mm to 2.44 mm, as reported by Fledelius and Fledelius [30]. The depth of the anterior chamber in full-term infants at 3 months is 2.76+/-0.27 mm [18]. Other authors also report lower values (2.24+/-0.31D) (31). As reported in the studies by Anna Cook et al. [23] at 3 months of age the depth of the anterior chamber in preterm infants is smaller than in full-term infants, especially in the case of ROP. Its mean value in preterm infants without ROP is 2.8 mm and with ROP 2.7 mm at that age.

The mean value of the depth of the anterior chamber of both eyes was somewhat lower in children with ROP at 12 months but without any statistical significance in comparison to children without ROP. The depth of the anterior chamber was 3.01 mm in the right and 2.99 mm in the left eye in the group with ROP and 3.10 mm in both eyes in the group of children without ROP. The depth of the anterior chamber increases gradually in the first months after birth and its value in full-term children reaches 3.03+/-0.35 mm at 9 months [18], but at 12 months of age the anterior chamber depth of full-term children is around 2.8+/-0.26 mm [31].

The mean lens thickness at the first examination of infants with ROP in our study was 4.04 mm and 3.96 mm and in infants without ROP it was 4.16 mm and 4.14 mm. This difference had no statistical significance and in addition, there was no statistical difference between the values of the right and the left eye. The homogeneity of the two groups studied increased between 3 and 12 months, especially in the group with ROP. The thickness of the lens of full-term infants at birth ranges from 3.40 mm to 3.96 mm, while in preterm infants this value ranges from 3.89 mm to 4.04 mm [30]. However, in contrast to the axial length and depth of the anterior chamber, the thickness of the lens in full-term children decreases [18]. At 3 months of age the reported thickness of the lens in full-term children is 3.92+/-0.17 mm [18] or 3.65+/-0.25 mm [31], at 9 months it is 3.86+/-0.18 mm (19) and at 12 months approximately 3.65+/-0.14 mm [31]. As for preterm infants, Cook et al. [23] report that the mean value of lens thickness of premature infants without ROP is 4.0 mm and with ROP 3.96 mm at 3 months, which corresponds to the results of our study.

The mean values of the lens thickness of the right and the left eye in the group of children without ROP at 12 months were 4.37 mm and 4.36 mm and with ROP 4.28 mm and 4.29 mm. The values obtained bear no statistically significant difference between the patients with and without ROP. It is important to notice that, in contrast to full-term children, in preterm infants the thickness of the ocular lens increases during emmetropization, which also corresponds to the data reported in the literature [15, 22, 30, 32].

Conclusion

At 3 and 12 months of age, the axial length of both eyes is significantly smaller in preterm infants who develop retinopathy of prematurity at the earliest age, than in those infants who do not. However, the depth of the anterior chamber and the lens thickness of both eyes are not statistically significantly different in the two groups of preterm born infants. We can conclude that during the first year of life retinopathy of prematurity significantly affects the growth of the eye on the whole, but not the growth of the components of its anterior segment compared to the other premature population.

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5'-Nucleotidase and adenosine deaminase in patients with rheumatoid arthritis

5'-nukleotidaza i adenozin dezaminaza kod bolesnika sa reumatoidnim artritisom

Nela Živković*, Boris Djindjić†, Sonja Stojanović†‡, Ivana Krstić‡, Ivana Ćirić†, Tomislav Kostić†§, Gordana Kocić†

Clinical Center Niš, *Clinic of Pediatrics, §Clinic of Cardiology, Niš, Serbia; University of Niš, †Faculty of Medicine, Niš, Serbia; ‡Institute for Treatment and Rehabilitation "Niška Banja", Niš, Serbia

Abstract

Background/Aim. The essence of rheumatoid arthritis (RA) pathogenesis is inflammation, modification of immune system and cell damage. 5'-nucleotidase (5'-NT) and adenosine deaminase (ADA) have a significant role in the process of inflammation-caused tissue damage. The aim of the study is to define 5'-nucleotidase and adenosine deaminase activity in serum of patients with RA treated with methotrexate (MTX) and patients with RA who were not treated with methotrexate, as well as to determine the correlation between the enzymes' activities and the disease activity. **Methods**. The study included 160 patients suffering from RA, 60 of them were not treated with methotrexate (average age 56.8 years; 68.3% female) and 100 patients were treated with methotrexate (average age 59.8 years; 88% female), as well as 60 healthy controls (average age 58.8; 66.6% female). Patients suffering from chronic inflammatory diseases, chronic respiratory, cardiac and kidney insufficiency, severe acute diseases and other diseases which might modify inflammatory response were not included in the study. **Results**. There was no statistically significant difference in 5'-NT values among groups. ADA values were significantly different in all tested groups. Post-hoc analysis (Dunnett's T3 test) showed that ADA activity in RA groups was significantly higher as compared to that in the control group ($p < 0.001$), and that ADA activity in the RA group with MTX was significantly smaller as compared to RA group without MTX ($p < 0.001$). There was not significant correlation between the disease activity and activities of tested enzymes. **Conclusion**. We concluded that adenosine deaminase activity was increased in patients with rheumatoid arthritis, as well as that the application of methotrexate led to the decrease of this enzyme activity in the serum of patients with rheumatoid arthritis. The activity of 5'-nucleotidase is not increased in patients with rheumatoid arthritis and did not depend on methotrexate treatment. Serum adenosine deaminase and 5'-nucleotidase activities are not good indicators of rheumatoid arthritis activity.

Key words:
arthritis, rheumatoid; adenosine deaminase; 5'-nucleotidase.

Apstrakt

Uvod/Cilj. U patogenetskoj osnovi reumatoidnog artritisa (RA) su inflamacija, promena imunog sistema i oštećenje ćelija. Značajnu ulogu u procesu oštećenja tkiva posredovanog inflamacijom imaju 5'-nukleotidaza (5'-NT) i adenozin dezaminaza (ADA). Cilj rada bio je određivanje aktivnosti adenozin dezaminaze i 5'-nukleotidaze u serumima obolelih od RA lečenih uz pomoć ili bez terapije metotreksatom (MTX), kao i utvrđivanje povezanosti ovih enzima sa aktivnošću bolesti. **Metode**. Ispitivanjem je bilo obuhvaćeno 160 bolesnika obolelih od RA, od kojih 60 nije bilo lečeno MTX (prosečne starosti 56,8 godina; 68,3% žena) i 100 bolesnika na terapiji MTX (prosečne starosti 59,8 godina, 88% žena), kao i 60 ispitanika kontrolne grupe (prosečne starosti 58,8 godina; 66,6% žena). Bolesnici sa hroničnim inflamatornim oboljenjima, hroničnom respiratornom, srčanom i bubrežnom insuficijencijom, težim akutnim oboljenjima, i drugim bolestima od značaja koje bi mogle da modifikuju inflamatorni odgovor bili su isključeni iz istraživanja. **Rezultati**. Nije zabeležena razlika u aktivnosti 5'-NT između ispitivanih grupa. Vrednosti ADA su se značajno razlikovale između ispitivanih grupa. *Post- hoc* analizom (Dunnett-ov T3 test) pokazano je da je aktivnost ADA u grupama značajno viša u odnosu na kontrolnu grupu bolesnika ($p < 0,001$), kao i da je aktivnost ADA u grupi bolesnika sa RA lečenih MTX bila značajno niža u odnosu na aktivnost u grupi bolesnika sa RA koji nisu primili MTX ($p < 0,001$). Nije bilo statistički značajne korelacije između DAS28 skora i aktivnosti ispitivanih enzi-

Correspondence to: Nela Živković, Clinical Center Niš, Clinic of Pediatrics, Bulevar dr Zorana Djindjića 48, 18 000 Niš, Serbia.
E-mail: nelinazivkovic@gmail.com

ma. **Zaključak**. Aktivnost adenozin dezaminaze povećana je kod bolesnika sa reumatoidnim artritisom. Primena metotreksata dovodi do smanjenja aktivnosti ovog enzima u serumu bolesnika sa reumatoidnim artritisom. Aktivnost 5'-nukleotidaze nije povećana kod bolesnika sa reumatoidnim artritisom i ne zavisi od terapije metotreksatom. Aktivnosti adenozin dezaminaze i 5'-nukleotidaze u serumu nisu dobri pokazatelji aktivnosti reumatoidnog artritisa.

Ključne reči:
artritis, reumatoidni; adenozin dezaminaza; 5'-nukleotidaza.

Introduction

Rheumatoid arthritis (RA) is a frequent chronic inflammatory arthropathy which is present in 1% of the world population. Even though the etiology of the disease is not well known the present information indicates that RA is a result of the simultaneous influence of genetic risk factors, hormone factors, immunology and external factors [1]. The disease is characterized by the proliferation of autoreactive clones of T and B lymphocytes with the proliferation of synovial cells, the formation of pannus, activation of chondrocytes and metalloproteinases, which leads to the destruction of joint cartilage, bones and the surrounding structures [2]. The main characteristics of early immune reaction in RA are T lymphocytes, especially CD4 cells which initiate the entire sequence of further events.

Rheumatoid arthritis is followed by the increased activity of enzymes which take part in nucleic acid metabolism [3]. Adenosine deaminase (ADA) plays a significant role in the process of inflammation-caused tissue damage. ADA is an enzyme which regulates cellular and extracellular concentration of adenosine and deoxyadenosine along with 5'-nucleotidase (5'-NT) and adenosine kinase. The increase of ADA in serum of RA patients is related to its release from damaged cells [4]. This enzyme has the metabolic significance due to its role in the catabolism of purine derivatives of adenosine and deoxyadenosine, as well as in proliferative activity of immunocompetent cells [5]. ADA is a ligand of CD26 protein (ADA binding protein-ADAbp) which, in interaction with CD26, has a stimulating effect on T cell receptor (TCR), i.e. acts as a mediator in T cell activation [6]. Activation of T-cell response initiates a sequence of reactions which result in increased inflammation, thickening of synovial membrane and deterioration of cartilage and bones.

The central role of 5'- nucleotidase is the production of extracellular nucleotides, adenosine being the most important one. Ecto-5'-NT is believed to be the marker of human B-lymphocyte maturation, having in mind that it is increased during normal development, and reduced in immunodeficiency conditions [7]. Even though circulating monocytes show the low activity of ecto-5'-NT, differentiation of monocytes results in increased activity of this enzyme. Additionally, increased values of this enzyme in synovial fluid are significant in terms of diagnostics [8].

Clinical presentation of RA is characterized by numerous symptoms and signs which refer to joints, periarticular structures and internal organs. The basic features of RA are a symmetrical pain, swelling, morning stiffness and specific radiological changes. Clearly defined parameters of clinical presentation and complex composite indices, obtained by the combination of subjective and objective parameters, are used for the presentation of the disease activity.

In the era of biological targeted therapies, MTX is still the gold standard in the treatment of RA [9]. After absorption, 10% of this drug is converted in 7-hydroxy MTX in the liver, and afterwards, both are excreted through kidneys [10–12]. Several pharmacological mechanisms of MTX effects are explained, including anti-inflammatory effect mediated by stimulating adenosine receptors [13, 14]. MTX causes reduction of ADA level in three ways: firstly, MTX can directly inhibit ADA; secondly, MTX inhibits ADA indirectly through aminoimidazole carboxamide ribose-5-phosphate (AICAR) and its metabolites; and thirdly, ADA can be inhibited indirectly to compensate the adenosine reduction. The reduction of ADA causes an increase in adenosine level and consequently anti-inflammatory effect [14].

Biomarkers which refer to synthesis and degradation of cartilage and bones, inflammation and autoimmune processes which might have clinical significance in the assessment of the diseases' presentation have been the subject of interest in the past ten years. Purine metabolism enzyme testing has become more frequent, having in mind that understanding pathophysiological role these enzymes have in the progression of RA may be useful, not only in diagnostics but also in the process of monitoring the clinical course of the disease and therapy effects.

The aim of the study was to define 5'-nucleotidase and adenosine deaminase activity in the serum of patients with rheumatoid arthritis treated with methotrexate and patients with rheumatoid arthritis who were not treated with methotrexate, as well as to determine the correlation between the enzymes' activities and the disease activity.

Methods

The study included 160 patients suffering from RA; 60 of them were not treated with methotrexate (average age 56.8; 68.3% female patients) and 100 patients were treated with methotrexate (average age 59.8 years; 88% female patients), as well as 60 healthy controls (average age 58.8; 66.6% female patients). All included patients were treated at the Clinic for Rheumatology of the Institute for Treatment and Rehabilitation" Niška Banja", and the study was approved by the local Ethics Committee.

Diagnosis and classification of RA were performed based on revised American College of Rheumatology (ACR) classification criteria from 1987 [15]. Patients suffering from chronic inflammatory diseases (connective tissue diseases,

other systemic diseases), chronic respiratory, cardiac and kidney insufficiency, severe acute diseases and other diseases which might modify inflammatory response were not included in the study. General methodological approach implied a prospective analysis of medical history, as well as clinical and laboratory indicators.

Clinical assessment

Clinical assessment of patients implied physical examination with special emphasis on locomotor system. The number of painful and swelling joints was recorded. Subjective assessment of general health was defined by means of visual analogue scale (VAS) – 100 mm.

Disease activity score-sedimentation rate (DAS28 SE), a combined index which included palpation sensitivity and swelling of 28 joints: shoulders, elbows, metacarpophalangeal (MCP) joints, proximointerphalangeal (PIP) joints and knees, sedimentation rate and patients' assessment of general health, was used for determining disease activity. DAS28 SE score was a number on a scale from 0 to 10 which showed current disease activity. Disease activity level was considered low in case DAS 28 ≤ 3.2, medium in case 3.2 < DAS 28 ≤ 5.1 or high if DAS 28 > 5.1. In case of DAS 28 < 2.6 the disease was considered to be in remission [16].

Laboratory assessment

Patients were taken a blood sample from cubital vein early in the morning before they had breakfast (no food intake for 12 hours). Blood samples for laboratory analysis were kept in heparinized blood collection tubes. Peripheral blood was taken by means of venipuncture and the serum was separated by centrifugation at room temperature at 3500 cycle/min for 15 minutes. The samples were used for determining: erythrocytes (Er), hemoglobin (Hb), thrombocytes (Tr), leukocytes (Le), erythrocyte sedimentation rate (ESR), 5'-NT and ADA.

The activity of 5'-NT was defined by means of spectrophotometry, using Wood and Williams' method [17]. The method was based on defining released inorganic phosphorus from adenosine monophosphate (AMP). Aliquot of 0,5 ml of serum was incubated for 30 minutes at 37°C in the presence of barbiturate buffer pH = 7.8 with manganese as an activator and 10 μmol Mn-AMP as a substrate. After adding hydrazine sulfate-tin chloride solution and ammonium molybdate, released inorganic phosphorus was defined by means of spectrophotometry at 618 nm.

Adenosine deaminase assay kit produced by Diazyme was used for determining ADA activity in the serum. ADA determination was based on the enzymatic conversion of adenosine to inosine which was converted to hypoxanthine with the aid of purine nucleoside phosphorylase (PNP). Hypoxanthine was then converted to uric acid and hydrogen peroxide (H_2O_2) with the assistance of xanthine oxidase (XOD). One ADA unit was defined as the quantity of enzyme necessary for releasing 1 μmol/min of inosine from adenosine at 37°C. Adenosine deaminase activity was expressed as unit *per* liter.

Statistical assessment was carried out in Excel 7.0 and SPSS 11.0 in Windows 98 environment. The results were shown in tables. Comparison of mean values of continuous variables of the tested groups was carried out by means of ANOVA test with additional *Post-hoc* analysis (Dunnett's T3 test). Bivariate correlation analysis [(Spearman's correlation coefficient (r) for nominal and ordinal data and Pearson's correlation coefficient (C) for continuous numerical data] was used for the analysis of interrelation between the disease activity and activities of 5'-NT and ADA. Significance level $p < 0.05$ was accepted as significant.

Results

The study included 160 patients with RA; 60 of them were not treated with MTX (41 female, 19 male) and 100 patients were treated with MTX (80 female, 20 male), as well as 60 healthy controls (40 female, 20 male). The average age of the patients was similar for all groups. The average duration of the disease in patients who were not treated with MTX was 12 months, and 120 months in patients treated with MTX.

Basic characteristics of the tested groups of RA patients and the controls were shown in Table 1.

Table 1

Demographic and clinical characteristics of patients with rheumatoid arthritis (RA) and controls

Characteristics of patients	RA without MTX treatment (n = 60)	RA with MTX treatment (n = 100)	Control (n = 60)
Age (years), $\bar{x}$ ± SD	56.86 ± 12.96	59.81 ± 11.82	58.86 ± 18.05
Gender (female), n (%)	41 (68.3)	88 (88)	40 (66.6)
Disease duration (months), $\bar{x}$ ± SD	12.5 ± 7.2**	124.4 ± 81.0	-
No. of painful joints, $\bar{x}$ ± SD	12.2 ± 7.5	12.3 ± 7.6	-
No. of swollen joints, $\bar{x}$ ± SD	4.1 ± 3.0	3.2 ± 2.0	-
ESR (mm/h), $\bar{x}$ ± SD	35.8 ± 27.8	29.9 ± 20.1	8.6 ± 4.3**
VAS general health score (0–100 mm scale), $\bar{x}$ ± SD	32.86 ± 19.9	40.02 ± 22.7	17 ± 16,5**
DAS28 score, $\bar{x}$ ± SD	5.13 ± 1.3	4.66 ± 1.4	1.02 ± 1.23**

$\bar{x}$ – mean; SD – standard deviation; ESR – erythrocyte sedimentation rate; VAS – visual analogue scale; DAS – disease activity score; MTX – methotrexate; **$p < 0.01$ as compared to other groups.

There was no statistically significant difference in the level of the disease activity assessed by DAS28 score in the above mentioned groups of patients. However, numerical results showed that the value of DAS28 score in patients not treated with MTX was 5.11 which pointed to the high activity of the disease. The average DAS28 score in patients treated with MTX was 4.66 which pointed to moderate activity of the disease. χ^2-test showed that the controls had a significantly lower DAS28 score ($p < 0.01$).

Values of the 5'-NT and ADA are shown in Table 2.

ADA values were significantly different in all tested groups. *Post-hoc* analysis (Dunnett's T3 test) showed that ADA values in the control group were significantly smaller as compared to those in the RA groups ($p < 0.001$), and that ADA value in the MTX group was significantly smaller as compared to that in the no MTX group ($p < 0.001$). There was no statistically significant difference in 5'-NT values among groups (Table 2).

The correlation between DAS28 score and the serum activities of 5'-NT and ADA by groups is shown in Table 3.

The connection between the activities of the tested enzymes and MTX treatment was analyzed by means of binary logistic regression. The analysis showed that the entered model could explain 47% of the variance of the dependent variable, which pointed to the strong predictive value of the model. The increase of ADA activities was found in the RA group without MTX treatment (OR = 0.98, 95% CI 0.97–0.99).

Having in mind that we recorded significant difference in the diseases duration for the MTX and no MTX groups, we tested the correlation between the disease duration and the serum activity of ADA and 5'-NT and recorded mean negative correlation between ADA activity and the disease duration, i.e. the longer the disease, the less ADA activity. However, if we disregarded the effects of MTX treatment, we could see the stronger negative correlation between the two variables (significance level of 0.001).

Discussion

Previous research indicates that activities of some purine enzymes in rheumatoid arthritis differ from those in healthy controls. However, the question which referred to the way these enzymes affected the RA activity remained unanswered.

We did not record the significant difference in 5'-NT activity in RA patients and the controls. One study showed that the activities of serum 5'-nucleotidase were increased in patients with RA, i.e. increase of serum 5'-NT appeared at 30% to 66% of patients with RA [18]. Some researchers pointed that this enzyme was present in synovial fluid at 58% of RA patients and that its concentration was much larger than in the serum. Therefore, testing 5'-NT in synovial fluid could have significant diagnostic value for this type of patients [19]. Literature data on role and origin of 5'-NT in RA was quite contradictory. Additionally, they believed that 5'-NT activities in synovial fluid represented a useful indicator of inflammatory activity in synovial membrane, but that 5'-NT in serum did not have the same significance. The majority of other researchers believed that the importance of this enzyme in RA inflammatory response was negligible, because the increase of 5'-NT in synovial fluid was not statistically significant even though it was verified. The goal of our study was to test the activity of this enzyme in the serum. However, our

Table 2

Values of 5-nucleotidase (5'-NT) and adenosine deaminase (ADA) serum activities in tested groups of patients

Enzyme	RA without MTX treatment	RA with MTX treatment	Control
ADA (IJ/L), $\bar{x} \pm SD$	19.32 ± 5.43**	12.05 ± 6.57**	5.09 ± 1.51**
5'NT (IJ/L), $\bar{x} \pm SD$	37.63 ± 23.71	39.25 ± 15.01	39.15 ± 21.25

$\bar{x}$ – mean; SD – standard deviation; **$p < 0.01$ as compared to other groups; ANOVA and *Post-hoc* Dunnett's T3 test; RA – rheumatoid arthritis; MTX – methotrexate.

Table 3

Correlation coefficients between disease activity score (DAS28) and enzyme activity in patients with rheumatoid arthritis (RA) and controls

Groups of patients	5'-NT	ADA
RA without MTX treatment	0.108	-0.115
RA with MTX treatment	0.128	0.170
Control	-0.033	0.105

5'-NT – 5-nucleotidase; ADA – adenosine deaminase; MTX – methotrexate.

Even though the groups were of similar age structure, we tested the effect of the age on the activities of the tested enzymes. The results obtained by ANOVA analysis showed no statistically significant difference in ADA and 5'-NT enzymes serum activities in terms of age.

The result obtained by *t*-test of independent samples did not show statistically significant difference in serum activities of ADA and 5'-NT in terms of genders.

further research would most definitely test synovial fluid in patients with RA.

The analysis showed statistically significant higher values of ADA in patients with RA than in healthy controls. Mean value of ADA in the serum of RA patients with MTX therapy was 12.05 ± 6.57 U/L, the value of analyzed enzyme in patients with RA without MTX was 19.32 ± 5.43, while the recorded value for healthy controls was 5.09 ± 1.51 U/L. The results of most previous studies showed higher values of ADA - for instance, one group of authors [20] stated that patients with RA had ADA of 26.67 U/L ± 8.74 U/L and healthy controls 19.79 ± 5.63. Surekha et al. [21] presented high serum activity of ADA in patients with RA (59.79 U/L ± 21.09 U/L), while the value in healthy controls' was 20.71 ± 5.63. Aside from the disagreement between the activities of the enzyme, this analysis confirmed the results of the above mentioned analyses [20, 21] which stated that patient with RA showed higher activity of ADA in the serum. Unlike the results that we obtained, other studies [22, 23] showed similar results of ADA activity in patients with RA and Mn control group.

The results of previous research, which referred to the interrelation between purine cycle enzymes and clinical presentation of RA, were contradictory, especially those which referred to the interrelation between RA activity and purine enzyme activity. The majority of studies showed a positive correlation between ADA activity in serum and the disease activity. A group of authors [24] tested the total serum of activity ADA in RA patients in different disease stages and showed that ADA activity was in significant correlation with DAS28. Some authors stated that ADA activity in the serum could predict RA activities [25]. Results of several studies [24, 26] showed that ADA and its isoenzymes could be used as alternative parameters for showing the disease activity. Namely, they recorded clear correlation between DAS28 score in RA and catalytic activity of total ADA and ADA2 isoenzymes. Additionally, they stated that isoenzyme form of ADA 2 was increased along with the disease progression.

Unlike the above-stated results, one study [27] showed no positive correlation between ADA activity and parameters of RA activities, especially in patients who had been treated with the nonsteroidal anti-inflammatory drug (NSAIL) and anti-TNF inhibitors. The latest research [28] which included 110 RA patients and 55 controls did not record any positive correlation between ADA values and the disease activity. However, the research confirmed increased activity of ADA in the serum of RA patients as compared to that in the control group.

Attempting to continue previous research, we analyzed the correlation between serum ADA and the disease activities and showed that the disease activity measured by DAS28 score was not in correlation with serum ADA activity. Even though our results showed increased serum ADA activity in the RA group without MTX (Table 2), we did not obtain a positive correlation between the disease activity and ADA activity in the serum.

Literature data on role and origin of 5'-NT in RA were also contradictory. Most authors agreed that increased levels of this enzyme were present in the active disease, while lower levels were expected in remission stage. Erer et al. [27] showed that the level of 5'-NT was increased with the disease activity increase and stated that synovial 5'-NT was probably isoenzyme of the serum 5'-NT. Our results showed that there was no significant correlation between DAS28 score and 5'-NT activity in the MTX and no MTX groups. Additionally, we did not record the change of 5'-NT activity in terms of age. Contrary to our results, one extensive research showed that the level of 5'-NT dropped with age, as well as that the values of 5'-NT were by 27% lower at male than at female patients [29].

It would be very hard to compare the results of our study with previous ones because the enzymes were tested on different enzyme kits. Additionally, tests were carried out on different groups of patients (in terms of gender and age distribution) with different disease duration and therapeutic approach. Stolk et al. [29] pointed out that majority of RA patients who participated in the research at medical and research centers had already been treated with NSAIL, while large number of them was treated with some of the disease-modifying antirheumatic drugs (DMARD) and therefore the effects that such treatment had on ADA and 5'-NT activity cannot be excluded.

It should be emphasized that almost all of our patients used NSAIL, while three patients were treated with paracetamol. Patients with RA and controls did not use corticosteroids during the month they were included in the study. The average dose of MTX applied in patients with RA was 14,5 mg a week.

It is well-known that reduction of the local concentration of adenosine, by ADA contributes to joint inflammation in RA. MTX increases the concentration of extracellular adenosine at inflammation area [30, 31]. Analysis of results confirmed that activity of ADA was significantly lower in RA patients treated with MTX therapy than in patients who were not treated with MTX. These results are in accordance with the mechanism of MTX action on adenosine metabolism, i.e. ADA inhibition. Previously, researchers [22, 32, 33] also presented a significant difference between the ADA activity in patients with RA treated with MTX and in patients who did not receive MTX in their therapy. On the other hand, we did not find any positive correlations between the activity of 5'-NT and MTX treatment.

The disease duration in patients with no MTX treatment was 12 months, which was significantly less as compared to a group treated with MTX (disease duration approximately 120 months). Our results indicated that the longer the disease, the less serum ADA activity. However, if we disregarded the effects of MTX treatment, we could see the stronger negative correlation between the two variables. The activity of 5'-NT in the tested groups did not depend on the disease duration. A study [13], which monitored the changes of purine cycle enzyme activities during MTX treatment of RA patients, showed similar results. There were no differences in enzyme activities after 6 weeks of MTX treatment. However, the study recorded a significant drop in the ADA activity and no changes in 5'-NT activity after 48 weeks.

Recent research [13] did not show mutual dependence between 5'-NT and ADA activities, and gender. We did not find the changes in 5'-NT and ADA activities in terms of

gender. However, Stolk et al. [29] showed that the activity of 5'-NT was by 27% lower in male than in female patients.

Our research did not record the significant effect of age on ADA and 5'-NT activities. It should be emphasized that some researches showed the decrease of 5'NT with age increase [29, 34].

Conclusion

We demonstrated that serum adenosine deaminase activity was increased in patients with rheumatoid arthritis, as well as that the application of methotrexate led to its decrease. Serum activity of 5'-nucleotidase was not increased in patients with rheumatoid arthritis and did not depend on methotrexate treatment. Serum adenosine deaminase and 5'-nucleotidase activities were not good indicators of rheumatoid arthritis activity.

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Two-dimensional echocardiographic evaluation in liver cirrhosis patients in prediction of cirrhotic cardiomyopathy

Dvodimenzionalna ehokardiografska evaluacija obolelih od ciroze jetre u predviđanju cirotične kardiomiopatije

Violeta A. Dinić-Radović*, Aleksandar V. Nagorni*†, Milan M. Radović†‡, Svetlana Apostolović‡§, Goran B. Bjelaković*†, Biljana Radovanović-Dinić*†, Snežana Tešić-Rajković*

Clinical Center Niš, *Clinic for Gastroenterology and Hepatology, ‡Clinic for Lung Diseases, §Clinic for Cardiovascular Diseases, Niš, Serbia; University of Niš, †Faculty of Medicine, Niš, Serbia

Abstract

Background/Aim. Cirrhotic cardiomyopathy (CCM) is a clinical syndrome in liver cirrhosis (LC) patients, which is characterized by the abnormal cardiovascular (CV) response to physiologic, pathologic, or pharmacologic stress provocation, but normal to increased cardiac output and contractility at rest. The aim of the study was to identify the structural and functional myocardial changes in the prediction of CCM in patients with LC of various origins in advanced stages. **Methods.** The research was performed as a prospective, nest case-control study, on carefully selected 40 patients in the advanced stage of LC and negative personal medical history on previous CV disease and 40 healthy subjects as the control, from January 2012–December 2014. Echocardiographic parameters significant for prediction of the development and/or presence of CCM were determined by trans-thoracic two-dimensional Doppler echocardiography imaging. **Results.** Most of the LC patients were alcoholic (80%), dominantly in Child-Pough C stage of the disease (70%). The average value of QT interval in the LC patients was significantly higher (0.44 ± 0.03 ms vs 0.42 ± 0.01 ms; $p < 0.001$), as well as brain natriuretic peptide (BNP) serum level (284.61 ± 181.44 ng/L vs 69.41 ± 31.08 ng/L; $p < 0.001$) compared to those in the healthy subjects. A significant association with serum BNP level in LC patients was shown with left atrial diameter ($p = 0.031$), left ventricular ejection fraction ($p = 0.014$), pulmonary artery systolic pressure (PASP) ($p = 0.000$) and the presence of tricuspid valve regurgitation of 2+ ($p = 0.000$), affecting its change of 41.6%. **Conclusion.** The obtained results suggest that LC patients have significant echocardiographic signs of myocardial dysfunction, as well as the increased BNP serum level. Left atrial diameter, left ventricular ejection fraction, PASP and tricuspid valve regurgitation are valuable echocardiographic predictors of CCM.

Key words:
liver cirrhosis; cardiomyopathies; echocardiography, doppler; natriuretic peptide, brain.

Apstrakt

Uvod/Cilj. Cirotična kardiomiopatija predstavlja klinički sindrom obolelih od ciroze jetre, koji karakteriše nenormalno usporen odgovor na fiziološke, patološke, ili farmakološke draži, uz normalno povećan minutni volumen srca i kontraktilnost u mirovanju. Cilj istraživanja je bio identifikacija strukturnih i funkcionalnih promena miokarda, kao prediktora cirotične kardiomiopatije, kod bolesnika sa cirozom jetre različite etiologije u odmaklim fazama bolesti. **Metode.** Istraživanje je sprovedeno po tipu prospektivne studije slučaja, na odabranih 40 bolesnika u odmakloj fazi ciroze jetre sa negativnom ličnom medicinskom istorijom na kardiovaskularne bolesti i 40 zdravih ispitanika kao kontrolom, u periodu od januara 2012. do decembra 2014. godine. Pomoću transtorakalne dvodimenzionalne dopler ehokardiografije određivani su parametri od značaja za razvoj i/ili prisustvo cirotične kardiomiopatije. **Rezultati.** Većina obolelih bila je sa alkoholnom cirozom jetre (80%), dominantno u Child-Pough C stadijumu bolesti (70%). Prosečne vrednosti QT intervala kod obolelih od ciroze bile su značajno više (0,44 ± 0,03 ms *vs* 0,42 ± 0,01 ms; $p < 0,001$), kao i serumske vrednosti moždanog natriuretskog peptida (BNP) (284,61 ± 181,44 ng/L *vs* 69,41 ± 31,08 ng/L; $p < 0,001$), u poređenju sa onima kod zdravih ispitanika. Oboleli od ciroze jetre imali su značajnu povezanost između serumskih vrednosti BNP i dijametra leve pretkomore ($p = 0,031$), ejekcione frakcije leve komore ($p = 0,014$), sistolnog pritiska u pulmonalnoj arteriji

Correspondence to: Violeta Dinić-Radović, Clinical Center of Niš, Clinic for Gastroenterology and Hepatology, Bul. Dr Zorana Djindjića 48, 18 000 Niš, Serbia. Phone: + 381 18 23 37 88; +381 184 537 343. E-mail: violeta2807@gmail.com

(p = 0,000) i prisustva regurgitacije trikuspidne valvule od 2+ (p = 0,000), koji su uticali na promenu vrednosti BNP za 41,6%. **Zaključak.** Rezultati ovog istraživanja sugerišu da oboleli od ciroze jetre imaju značajne ehokardiografske znake miokardne disfunkcije, uz povećan serumski nivo BNP. Dijametar leve pretkomore, ejekciona frakcija leve komore, pritisak u pulmonalnoj arteriji i prisustvo regurgitacije trikuspidalne valvule, predstavljaju važne ehokardiografske prediktore cirotične kardiomiopatije.

Ključne reči:
jetra, ciroza; kardiomiopatije; ehokardiografija, dopler; natriuretski peptid, moždani.

Introduction

The heart and liver may interact in several different ways. Acute or chronic heart failure (HF), especially in case of the right HF, may lead to a spectrum of many liver disorders, including the cardiac cirrhosis, or congestive hepatopathy [1]. On the other hand, chronic liver disease such as cirrhosis may affect the heart and the whole cardiovascular system, leading to a clinical syndrome of cirrhotic cardiomyopathy (CCM) [2].

Cirrhotic cardiomyopathy was first defined in 2005 on the annual expert consensus meeting of the World Gastroenterology Organisation in Montreal as: "a cardiac dysfunction in patients with liver cirrhosis characterised by impaired contractile responsiveness to stress and/or altered diastolic relaxation with electrophysiological abnormalities in the absence of other known cardiac disease" [3, 4]. Although this term had a significant lack of strict criteria, the prevalence of CCM is reported to be between 40% to 50% in cirrhosis patients, while its development seems to be independent of the etiology of liver disease [5, 6].

The different myocardial adaptations seen in liver cirrhosis (LC) can be classified in structural, electrophysiological and functional changes. Structural changes include enlarged cardiac chambers and increased myocardial mass [7]. A prolonged QT interval and an abnormal chronotropic response to stress, are the main electrophysiological changes. Functional changes mainly consist of an impaired cardiac response to exercise or diastolic dysfunction [1, 7, 8]. The most common cardiac abnormalities on autopsy were left or right ventricular dilatation and left ventricular hypertrophy, which are encountered in more than 30% of the patients [9]. Some echocardiographic studies show conflicting data, but left atrial (LA) dilatation and mild left ventricular (LV) hypertrophy are the most common reported abnormalities [10, 11]. These changes probably reflect a combination of mechanical overload due to hyperdynamic circulation and neurohormonal activation [2, 12, 13].

Diastolic dysfunction is the most prominent functional alteration seen in CCM [3, 4]. It is due to a combination of myocardial hypertrophy, fibrosis due to increased aldosterone levels and subendothelial oedema. The reported prevalence is between 45% and 56% [8]. There are no strict diagnostic criteria but impaired cardiac response to stress, LV diastolic dysfunction, and prolonged QT are the most prominent features [14].

Cirrhotic cardiomyopathy is diagnosed if there is any evidence of systolic or diastolic myocardial dysfunction, along with associated criteria such as electrophysiological abnormalities and/or changes in serum markers levels [4, 14]. Many studies have made the link between the decrease in LV function and increased concentrations of natriuretic peptides in plasma, which opened the possibility of biochemical confirmation of heart failure [15, 16]. The most reliable results are obtained with the N-terminal atrial natriuretic peptide (NT ANP), brain natriuretic peptide (BNP) and its precursor N-terminal pro BNP (NT pro BNP) [13]. Analysis of these peptides is clinically significant as a test to exclude the diagnosis of heart failure due to their high negative predictive value [12].

Data on the clinical significance of CCM are scarce but several publications indicate a poor prognosis for these patients. In patients prior to performing a transjugular portosystemic shunt (TIPS), diastolic dysfunction expressed as an E/A ratio ≤ 1, was a predictor of ascites persistence and death [17]. The presence of the cardiomyopathy should be suspected in patients with worsening hemodynamics. Such patients may benefit from more aggressive monitoring and treatment of the underlying pathology leading to decompensation, and from close monitoring during procedures that could cause decompensation i.e., TIPS, paracentesis, liver transplantation (LT) [18, 19].

The aim of the study was to identify the structural and functional myocardial changes in patients with liver cirrhosis of various origin in advanced stages of the disease, by two-dimensional echocardiographic evaluation, assessing its significance and correlation with serum levels of BNP in the prediction of CCM.

Methods

Patients

The research was performed as a prospective, nest case-control study, in the Clinic for Gastroenterology and Hepatology and Clinic for Cardiovascular Disease, Clinical Center of Niš, on carefully selected 40 patients with verified liver cirrhosis in the advanced stage of the disease (Child-Pough B and C stage) and the negative personal medical history on previous cardiovascular disease, as an experimental group, and 40 healthy subjects as the control one, in the period between January 2012 and December 2014. All the patients gave informed and written consent for the participation in the study, while the ethical approvement for the research was obtained from the Academic Council of Faculty of Medicine, University of Niš (N^{o} 04-828/12).

Diagnosis and staging of liver cirrhosis

The diagnosis of cirrhosis was based on clinical, echocardiographic (Toshiba Ecossee 96, 3.75 MHz convex probe, 1996, Japan) and laboratory parameters of liver damage, and in some

cases histologically by parenchymal biopsy. The severity degree of cirrhosis was evaluated by "scoring" system of functional liver damage by using the Child-Pough classification [20].

Cardiovascular examination

In all of the observed patients was done: a) standard electrocardiogram in 12 leads, b) arterial blood pressure measurement (mmHg), c) heart rate measurement, d) serum values of brain natriuretic peptide (ng/L) (Olympus, AU 400, 2003, Japan) and e) echocardiography examination determining the values of left ventricular end-diastolic and end-systolic diameter, LV ejection fraction and LA diameter, E/A ratio, deceleration time (DT) and isovolumic relaxation time (IVRT), right ventricular diameter, presence of tricuspid valve regurgitation and pulmonary artery systolic pressure (PASP). Echocardiographic study was made in M-mode technique, by two-dimensional Doppler echocardiographic examination (ACUSON X 300, KT-LM 150 HD, Siemens, Germany) [21].

Follow-up of the patients

At the moment of hospitalization, patients underwent a standard clinical, echosonographic and laboratory processing in order to verify the cause and severity of liver cirrhosis, and then performed cardiovascular analysis in accordance with inclusion and exclusion criteria for the entry into the study, whereupon were placed in the experimental subjects group. All subjects of the experimental group were treated by hepatoprotective and substitution therapy in accordance with the stage of liver cirrhosis. The control group subjects made only the cardiovascular analysis.

Statistical analysis

Statistical analysis was performed on the personal computer. Excel program from Microsoft Office 2007 software package was used for entering, ranking, clustering, tabular and graphical display of data. All calculations were performed using SPSS program ver.18.0. In all analysis, the limit of statistical significance as the default error estimates of 0.05.

The mean ($\bar{x}$), standard deviation (SD), structure (%) and 95% confidence interval (95% CI) were shown in the description analysis. Comparison of mean values of numerical characteristics between two examined groups of patients was done by Student's-*t* test or Mann-Whitney U test in cases where the distribution of values did not meet the requirements of the normal distribution. Comparison of the frequency of attribute characteristics between groups was performed by Mantel-Haenszel χ^2 test or Fisher exact test's probability of the null hypothesis, in cases where some of the features expected frequency was less than five [22].

Analysis of the relationship of investigated factors and indicators of cardiac function were done by the linear regression analysis. There were calculated values of the regression coefficients (β) and the boundaries of their 95% confidence intervals (95% CI). All the examined factors were involved in a univariate model of analysis. Those factors which shown significant association with the dependent variables in univariate models, were included in the multivariate models, and then, applying the backward method of multivariate model, were excluded under the control of the influence of other factors involved, which did not show a significant impact on the dependent variable, as long as the model did not contain only major factors and constant of regression [23].

Results

The observed groups of patients were homogenous in relation to the average distribution of general demographic characteristics (age, gender, social status, living and working place). In relation to ethiology of liver cirrhosis, most of the patients in experimental group were with alcoholic liver cirrhosis (32/80%). According to Child-Pough classification, there was registered a significantly higher number of patients in stage C of disease (28/70%), in relation to those in stage B of disease (12/30%). From typical clinical symptoms and signs of cirrhosis deterioration, which were decisive parameters in the further assessment for the effects on cardiovascular function in observed patients of the experimental group, there were registered: ascites in 29 (72.5%), encephalopathy in 19 (47.5%), jaundice in 21 (52.5%) and the presence of esophageal varices in 30 (75%) of the patients.

Results of examined cardiovascular system parameters in liver cirrhosis patients (experimental group)

The average values of arterial blood pressure in cirrhosis patients were within normal limits and amounted to 113.38 ± 14.82 mmHg for systolic blood pressure, and 70.13 ± 10.03 mmHg for diastolic one. For these subjects, electrocardiogram registered an average moderately elevated heart rate frequency from 86.9 ± 14.45 beats *per* minute, while in 22 (55%) of respondents, the extended value of the QT interval (> 0.44 s) was registered, an average of 0.44 ± 0.03 s. In 34 (85%) patients with cirrhosis, an average increased serum level of BNP of 284.61 ± 181.44 ng/L was registered. The presence of pathologically altered values of echocardiographic parameters in patients with liver cirrhosis is shown in Figure 1.

Only in 5% of cirrhosis patients, a reduced LV ejection fraction (<55%), with average values at the group level of 64.43 ± 4.12% was registered. Increased LA diameter (> 40 mm) was registered in 40% of respondents, the average at the group level of 38.28 ± 6.17 mm. Reduced E/A ratio (< 1 ms), was registered in 29 (72.5%) cirrhosis patients, with mean values at the group level of 0.93 ± 0.28 ms. Extending values of DT (> 200 ms), were registered in 38 (95%) patients, with average values, at the group level of 275.65 ± 71.25 ms, as well as IVRT (> 80 ms) in 30 (75%) with an average value at the group level of 90.52 ± 22.82 ms. Increased right ventricular diameter (RVD) (> 25 mm), was registered in 31 (77.5%) of cirrhosis patients, with average values of 26.63 ± 2.93 mm and tricuspid valve regurgitation was present in 24 (60%), mostly of the first degree (55%). The average value of the inferior vena cava diameter in in-

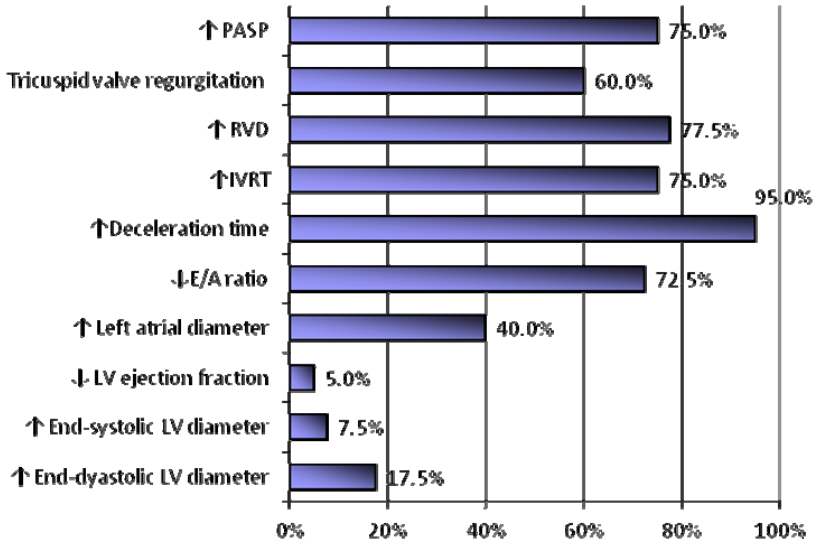

Fig. 1 – The presence of pathologically altered values of echocardiographic parameters in patients with liver cirrhosis PASP – pulmonary artery systolic pressure; RVD – right ventricular diameter; IVRT – isovolumic relaxation time; LV – left ventricle.

spirium amounted to 2.27 ± 3.69 cm. In 30 (70%) an increased PASP (>25 mm) was registered, in an average of 31.75 ± 7.89 mm, as shown in Figure 1.

Comparison of the cardiovascular system parameters in the assessment of myocardial functions between two observed groups of patients

The average value of the arterial systolic blood pressure in the cirrhosis patients was 113.38 ± 14.82 mmHg, which was significantly lower compared to the healthy subjects (125.38 ± 8.65 mmHg) ($p < 0.001$). Mean arterial diastolic blood pressure was not significantly different between the observed groups and was within normal range (70.13 ± 10.03 *vs* 72.0 ± 8.83 mmHg; $p = 0.378$). From the examined electrocardiographic parameters, the average value of heart rate was statistically significantly higher in the cirrhosis patients compared to that in healthy subjects (86.9 ± 14.45 *vs* 70.97 ± 6.07 n/min; $p < 0.001$), as well as the average value of QT interval (0.44 ± 0.03 *vs* 0.42 ± 0.01; $p < 0.001$). In patients with liver cirrhosis, the average value of BNP was 284.61 ± 181.44 ng/L, being significantly higher compared to the value of healthy subjects ($p < 0.001$).

Echocardiographic examination of morphological and functional characteristics of the heart cavities, as well as all tested parameters of myocardial dysfunction in patients with liver cirrhosis, verified significant differences in the average values, compared to values in healthy subjects, as shown in Table 1.

Analysis of relationship between the values of echocardiographic characteristics and serum brain natriuretic peptide in cirrhosis patients for predicting cirrhotic cardiomyopathy

Univariate linear regression analysis as significant predictors of serum levels of BNP, among the examined echocardiographic characteristics of heart cavities and the parameters of diastolic dysfunction in liver cirrhosis patients, confirmed: LA diameter, LV ejection fraction, DT, RVD, PASP and the presence of tricuspid valve regurgitation, as shown in Table 2.

Multivariate regression analysis as the most important predictors of serum levels of BNP, among the investigated echocardiographic characteristics of the cirrhosis patients, allocated values of : LA diameter, LV ejection fraction, PASP and the presence of tricuspid valve regurgitation of 2+, as shown in Table 3.

These echocardiographic parameters in common analysis model of association with serum levels of BNP, affecting its change of 41.6%.

Discussion

Liver cirrhosis of various etiology manifests as a state of low systemic vascular resistance, high peripheral volume with low central blood volume, precipitating a state of neurohormonal activation and high cardiac output, which may adversely affect cardiac reserve and lead in clinical syndrome of CCM [14]. In its most benign form, CCM manifests as subtle electrocardiographic (ECG) and echocardiographic abnormalities, most commonly as prolonged QT interval and diastolic dysfunction, but typically with preserved systolic function by two-dimensional ultrasound imaging in the resting and pre-transplant vasodilated state [24–25]. At the extreme, CCM progresses to the heart failure under the hemodynamic stress, unmasked by the demands of variceal bleeding, high doses of vasopressors, reversal of low to high afterload, withdrawal of cardioprotective medications used in the treatment of end stage LC (beta-blockers and mineralocorticoid antagonists), worsened by concomitant sepsis or systemic inflammatory response syndrome [24]. Recently, there is increasing data on the role of cardiac biomarkers, such as

Table 1

Comparison of the average echocardiographic parameters values between liver cirrhosis patients (experimental group) and healthy subjects (control group)

Echocardiographic parameters	Cirrhosis patients (n = 40)	Health subjects (n = 40)	Significance (*p*-value)
Left heart cavities			
left atrial diameter (mm), $\bar{x} \pm SD$	38.28 ± 6.17	34.73 ± 1.91	0.001*
end-systolic left ventricular (LV) diameter (mm) , $\bar{x} \pm SD$	29.48 ± 5.35	27.33 ± 1.69	0.018*
end-diastolic LV diameter (mm), $\bar{x} \pm SD$	47.78 ± 5.48	46.68 ± 3.32	0.281
LV ejection fraction (%), $\bar{x} \pm SD$	64.43 ± 4.12	69.68 ± 3.29	0.001*
Diastolic dysfunction			
E/A ratio (ms), $\bar{x} \pm SD$	0.93 ± 0.28	1.16 ± 0.07	0.001*
deceleration time (ms), $\bar{x} \pm SD$	275.65 ± 71.25	185.15 ± 8.34	0.001*
isovolumic relaxation time (ms), $\bar{x} \pm SD$	90.52 ± 22.82	76.95 ± 2.29	0.001*
Right heart cavities			
right ventricular diameter (mm), $\bar{x} \pm SD$	26.63 ± 2.93	23.38 ± 3.03	0.001*
pulmonary artery systolic pressure (mm Hg), $\bar{x} \pm SD$	31.75 ± 7.89	23.35 ± 2.66	0.001*
inferior vena cava diameter in inspirium (cm), $\bar{x} \pm SD$	2.27 ± 3.69	1.59 ± 0.14	0.253
Tricuspid valve regurgitation, n (%)			
1+	22 (55)	6 (15)	0.001*
2+	2 (5)	0 (0)	0.494

$\bar{x}$ – arithmetic mean; SD – standard deviation; *statistically significant difference.

Table 2

Correlation between echocardiographic parameters of the liver cirrhosis patients with the values of serum brain natriuretic peptide (results of univariate linear regression analysis)

Echocardiographic parameters	ß	Boundaries of 95% CI for ß		Significance (*p*-value)
		lower limit	upper limit	
Left heart cavities				
left atrial diameter (mm)	12.927	5.698	20.156	0.001*
end-systolic left ventricular diameter (mm)	-3.021	-14.41	8.371	0.598
end-diastolic left ventricular diameter (mm)	6.058	-3.997	16.112	0.233
left ventricular ejection fraction (%)	-13.528	-21.30	-5.748	0.001*
Diastolic dysfunction				
E/A ratio (ms)	53.522	-120.0	227.046	0.540
deceleration time (ms)	0.733	0.199	1.268	0.008*
osovolumic relaxation time (ms)	0.996	-1.436	3.428	0.417
Right heart cavities				
right ventricular diameter (mm)	12.998	2.146	23.850	0.020*
pulmonary artery systolic pressure (mm Hg)	11.977	4.631	19.323	0.002*
inferior vena cava diameter (inspirium) (cm)	2.058	-16.03	20.146	0.821
Tricuspid valve regurgitation				
1+	152.444	81.066	223.822	0.000*
2+	404.293	722.3	86.380	0.013

ß – regression coefficient; CI – confidence interval; * statistically significant difference.

Table 3

Correlation between echocardiographic parameters of the liver cirrhosis patients with the values of serum brain natriuretic peptide (results of multivariate regression analysis)

Echocardiographic parameters	ß	Boundaries of 95% CI for ß		Significance (*p*-value)
		lower limit	upper limit	
Left atrial diameter (mm)	7.368	0.711	14.025	0.031*
Left ventriculary ejection fraction (%)	-8.752	-15.675	-1.829	0.014*
Pulmonary artery systolic pressure (mm Hg)	12.940	7.405	18.474	0.000*
Tricuspid valve regurgitation 2+	477.794	714.254	241.334	0.000*
Constant of regression	150.344	-424.484	725.172	0.604

ß – regression coefficient; CI – confidence interval; * statistically significant difference.

BNP, and advanced echo imaging techniques using tissue Doppler imaging (TDI) and strain imaging, to advance the better understanding of this clinical entity.

Prolongation of the QT interval is the most common ECG finding in LC, seen in up to 50% of patients and is associated with sudden cardiac death [26]. Chronic hyperactivation of the sympathetic nervous system and delayed repolarization of cardiomyocytes secondary to defects in K^+ channel function, have been observed in cirrhotic patients with the associated QT interval prolongation, which

may reverse after LT, although may persist in up to 50% of patients [27]. On the other hand, the use of beta-blockers for the varices treatment is associated with QT prolongation reduction [28, 29]. There is also a strong evidence of electromechanical uncoupling, with dysregulation in the normal sequence of cardiomyocyte depolarization and contraction [30, 31]. In our study, 55% of LC patients had prolonged QT interval values, significantly higher compared to the healthy subjects on average ($p < 0.001$).

In addition to ECG testing, echocardiography provides more valuable information regarding the development of clinically important systolic and diastolic dysfunction. Two-dimensional transthoracic echocardiography with TDI is an important imaging technique for better understanding of the dynamic myocardial changes that may occur as cirrhosis worsens, while volume overload becomes more severe, developing a hepatorenal syndrome. The American Association for the Study of Liver Diseases recommends this method as part of the evaluation of liver transplantation cirrhosis candidates to assess for systolic and diastolic dysfunction, outflow gradients, hypertrophy, chamber sizes, and non-invasive assessment of pulmonary pressures [32]. Many echo studies in this patient population have been done, demonstrating variable findings, most notably the presence of diastolic dysfunction, using both two-dimensional imaging and TDI assessments, demonstrating the association of diastolic dysfunction with worsening of cirrhosis [4, 8, 18]. In a recent autopsy study of 133 patients with LC, cardiomegaly and left ventricular hypertrophy were found in up to 43% of patients [9].

Diastolic dysfunction is common in LC patients and has been widely reported in many clinical studies, most commonly evaluating abnormalities in E/A ratio [17, 24]. Using basic two-dimensional echocardiography indices of diastolic dysfunction, pulsed-wave Doppler at the mitral valve leaflet tips provide information about early and late diastolic filling in normal sinus rhythm, with rapid passive filling followed by atrial contraction. Measurements of DT, along with measurement of isovolumic relaxation time by pulsed-wave Doppler at the septal insertion of the mitral valve, provide estimates of diastolic parameters and can guide in categorizing patients on the spectrum of diastolic abnormalities using a validated grading system [33]. However, E/A ratio is load-dependent, making its use in cirrhosis problematic, as the fluid shift is a prominent physiologic derangement in this condition. Diastolic dysfunction parameters may change based on weight, whether or not measurements were obtained before or after paracentesis, or whether obtained before or soon after other major interventions such as TIPS, which precipitates a marked increase in preload and can precipitate fulminant heart failure from latent or subclinical CCM. TDI is an increasingly attractive modality to assess diastolic dysfunction in the setting of suspected CCM given both angle-independence and load-independence [34]. In our investigation, reduced E/A ratio (< 1 ms) has been registered in 72.5% cirrhosis patients, significantly lower compared to healthy subjects ($p < 0.001$). Extending values of DT (> 200 ms), were registered in 95% of patients, as well as prolonged IVRT (> 80 ms) in 75% LC patients, significantly higher compared to values in healthy subjects ($p < 0.001$). On the other hand, we registered a reduced LV ejection fraction (< 55%) in only 5 LC patients, generally significantly lower average values compared to those in the healthy subjects and increased LA diameter (> 40 mm) at 40% of patients. Increased RVD (> 25 mm) was registered in 77.5% of LC patients, while the tricuspid valve regurgitation was present in 60.0%, mostly of the first degree. In 70.0% of investigated LC patients an increased PASP was registered, significantly higher in average compared to values found in the healthy subjects, which is corresponding to the previously mentioned literature data.

Nowadays, a serial use of biomarkers is an increasingly important strategy for the diagnosis and management of patients with CCM. Both BNP and NT-proBNP have been reported to be significantly increased in patients with advanced cirrhosis compared to controls. This increase is probably secondary to increased cardiac production of natriuretic peptides since hepatic degradation does not seem to be affected. Increased BNP and NT-proBNP were associated with the severity of both cirrhosis and cardiac dysfunction [13]. Our investigation showed a significantly higher the average BNP value in LC patients in Child-Pough stage B and C of the disease, compared to values in the healthy subjects ($p < 0.001$). BNP has been studied in cirrhotic patients as a surrogate for cirrhotic cardiomyopathy, with recent predictive data regarding the incidence of renal failure and mortality after liver transplantation, and its association with a model for the end stage liver disease (MELD) and Child-Pough scores, the severity of LC, and diastolic or systolic dysfunction [13, 35, 36]. In our study, the most important predictors of serum levels of BNP, among the investigated echocardiographic characteristics of the LC patients, were values of: LA diameter, LV ejection fraction, PASP and the presence of tricuspid valve regurgitation of 2+. Therefore, an increasing BNP levels in the setting of dyspnea, exertional intolerance, and progressive renal dysfunction in LC patients may alert clinicians to reassess myocardial systolic and diastolic function.

Conclusion

Data obtained in this study indicate that in patients with liver cirrhosis, morphological and functional myocardial damages in the form of cirrhotic cardiomyopathy, are created and developed in the field of the advanced stage of the disease, mostly as a consequence of altered systemic hemodynamics. Patients with liver cirrhosis had significant echocardiographic signs of myocardial dysfunction, as a prolonged deceleration time and isovolumic relaxation time, together with reduced E/A ratio, as well as the increased BNP serum level. Left atrial diameter, LV ejection fraction, pulmonary artery systolic pressure and tricuspid valve regurgitation, were strongly associated with BNP serum levels, which suggests their importance as predictors in the assessment of the origin and development of cirrhotic cardiomyopathy.

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Etiology and resistance patterns of bacteria causing ventilator-associated pneumonia in a respiratory intensive care unit

Uzročnici pneumonije udružene sa ventilatornom potporom bolesnika i njihova rezistencija na antibiotike u pulmološkoj jedinici intenzivnog lečenja

Vlada Injac*, Uroš Batranović†, Jovan Matijašević†‡, Marija Vukoja§, Mirjana Hadnadjev‖, Zoran Bukumirić¶, Goran Trajković¶, Slobodan Janković**

Hemofarm AD, *Research and Development Department, Belgrade, Serbia; Institute for Pulmonary Diseases of Vojvodina, Clinic for Urgent Pulmonology, †Intensive Care Unit, §Center for Pathophysiology of Breathing and Sleep Medicine, ‖Center for Microbiology, Virology and Immunology, Sremska Kamenica, Serbia; University of Novi Sad, ‡Faculty of Medicine, Novi Sad, Serbia; University of Belgrade, Faculty of Medicine, ¶Institute for Medical Statistics and Informatics, Belgrade, Serbia; University of Kragujevac, **Faculty of Medical Sciences, Kragujevac, Serbia

Abstract

Background/Aim. Ventilator-associated pneumonia (VAP) incidence, causative pathogens, and resistance patterns are different among countries and intensive care units (ICUs). In Europe, resistant organisms have progressively increased in the last decade. However, there is a lack of data from Serbian ICUs. The aims of this study were to evaluate etiology and antimicrobial resistance for pathogens causing VAP in ICU patients, to examine whether there were differences among pathogens in early-onset and late-onset VAP and to identify mortality in patients with VAP after 30 and 60 days of hospitalization. **Methods.** A retrospective cohort study was conducted in the respiratory ICU and all adult patients diagnosed with VAP from 2009 to 2014 were included. **Results.** Gram negative organisms were the major pathogens (80.3%). The most commonly isolated was *Acinetobacter* spp (59.8%). There was a statistically significant increase in the incidence of infection with *Klebsiella pneumoniae* (8.9% vs 25.6%; $p = 0.019$). Extensively drug-resistant strains (XDR) were the most common (78.7%). Late-onset VAP was developed in 81.1% of patients without differences among pathogens in comparison with early-onset VAP. *Acinetobacter* spp was susceptible to tigecycline and colistin with a significant increase in resistance to ampicillin/sulbactam (30.2% vs 58.6%; $p = 0.01$). Resistance rate of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* to carbapenems was 38% and 11%, respectively. In methicillin-resistant *Staphylococcus aureus* no resistance was observed against vancomycin and linezolid. There was no difference in mortality rate between patients with early-onset and late-onset VAP after 30 and 60 days of hospitalization. **Conclusion.** Gram negative organisms were the primary cause of bacterial VAP of which the most common was the XDR strain of *Acinetobacter* spp. Patients with early- and late-onset VAP had the same pathogens. There was no difference in mortality between this two group of patients during 60 days of hospitalization.

Key words:
pneumonia; cross infection; anti-bacterial agents; drug resistance, bacterial; respiration, artificial; mortality.

Apstrakt

Uvod/Cilj. Incidencija pneumonije udružene sa ventilatornom potporom bolesnika (VAP), njeni uzročnici i njihova rezistencija razlikuju se između zemalja i jedinica intenzivne nege (JIN). U Evropi je u poslednjih deset godina došlo do progresivnog porasta rezistentnih bakterija. Međutim, ne postoji dovoljno podataka za JIN u Srbiji. Ciljevi rada bili su da se ispita etiologija i rezistencija uzročnika VAP na antibiotike u JIN, da se ispita da li postoji razlika između uzročnika ranog i kasnog VAP i da se utvrdi letalitet kod bolesnika sa VAP nakon 30 i 60 dana hospitalizacije. **Metode.** Retrospektivno kohortno ispitivanje je bilo sprovedeno u pulmološkoj JIN. Bili su uključeni svi odrasli bolesnici sa dijagnostikovanim VAP od 2009. do 2014. godine. **Rezultati.** Glavni uzročnici VAP bili su gram negativne bakterije (80,3%). Najčešće je bio izolovan *Acinetobacter* spp (59,8%). Zabeležen je statistički značajan porast incidencije oboljevanja usled *Klebsiella pneumoniae* (8,9% vs 25,6%; $p = 0,019$). Najzastupljeniji su bili ekstremno rezistentni (XDR) sojevi bakterija (78,7%). Kasni VAP je dijagnostikovan kod 81,1% bolesnika bez razlike u patogenima u poređenju sa ranim VAP. *Acinetobacter* spp je bio osetljiv na tigeciklin i kolistin uz statistički značajan porast rezistencije na ampicilin/sulbaktam (30,2% vs 58,6%; $p = 0,01$). Rezistencija *Pseudomonas aeruginosa* i *Klebsiella pneumoniae* na karbapeneme iznosila

Correspondence to: Vlada Injac, Hemofarm AD, Research and Development Department, Prote Mateje 70, 11 000 Belgrade, Serbia.
E-mail: vlada.cekic@yahoo.com

je 38%, odnosno 11%. Kod meticilin-rezistentnog *Staphylococcus aureus* nije postojala rezistencija na vankomicin i linezolid. Nisu utvrđene razlike u letalitetu između bolesnika sa ranim i kasnim VAP posle 30 i 60 dana hospitalizacije. **Zaključak.** Gram negativne bakterije bile su glavni uzročnici VAP, od kojih je najzastupljeniji bio XDR soj *Acinetobacter* spp. Bolesnici sa ranim i kasnim VAP imali su iste uzročnike. Nije bilo razlike u letalitetu između te dve grupe bolesnika tokom 60 dana hospitalizacije.

Ključne reči:
pneumonija; infekcija, intrahospitalna; antibiotici; lekovi, rezistencija bakterija; disanje, mehaničko; mortalitet.

Introduction

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection in patients receiving mechanical ventilation. VAP remains a major cause of morbidity and mortality among critically ill patients and accounts for more than one-half of all antibiotic use in an Intensive Care Unit (ICU) [1]. The estimated incidence of VAP is 9% to 27%, with a mortality rate of 25% to 50% [2]. The appropriateness of empirical antimicrobial therapy for VAP is a key determinant of patient outcome [2]. Changes in pathogen distribution and patterns of antibiotic resistance complicate antibiotic treatment and care of the patients. Duration of mechanical ventilation was found to be one of the most important factors determining the composition of offending VAP pathogens [3]. Whereas early-onset VAP is more likely to be caused by antibiotic-sensitive bacteria, late-onset VAP is more likely to be caused by multi-drug resistant (MDR) pathogens. However, studies with opposite results were published recently [4–7], which showed that bacteriology of VAP may not follow a pattern of early *versus* late infection, particularly in patients that are at risk for MDR infections [8].

The incidence of infection with specific pathogens with different susceptibility patterns causing VAP may not only vary from hospital to hospital but also within the same hospital or ICU over time [8]. This is the reason why empiric initial antibiotic treatment for VAP should be based on general guidelines, but also on up-to-date information on local epidemiology [8]. There is a lack of National registry consisting data from Serbian ICUs in regard to the local microbiological profile of pathogens causing VAP as well as to their antibiotic susceptibility and resistance patterns.

Given the scarcity of data, the primary aim of the present study was to evaluate etiology and antimicrobial resistance trends for nosocomial pathogens causing VAP in respiratory ICU patients and to examine whether there were differences among pathogens in early-onset and late-onset VAP. The aim of the study was also to identify mortality in patients with VAP after 30 and 60 days of hospitalization.

Methods

Study design

A retrospective cohort study was conducted in the 5-bed respiratory ICU of the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica (Serbia). All adult patients diagnosed with VAP from January 2009 to December 2014, were included. This study was approved by the Hospital Ethics Committee. In order to evaluate changes in the incidence of infection with pathogens and changes in their resistance pattern, 6 year period was divided into two separate periods: period I (January 2009 to December 2011) and period II (January 2012 to December 2014).

Definitions

VAP was diagnosed in the presence of a new or persistent (≥ 48 hours) and progressive radiographic infiltrate plus at least 2 of the following: temperature of ≥ 38ºC or < 35ºC; purulent tracheal secretions or a change in characteristics of sputum; or leucocytosis (> 10.000 white blood cells/mm^3) or leucopenia (< 4.000 white blood cells/mm^3) [9, 10]. After clinical diagnosis of VAP had been established, quantitative culture of endotracheal aspirate (ETA) was performed to identify VAP pathogens. Only pathogens isolated at a concentration of > 10^6 colony-forming units (CFU/mL) was considered causative of VAP [9]. VAP was classified by the onset of the disease as early-onset VAP, which occurred within the first 4 days and late-onset VAP, which developed more than 4 days after starting mechanical ventilation (MV) [3].

If patients met clinical criteria for VAP, antibiotic therapy was initiated empirically according to primary diagnosis, comorbidities, prior antibiotic exposure, duration of the previous hospitalization of the patient, and the result of the surveillance cultures.

Multidrug resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Extensively drug-resistance (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories). Pandrug-resistance (PDR) was defined as non-susceptibility to all agents in all antimicrobial categories [11]. Standardized definitions were used to determine the presence or absence of ICU complications including acute respiratory distress syndrome (ARDS) [10], severe sepsis and septic shock [12].

Data collection

Demographic data, comorbidities, Charlson comorbidity score, Simplified Acute Physiology Score II (SAPS II), Acute Physiology and Chronic Health Evaluation II (APACHE II), the Sequential Organ Failure Assessment (SOFA) score at ICU admission, admission diagnosis of the patients, reason for endotracheal intubation, prior antibiotic use, microorganisms of VAP, antibiotic susceptibility, lengths of ICU and hospital stays and duration of MV prior to VAP onset were recorded. All patients were followed-up for survival status until 60 days after the initial onset of VAP or until death (if patients died within 60 days). The overall 30-day and 60-day mortalities were recorded. Only the first VAP episode was evaluated.

Microbiology

The antibiotic susceptibility of clinical isolates was determined by the Kirby-Bauer disk diffusion method and, if required, E-test, and analyzed according to the Clinical and Laboratory Standards Institute 2013 document. Identification of pathogens and the antibiotic susceptibility was performed in the Center for Microbiology, Virology and Immunology, Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia.

Statistical analysis

Data entry and analysis were performed using IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA). A descriptive analysis was used to investigate patient demographics and ICU data. The median with range was employed for not-normally distributed continuous variables, and mean ± standard deviation (SD) was used for normally distributed continuous variables. Numbers and percentages were used when applicable. The χ^2 test and Fisher's exact test were employed for testing differences in values of dichotomous variables among study groups, Kruskal-Wallis and Mann-Whitney U tests were used for continuous variables with not-normal distribution. The level of significance was set at $p < 0.05$.

Results

During the study period, 844 adult patients were admitted to the ICU. VAP developed in 144 (17%) patients during the ICU stay. Twenty two patients were excluded from the analysis (lack of data). So, the final analysis of bacterial etiology included 122 patients/ETA samples (78 males and 44 females) with 141 bacterial species isolated. The incidence of VAP was 47.3 cases per 1,000 ventilator days. The mean age of the patients was 56.8 ± 14.6 years. The most common comorbidities of the VAP patients were hypertension (50.8%), other cardiovascular diseases (41.8%) and the chronic obstructive pulmonary disease (COPD) (28.7%). The major reasons for intubation were as following: ARDS (39.3%), pneumonia (34.4%), cardiac arrest (8.2%), and exacerbation of COPD (6.6%). Clinical characteristics of analyzed patients on admission at ICU are shown in Table 1.

Median length of ICU stay was 19 days (4–85) and of hospital stay 30 (4–112) days. Out of 122 patients that developed VAP, 50 (41.0%) died. The patients who did not survive after 60 days were significantly older than the patients who survived (60.6 ± 14.5 years and 53.2 ± 13.8 years respectively; $p = 0.004$). Monomicrobial infection occurred in 103 of 122 (84.4%) patients. Isolated pathogens and mortality rates are shown in Table 2. Almost all isolate were bacteria; the fungal infection (*Candida albicans*) was reported in one patient and *Stenotrophomonas maltophilia* was isolated in one patient (0.8%) in this study (Table 2).

Early-onset VAP was diagnosed in 23 patients (18.9%) and median of onset was 4 (2–4) days of mechanical ventilation. Late-onset VAP was diagnosed in 99 (81.1%) patients and median of onset was 9 (5–27) days of MV. Mortality after 30 days (47.8% vs 39.4%; $p = 0.46$) and 60 days (47.8% vs 49.5%; $p = 0.89$) was not different in patients with early-onset and late-onset VAP, respectively. The pattern of isolates according to the type of VAP is shown in Table 3.

Figure 1 demonstrates the proportion of drug susceptibility of each microorganism.

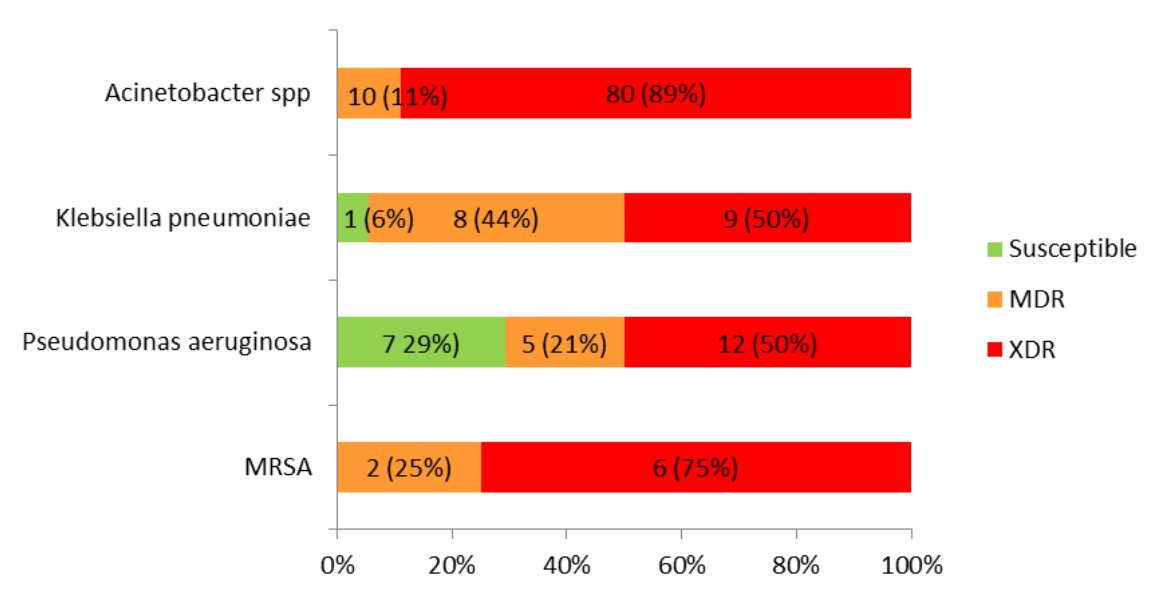

Fig. 1 – Percentage of drug susceptibility patterns for each microorganism.
MDR – multidrug resistant; XDR – extensively drug resistant; MRSA – methicillin-resistant *Staphylococcus aureus*.

Table 1

Characteristics of ventilator-associoted pneumonia (VAP) patients at intensive care unit (ICU) admission

Parameters	Value
Number of patients (n)	122
Age (year), mean ± SD	56.8 ± 14.6
Gender, n (%)	
male	78 (63.9)
Body mass index (kg/m^2), n (%)	
underweight (< 18.5)	5 (4.5)
normal weight (18.5–24.9)	44 (39.3)
overweight (25–29.9)	35 (31.3)
obese (> 30)	28 (25)
Smoking habit, n (%)	61 (63,5)
APACHE II score, mean ± SD	23.34 ± 7.03
SAPS II score, mean ± SD	47.92 ± 13.87
SOFA score, mean ± SD	8.39 ± 3.03
Patient's location before ICU, n (%)	
emergency room	22 (18.0)
ward in same hospital	55 (45.1)
other hospital	45 (36.9)
Duration of previous hospital stay if no direct ICU: admission (days), median (range)	2 (0–65)
Comorbidities, n (%)	
hypertension	62 (50.8)
ischemic heart disease	16 (13.1)
other cardiac diseases	51 (41.8)
cerebrovascular diseases	4 (3.3)
COPD	35 (28.7)
other chronic respiratory disease	22 (18)
diabetes mellitus	30 (24.6)
chronic renal disease	13 (10.7)
chronic liver disease	8 (6.6)
immunodeficiency[a] and cancer	17 (13.9)
gastrointestinal diseases	25 (20.5)
neurologic diseases[b]	10 (8.2)
psychiatric diseases and addiction disorders	26 (21.3)
other diseases	34 (27.9)
Diagnosis at ICU admission, n (%)	
CAP	68 (55.7)
HAP	15 (12.3)
HCAP	9 (7.4)
viral pneumonia	17 (13.9)
exacerbation of COPD	13 (10.7)
ARDS	53 (43.7)
pulmonary thromboembolism	4 (3.3)
severe sepsis	118 (96.7)
MODS	60 (49.2)
septic shock	44 (36.1)
acute intoxication	4 (3.3)
cardiorespiratory arrest	10 (8.2)
H1N1 infection	19 (15.6)
acute renal failure	16 (13.1)
Charlson comorbidity index, median (range)	3 (0–11)
Reason for MV, n (%)	
pneumonia	42 (34.4)
ARDS	48 (39.3)
COPD	8 (6.6)
cardiorespiratory arrest	10 (8.2)
pulmonary edema	3 (2.5)
thromboembolism	3 (2.5)
intoxication	4 (3.3)
septic shock	5 (4.1)
Prior antibiotic use[c], n (%)	79 (69.3)
cephalosporins	52 (45.6)
fluoroquinolones	24 (21.1)
macrolides	28 (24.6)
carbapenem (imipenem or meropenem)	10 (8.8)
aminoglycosides	14 (12.3)

APACHE II – Acute Physiology and Chronic Health Evaluation II; SAPS II – Simplified Acute Physiology Score II; SOFA – Sequential Organ Failure Assessment; COPD – Chronic Obstructive Pulmonary Disease; CAP – community acquired pneumonia; HAP – hospital acquired pneumonia; HCAP – health care associated pneumonia; ARDS – acute respiratory distress syndrome; MV – mechanical ventilaton; MODS – multiple organ dysfunction syndrome, SD – standard deviation.

[a]Immunodeficiency including acute rheumatoid arthritis, HIV+, Sjogren's syndrome and immunosuppressant; [b]Other commorbidities – fractures, thyroid gland diseases, osteoporosis and anemia; [c]Antibiotic treatment during the two weeks preceding ICU admission.

Table 2

Pathogens of ventilator-associated pneumonia (VAP) patients and their susceptibility pattern compared between survivor and non-survivor groups after 30 and 60 days

Organism types	Number of patients (n = 122)	Mortality after 30 days			Mortality after 60 days		
		non-survivors (n = 50)	survivors (n = 72)	*p*	non-survivors (n = 60)	survivor (n = 62)	*p*
Monomicrobial VAP, n (%)	103 (84.4)	42 (40.8)	61 (59.2)	0.91	52 (50.5)	51 (49.5)	0.50
Gram-positive (MRSA)	5 (4.1)	3 (60.0)	2 (40.0)	0.40	4 (80.0)	1 (20.0)	0.20
Gram-negative	98 (80.3)	39 (39.8)	59 (60.2)	0.59	48 (49.0)	50 (51.0)	0.93
Acinetobacter spp	73 (59.8)	31 (42.5)	42 (57.5)	0.69	38 (52.1)	35 (47.9)	0.44
Pseudomonas aeruginosa	14 (11.5)	4 (28.6)	10 (71.4)	0.32	5 (35.7)	9 (64.3)	0.28
Klebsiella pneumoniae	10 (8.2)	3 (30)	7 (70)	0.46	4 (40)	6 (60)	0.54
Polymicrobial VAP, n (%)	19 (15.6)	8 (42.1)	11 (57.9)	0.91	8 (42.1)	11 (57.9)	0.50
Acinetobacter spp *plus Pseudomonas aeruginosa*	8 (6.6)	3 (37.5)	5 (62.5)	1.00	3 (37.5)	5 (62.5)	0.72
Acinetobacter spp *plus Klebsiella pneumoniae*	5 (4.1)	3 (60.0)	2 (40.0)	0.40	3 (60.0)	2 (40.0)	0.68
Acinetobacter spp *plus* MRSA	3 (2.5)	1 (33.1)	2 (66.7)	1.00	1 (33.1)	2 (66.7)	1.00
Klebsiella pneumoniae plus Pseudomonas aeruginosa	2 (1.6)	0 (0)	2 (100)	0.51	0 (0)	2 (100)	0.50
Susceptibility pattern							
susceptible	5 (4.1)	2 (40.0)	3 (60.0)	1.00	2 (40.0)	3 (60.0)	1.00
MDR organisms	21 (17.2)	6 (28.6)	15 (71.4)	0.20	9 (42.9)	12 (57.1)	0.52
XDR organisms	96 (78.7)	42 (43.8)	54 (56.2)	0.23	49 (51.0)	47 (49.0)	0.43

MRSA – methicillin-resistant *Staphylococcus aureus;* MDR – multi-drug resistant pathogens; XDR – extensively drug-resistant pathogenus.

Table 3

Bacterial species isolated from endotracheal aspirate (ETA) samples in ventilator-associated pneumonia (VAP) patients

Bacterial species	Total (n = 122)	Early-onset VAP (n = 23)	Late-onset VAP (n = 99)	*p*
Monomicrobial VAP, n (%)	103 (84.4)	18 (78.3)	85 (85.9)	0.37
Gram-positive (MRSA), n (%)	5 (4.1)	1 (4.3)	4 (4.0)	0.95
Gram-negative, n (%)	98 (80.3)	17 (73.9)	81 (81.8)	0.39
Acinetobacter spp	73 (59.8)	13 (56.5)	60 (60.6)	0.72
Pseudomonas aeruginosa	14 (11.5)	1 (4.3)	13 (13.1)	0.23
Klebsiella pneumoniae	10 (8.2)	3 (13.0)	7 (7.1)	0.35
Polymicrobial VAP, n (%)	19 (15.6)	5 (21.7)	14 (14.1)	0.37
Acinetobacter spp *plus Pseudomonas aeruginosa*	8 (6.6)	0 (0)	8 (8.1)	0.16
Acinetobacter spp *plus Klebsiella pneumoniae*	5 (4.1)	3 (13)	2 (2)	0.05
Acinetobacter spp *plus MRSA*	3 (2.5)	1 (4.3)	2 (2)	0.47
Klebsiella pneumoniae plus Pseudomonas aeruginosa	2 (1.6)	0 (0)	2 (2.0)	1.00

MRSA – methicillin-resistant *Staphylococcus aureus.*

The resistance of all *Acinetobacter* isolates was high to piperacillin/tazobactam (99%), ciprofloxacin (97%), carbapenems (95% to imipenem and 96% to meropenem), ceftazidime (92%), cotrimoxazole (88%) and gentamicin (87%). Among the 4th generation of cefalosporins, the resistance was lower to cefepime (72%) and among aminoglycosides to netilmicin (67%). *Acinetobacter* spp was not tested for amikacin in 52 (58%) cases, however, all isolates which were tested (38/90) were resistant to amikacin (42%). Thirty six percent of *Acinetobacter* spp isolates were resistant to ampicillin-sulbactam. Monitoring of total resistance of isolates showed that there was no resistance to colistin, while resistance to tigecycline was 7%. Seventy three percent of *Pseudomonas aeruginosa* isolates were resistant to gentamicin, 67% to ciprofloxacin, 57% to meropenem, 55% to piperacillin, 42% to piperacillin/tazobactam, 52% to ceftazidime, 41% to amikacin, 38% to cefepime and 35% to imipenem. The highest resistance of *Klebsiella pneumoniae* (*K. pneumoniae*) was observed against β-lactams (100% isolates were resistant to ampicillin and 94% to ampicilin/sulbactam); among 3rd and 4th generation cephalosporins 93% isolates were resistant to ceftriaxone, 89% to cefotaxime, 94% to ceftazidime and cefepime), and then against ciprofloxacin (94%) and cotrimoxazole (93%). However, it was relatively less resistant to piperacillin-tazobactam (47%), gentamicin (44%) and amikacin (22%). Among carbapenems, 38% isolates were resistant to ertapenem, 11% to imipenem and 11% to meropenem. In methicillin-resistant *Staphylococcus aureus* (MRSA) isolates no resistance was observed against vancomycin, teicoplanin and linezolid.

There were no differences in the incidence of infection with *Acinetobacter* spp, *Pseudomonas aeruginosa* and MRSA between period I (2009–2011) and period II (2012–

2014), but there was a statistically significant increase in incidence of infection with *K. pneumoniae* from 8.9% in period I to 25.6% in period II ($p = 0.019$). A significant increase in resistance rates of *Acinetobacter* spp to ampicillin/sulbactam in period II (2012–2014) was also found (30.2% vs 58.6%; $p = 0.01$).

The resistance of Acinetobacter spp to other tested antibiotics did not change significantly between the two studied periods. The resistance of *Pseudomonas aeruginosa* to cefepime, piperacillin/tazobactam and amikacin increased between the two studied periods, without a statistically significant difference, but it increased significantly to imipenem (20% vs 71.4% respectively; $p = 0.052$). The resistance of *K. pneumoniae* increased, especially to carbapenems (ertapenem: 0% vs 54.5%; $p = 0.09$), but due to the small sample statistical significance could not be detected. During the two studied periods there was no difference in incidence of infection with XDR (75.9% vs 83.7%; $p = 0.317$) and MDR strains (19% vs 14%; $p = 0.482$) in all isolated bacteria.

Discussion

Multiresistant bacteria pose a great threat and challenge in everyday clinical practice. That is why regular monitoring of susceptibility of isolated bacteria to antibiotics in each ICU is essential. These data, as well as data regarding patient's characteristics on admission to ICU will help determine the most efficient empirical therapy for VAP. Empirical therapy based on data on local resistance has an impact on lowering morbidity and mortality, shortening of hospitalization, lowering of treatment expenses, and prevents the development of MDR bacteria in patients with VAP [13, 14].

The overall incidence of VAP was 47.3 per 1,000 ventilator-days. The incidence rate of VAP ranges from 13.2 to 51 per 1,000 ventilator days [9]. In a study performed in surgical and medical ICUs in a tertiary hospital in China it was found that the incidence of VAP in the medical ICU was 29.7 cases per 1,000 ventilator days [15]. Differences in infection control practices and lack of established infection control programs may account for higher rates.

VAP caused mortality varies (25%–50%) and greater mortality has been noticed in infections caused by *Pseudomonas* and *Acinetobacter*, in medical ICUs compared to surgical ones, and with the administration of inadequate empirical antibiotic therapy [3]. In our study, mortality in patients with VAP was 41.0% after 30 days, and 49.2 % after 60 days. In a study by Song et al. [15] mortality in VAP patients in medical ICU was 41.9% after 30 days, and 53.5% after 60 days. One of the significant characteristics of our patients on admission to ICU was a high incidence of ARDS (43.7%). A number of clinical studies have shown that pulmonary infection is very frequent in patients with ARDS (34–70%) and often leads to sepsis, multiorgan dysfunction and death [16]. In a study by Forel et al. [17], mortality in patients with ARDS and VAP was 41.8%.

According to American Thoracic Society, empirical antibiotic therapy in VAP treatment should be based on VAP onset time (early-onset *vs* late-onset VAP) and on the presence of the risk factors for the development of MDR bacteria [3]. In our study, most of the patients had late-onset VAP (81.1%) and there was no difference among pathogens causing early-onset and late-onset VAP. These results were expected since most of our patients had a number of risk factors which predispose to the colonization with MDR bacteria (69.3% of patients had received antibiotic treatment prior to admission, 82% was previously hospitalized). Evidence suggests that resistance is a problem also in patients with early-onset VAP is growing. In a study conducted in Serbia in multidisciplinary ICU, no difference was shown among isolated bacteria in patients with early-onset and late-onset VAP [18]. It is interesting that prospective multicenter study in 27 ICUs in 9 European countries showed that even in patients who do not have classic risk factors for MDR bacteria, in 50.7% VAP was caused by multiresistant bacteria [5]. These results speak in favor of the fact that microbiology does no longer follow model "early-onset *vs* late-onset VAP" what presents a new problem for empirical therapy [19]. Our study also showed there was no difference in mortality in patients with early-onset and late-onset VAP after 30 days and 60 days suggesting that VAP onset time is not mortality predictor. Other researchers who compared mortality in patients with early-onset and late-onset pneumonia, came to the similar results [20–23].

In our study, gram negative bacteria were the main pathogens of VAP (80.3%) which is in correlation with the results from other recently published studies, especially from the developing countries. In a study by Chittawatanarat et al. [24] gram negative bacteria caused VAP in 94.7% of cases. During the past ten years, the significance of *Acinetobacter* spp has increased due to the rapid spreading of the strains resistant to the most of the antibiotics, which was noticed throughout the world [25]. One of the important features of *Acinobacter baumannii* (*A. baumannii*) is its ability to survive for a longer time period on the surfaces around patient from where is transmitted to patients in the direct or indirect way. In our study, *Acinetobacter* spp was the most frequent VAP pathogen (59.8%), and the most common XDR strain (89%). Studies in Asian countries have shown that *A. baumanii* was the most frequent pathogen in mixed medical-surgical ICUs where the incidence of infection was between 25% and 50% [24, 26]. In the neighboring countries, a high incidence of infection with MDR *A. baumannii* (85.6%) and XDR (14.4%) was also noticed [27]. Having in mind risk factors for infections caused by multiresistant *Acinetobacter* spp (ARDS, septic shock, severity of the disease, previous use of broad spectrum antibiotics, previous hospitalisation, duration of ICU stay and contamination of patient's surroundings) [28, 29], significant presence of *Acinetobacter* spp in our ICU (frequency of ARDS – 43.7% and septic shock on admission – 36.1%, previous antibiotic therapy – 69.3%, previous hospitalisation – 82%) can be partially explained. Although carbapenems are considered to be a basic therapy in the treatment of *A. baumannii*, during the last years reporting of strains resistant to carbapenems is increased throughout the world [30]. In our study, *Acinetobacter* spp showed a high degree of resistance to carbapenems (more than 95%). According to the Annual Report of the European Antimicrobial Resistance Surveillance Network for 2014, resistance to carbapenems was from

0% (the Netherlands) to 93.2% (Greece) [31]. Šuljagić et al. [32] in their study analyzed and compared the surveillance data on *Acinetobacter* spp nosocomial colonization/infection collected during the wartime with the data collected in peacetime in surgical clinics of the Military Medical Academy in Belgrade (Serbia). Their data showed that resistance of *Acinetobacter* spp to imipenem was 0% during wartime (1999) and 18.6% during peacetime (2001), and to meropenem 4.6% and 27.1%, respectively. Having in mind these data, pronounced increase of *Acinetobacter* spp resistance to carbapenems in our region during the last 15 years is obvious. In the study by Šuljagić et al. [32] *Acinetobacter* spp resistance to ampicillin/sulbactam was 30% what is similar to the results of our study. However, in our study the significant increase of resistance to ampicillin/sulbactam was noticed between two study periods (30.2% vs 58.6%; $p = 0.012$). The increase of resistance was also marked in the neighboring countries. In Croatia, resistance of *Acinetobacter* spp to ampicillin/sulbactam increased from 13% (2009) to 19% (2012) [33]. In our ICU resistance to colistin was not detected, although it was noticed during the last years in Europe (4%), and countries with the highest resistance to colistin were Greece and Italy [31]. The assumed reason that resistance to colistin was not detected in this study is that the colistin was just approved in Serbia for hospital use in 2013, and in this paper data analyzed was ending with the year 2014.

In our study the resistance of *Pseudomonas aeruginosa* to piperacillin/tazobactam, ciprofloxacin, ceftazidime and aminoglycosides was high in comparison with the latest data on resistance in other European countries. The highest resistance of *Pseudomonas aeruginosa* was marked in Romania (62.2% to piperacillin/tazobactam, 55.4% to ciprofloxacin, 59.1% to ceftazidime, 63.4% to aminoglycosides), and the lowest in Denmark, Iceland and Luxembourg (0%–4.4%) [31]. Besides, the resistance of *Pseudomonas aeruginosa* to imipenem was grown significantly between the two studied periods. The trend of resistance increase to carbapenems from 2011 to 2014 was also noticed in Germany, Hungary and Slovakia [31].

In our study significant increase in the incidence of infection with *K. penumoniae* as a pathogen of VAP between the two study periods was noticed (3.8% vs 16.3%; $p = 0.02$). This increase was due to the statistically significant increase of XDR strain of *K. pneumoniae* (3.8% vs 14.0%; $p = 0.040$). For the first time, XDR strain of *K. pneumoniae* (sensitive only to aminoglycosides and carbapenems) was noticed in December 2010. The resistance of *K. pneumoniae* has also increased to imipenem, meropenem i ertapenem between the two studied periods (0% vs 18.2%; 18.2% vs 54.5%, respectively), but statistically significant difference could not be detected. The three European countries with the highest marked resistance to carbapenems in 2014 were Greece (62.3%), Italy (32.9%) and Romania (31.5%). In these countries the greatest percent of *K. pneumoniae* resistant to polymyxins was noticed, indicating that the situation is very worrying [31].

All cases of MRSA caused VAP (5/122) were marked in 2009 and all were sensitive to linezolid and vancomycin. Reports from other European countries also show a decrease of MRSA from 2011 to 2014 from 18.6% to 17.4%, respectively [31].

Empirical treatment of VAP with the high probability of MDR pathogens is one of the greatest challenges met by the intensive care specialists. Having in mind the results of our study, adequate empirical therapy of VAP would be colistin (because of the high prevalence of XDR *Acinetobacter* spp) plus imipenem (because of *Pseudomonas aeruginosa* and *K. pneumoniae*) plus vancomycin (because of MRSA). But, due to the increased use of colistin PDR strains of *Acinetobacter* spp can develop, and that is why its use must be rational [29]. After the short duration of broad spectrum antibiotic therapy and after the antibiogram is obtained, quick deescalation of antibiotic therapy should follow [34].

The significance of this study is in a long monitoring period and therefore obtaining insight in resistance changes of VAP pathogens during the time. Besides, this is the first analysis of VAP pathogens in our ICU.

There are several limitations of this study. The first one is that the bacterial isolates from the VAP cases may not reflect the true etiologic pathogens because more specific diagnostic procedures, such as bronchoalveolar lavage, were not performed. The second limitation is that it was performed in one ICU of the tertiary health facility specialized in treating pulmonary patients. Therefore, the microbiological profile of the isolated strains of VAP pathogens reflects the local epidemiologic situation and it can not be extrapolated to other centres. That is why multicentric research that would help forming general recommendation is necessary. Also, it is necessary to continue regular monitoring of microbiological profile and susceptibility of the pathogens in our ICU in order to timely detect changes, especially today when PDR strains are marked in southeast Europe.

Conclusion

According to results obtained in this study, gram negative bacteria were the main pathogens of ventilator associated pneumonia, out of which the most common was XDR strain of *Acinetobacter* spp with a high resistance to all tested antibiotics except for colistin and tigecycline. There was no difference in pathogens in patients with early-onset and late-onset ventilator associated pneumonia. Mortality in patients with VAP was 41.0% after 30 days and 49.2 % after 60 days of hospitalization, and there was no difference in mortality between patients with early-onset and late-onset VAP.

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Evaluation of ghrelin and leptin levels in obese, lean and undernourished children

Ispitivanje nivoa leptina i grelina u serumu gojazne, normalno uhranjene i mršave dece

Mirjana Miljković*, Ljiljana Šaranac[†‡], Jelena Bašić[‡], Mirjana Ilić[†], Boris Djindjić[‡], Marija Stojiljković*, Gordana Kocić[‡], Goran Cvetanović*, Nebojša Dimitrijević*

***General Hospital Leskovac, Leskovac, Serbia; Clinical Centre Niš, [†]Pediatric Clinic, Niš, Serbia; University of Niš, [‡]Faculty of Medicine, Niš, Serbia**

Abstract

Background/Aim. Energy homeostasis is a balance between energy intake and energy expenditure. Leptin and ghrelin are two orexitropic hormones with opposite effect on energy homeostasis. We investigated fasting ghrelin and leptin serum levels in children with different nutritional conditions. **Methods**. In 30 obese children of both sexes, aged from 6 to 17.67 years (mean 13.04 ± 2.95 years), fasting ghrelin and leptin levels were determined in the serum, along with auxological assessment and pubertal staging. Obtained values were analyzed and compared with those of the same parameters in 33 lean and 25 undernourished (UN) children. **Results**. Mean ghrelin/body mass (BM) ratio was the lowest in obese children (21.75 ± 12.60 pg/mL/kg), which was significantly different in comparison with that in lean and UN subjects. Mean leptin/BM ratio of 0.62 ± 0.86 pg/mL/kg in obese children was significantly higher than that in lean and UN children ($p < 0.01$ and $p < 0.001$, respectively). Ghrelin and leptin levels showed different profiles in obese, lean and UN children. An inverse relationship was discovered among study groups in ghrelin/leptin and leptin/ghrelin ratios. **Conclusion**. Obese children, compared to other children, have significantly higher values of leptin, and UN children have significantly higher values of ghrelin per kilogram of body mass. The results also illustrate the inverse ratio of ghrelin and leptin, which has been demonstrated as a clinically reliable marker of the status of obesity or undernutrition in children, with significant implications concerning rather large variations in the concentration of these hormones in relation not only to the body mass but also to the children's age.

Key words:
nutritional status; child; leptin; ghrelin; obesity.

Apstrakt

Uvod/Cilj. Homeostaza energije je balans između energetskog unosa i potrošnje energije. Leptin i grelin su dva oreksitropna hormona sa suprotnim efektom na homeostazu energije. Ispitivane su vrednosti leptina i grelina u serumu dece različititog tipa uhranjenosti. **Metode**. Kod 30 gojazne dece oba pola, uzrasta od 6 do 17,67 godina (srednja vrednost 13,04 ± 2,95 godine), određivane su vrednosti leptina i grelina u serumu, uz određivanje auksoloških parametara i pubertetskog statusa. Dobijeni rezultati su analizirani i upoređivani sa vrednostima istih parametara kod 33 normalno uhranjene i 25 mršave dece. **Rezultati**. Vrednosti odnosa grelin/telesna masa (TM) bile su najniže kod gojazne dece (21,75 ± 12,60 pg/mL/kg) što je statistički značajno različito u odnosu na vrednosti kod mršave i normalno ishranjene dece. Vrednost odnosa leptin/TM bila je statistički značajno veća kod gojazne dece (0.62 ± 0.86) u odnosu na normalno uhranjene ($p < 0,01$) i mršave ($p < 0,001$). Vrednosti leptina i grelina pokazale su različit profil kod gojazne, normalno uhranjene i mršave dece. U ispitivanim grupama dokazane su inverzne vrednosti odnosa grelin/leptin i leptin/grelin. **Zaključak**. Gojazna deca imaju značajno više vrednosti leptina, a mršava deca značajno više vrednosti grelina po kilogramu telesne mase u odnosu na ostalu decu. Rezultati ukazuju i na inverzni odnos grelina i leptina koji se pokazao kao klinički pouzdan pokazatelj prisustva gojaznosti ili neuhranjenosti kod dece, što je od velikog značaja, uzimajući u obzir velike varijacije u koncentraciji ovih hormona, ne samo prema telesnoj masi, već i prema uzrastu dece.

Ključne reči:
uhranjenost; deca; leptin; grelin; gojaznost.

Correspondence to: Mirjana Miljković, General Hospital Leskovac, 16 000 Leskovac, Serbia. E-mail: mirjanamiljkovic@gmail.com

Introduction

The ghrelin-ghrelin receptor system is one of the most important mechanisms regulating energy balance and metabolism. Ghrelin, a 28-amino acid peptide, is mainly produced in the stomach from a distinct group of endocrine cells. It is discovered as potent growth hormone secretagogue and appetite stimulator by Kojima et al. [1] in 1999. Identified as a natural ligand for growth hormone secretagogue receptor (GHsR) the small peptide provoked a burst of new enthusiasm among scientists and clinicians. Many types of research were hunting for this hormone for years, but Kojima et al. [1] made great discovery by switching the search from the brain to the stomach [1–3].

Ghrelin quickly demonstrated its pleiotropic nature. Opposite to leptin, it stimulates food intake and rises body mass index (BMI) in rodents and humans. In fact, this hormone is one of the most important factors known for regulating appetite and energy expenditure. Ghrelin is also known as "starvation hormone", potent orexigenic signal acting *via* neuropeptide Y (NPY)/Agouti Related Peptide (AGRP) and orexin neurons stimulation in *nucleus arcuatus.* Both peripheral and central administration of ghrelin potently promotes body weight gain and adiposity through the stimulation of food intake while decreasing energy expenditure and body fat [4–8].

Ghrelin which operates as a signal of energy insufficiency and functional antagonist of leptin may play a physiological, and eventual pathophysiological role in the regulation of puberty onset and gonadal function [9–11].

Besides a role in energy homeostasis, growth and puberty, the influence of ghrelin on sepsis, atherogenesis, apoptosis, angiogenesis and addictional habits is among its most prominent effects with the potential of clinical application [8–12].

Since the discovery of leptin in 1994, it has been assumed that adipose tissue is not just fat storage organ, but plays a role in many physiological and pathological processes, including appetite regulation, glucose homeostasis, immune response, growth and differentiation, angiogenesis, hypertension, atherosclerosis and cancer. Finding that leptin signals to the brain that the stomach is full with consequent suppression of NPY production, a stimulator of food intake was among the first actions discovered in rats and confirmed in humans [13–18]. It controls the start of puberty [19], stimulates sympathetic nervous system activity and energy expenditure and influences thyroid, growth, and sex hormone axes [20, 21].

Taking into account the influence of both hormones on appetite regulation and energy expenditure, we found that it would be of importance to determine and compare levels of ghrelin and leptin in children and adolescents with different nutritional status. Also, it was intriguing to investigate ghrelin/leptin and leptin/ghrelin ratios in these groups and to explore correlations of two hormones with auxological data in children with different nutritional conditions.

Methods

The study was designed as cross-sectional and conducted in Pediatric Clinic of University Clinical Centre Niš, in the south-east region of Serbia.

Examinees

Our sample included 88 children and adolescents aged 6 to 17.67 years, stratified as obese (30 subjects, 11 females), undernourished – UN (25 subjects, 19 females) and lean (33 subjects, 24 females). Candidates for the study were selected from patients who were referred to the endocrinology examination due to obesity or consulted endocrinologist because of difficulties in gaining weight. Healthy and normal weight children, assigned as lean, with ideal weight for height, 33 of them, acted as controls. All these children underwent a complete physical examination by a pediatric endocrinologist in order to rule out organic disease or abnormalities in growth and development.

In all participants height and weight measurements were performed and calculation of height percentile (P), height standard deviation score (Height SDS) for chronological age (CA) and gender, body mass index (BMI kg/m²), percentiles of BMI and SDS of BMI for CA and gender, were established. Obesity was defined as BMI greater than P95 (+3SD) for their CA and gender. Undernutrition was defined as BMI-P < 3 (-2SD) for CA.

The informed consent was obtained from all participants and their legal representatives. The study was approved by the Ethical Committee of the Faculty of Medicine, University of Niš.

Hormone assays

Total ghrelin levels in the serum were measured using a commercial Human Ghrelin Elisa Kit Cusabio Biotech Co.,LTD. The assay sensitivity was 0.156 pg/mL, and the intra-assay coefficient of variation (CV) was < 8% and the inter-assay CV < 10%.

Serum leptin was measured using Quantikine Elisa Human Leptin Immunoassay USA&Canada. The assay sensitivity was 7.8 pg/ml with intra-assay CV of 3.3% and inter-assay CV of 5.4%.

Children fasted for at least 8 hours before specimen collection.

Statistical analysis

All statistical analyses were performed using SPSS version 12 software. The data are presented as mean ± SD and comparisons among groups were conducted by independent *t*-test, or Mann-Whitney test, depending on normality of variable distribution. The distributions of the continuous variables were assessed for normality by Shapiro-Wilk test. Pearson's correlation coefficients and Spearman's rho were calculated to evaluate the relationships between hormones values and auxological data $p < 0.05$ was considered to be statistically significant.

Results

The mean age of the obese group was 13.04 ± 2.95 (range 6–17.67) years and the mean puberty stage was 3.37.

Obese children had lipomastia, *genua valga*, and boys (with only two exceptions) pseudo-hypogenitalism.

The mean BMI was very high in obese children being 31.51 ± 4.78 kg/m², ranging from 23.95 kg/m² to 43.95 kg/m². The mean height percentile was P78.30 (1.17 ± 1.35 height SDS) and documented optimal growth in obese children. Mean height SDS of obese children was significantly higher than that of UN children. Children in the control group were also significantly taller than UN children. Overweight (body mass excess – BME), reached abnormal values: mean overweight being 27.45 (ranging from 10 to 52) kg (Table 1).

When absolute values of ghrelin levels in studied groups were compared we found statistical significance ($p < 0.05$). UN children (2,055.84 ± 579.37 pg/mL) and lean children (2,001.88 ± 598.75 pg/mL) had higher ghrelin levels than obese ones (1624.674.10 pg/mL). After adjusting ghrelin levels by calculation of ghrelin/kg BM the value of 72.25 pg/ mL/kg was significantly higher in the UN group in comparison with the control and the obese children ($p < 0.001$). Mean ghrelin/BM ratio was the lowest in the obese children, 21.75 (1.74–67.98) pg/mL/kg with high significance in comparison with the lean and UN subjects (Table 2).

The highest values of leptin were observed in the obese children (56.12 ± 96.94 pg/mL), which was statistically significantly higher compared to those in the controls ($p < 0.01$) and the UN children ($p < 0.001$). The values of leptin in normally nourished children (20.96 ± 12.74 pg/mL) were statistically significantly higher ($p < 0.001$) than those in the UN children (6.92 ± 8.10 pg/mL).

Table 1

Auxological data of investigated children

Parameters	Obese	UN	Control
Number	30	25	33
Sex (M/F)	19/11^{bc**}	6/19	9/24
Age (years), mean ± SD	13.04 ± 2.95	12.08 ± 3.70	13.23 ± 3.01
range	6.00 – 17.67	6.33 – 17.58	8.00 – 17.67
Height (cm), mean ± SD	160.66 ± 15.78^{b**}	144.16 ± 21.64	157.56 ± 13.92^{b*}
range	124.50 – 185.00	108.00 – 191.00	126.00 – 184.00
Height Percentile, mean ± SD	78.30 ± 22.22^{b***}	38.99 ± 33.23	66.98 ± 31.42^{b**}
range	4.60 – 99.90	0.70 – 99.90	0.80 – 99.90
Height SDS, mean ± SD	1.17 ± 1.35^{b***}	-0.35 ± 1.54	0.62 ± 1.23^{b**}
range	-1.70 – 6.50	-2.50 – 3.90	-1.28 – 3.00
BM (kg), mean ± SD	82.55 ± 21.72^{cb***}	31.69 ± 11.56	50.95 ± 16.43^{b***}
range	43.00 – 128.50	16.00 – 57.00	25.00 – 75.00
BME (kg), mean ± SD	27.45 ± 11.33^{bc***}	-11.71 ± 4.06	6.77 ± 9.61^{b***}
range	10.00 – 52.00	-2.00 – 18.00	-3.70 – 11.10
BMI (kg/m^2), mean ± SD	31.51 ± 4.78^{cb***}	14.65 ± 1.39	19.81 ± 3.37^{b***}
range	23.95 – 43.95	12.36 – 17.31	16.83 – 22.65
BMI z-score, mean ± SD	3.11 ± 0.18^{cb***}	-2.93 ± 0.49	0.9 ± 0.70^{b***}
range	3.00 – 4.70	-3.20 – 1.90	0.67 – 1.80
Puberty stage, mean ± SD	3.37 ± 1.35	2.88 ± 1.64	3.42 ± 1.46
range	1.00 – 5.00	1.00 – 5.00	1.00 – 5.00

SDS – standard deviation score; BM – body mass; BME – body mass excess; M – male; F – female; BMI – body mass index; BMI z-score – standard deviation of relative weight adjusted for child age and sex. UN – Undernourished group; a – vs Obese group; b – vs UN group; c – vs Control group; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 2

Orexitropic signaling proteins in investigated children

Parameters	Obese	UN	Control
Leptin (pg/mL), mean ± SD	56.12 ± 96.94^{c**b***}	6.92 ± 8.10	20.96 ± 12.74^{b***}
range	10.74 – 452.40	1.07 – 40.06	1.46 – 50.40
Leptin/BM (pg/mL/kg), mean ± SD	0.62 ± 0.86^{b***}	0.21 ± 0.17	0.37 ± 0.23^{b**}
range	0.15 – 3.93	0.04 – 0.80	0.02 – 0.90
Ghrelin (pg/mL), mean ± SD	1,624.93 ± 674.10	2,055.84 ± 579.37^{*a}	2,001.88 ± 598.75^{*a}
range	196.00 – 3195.00	838.00 – 3073.00	342.00 – 3124.00
Ghrelin/BM (pg/mL/kg), mean ± SD	21.75 ± 12.60	72.25 ± 32.82^{ac***}	38.68 ± 19.98^{a***}
range	1.74 – 67.98	37.36 – 180.59	5.23 – 96.21
Leptin/ghrelin ratio, mean ± SD	0.0582 ± 0.1536^{bc***}	0.0034 ± 0.0033	0.0116 ± 0.0079^{b***}
range	0.0042 – 0.8472	0.0004 – 0.0141	0.0008 – 0.0342
Ghrelin/leptin ratio, mean ± SD	59.69 ± 44.74	570.15 ± 507.42^{ac***}	194.36 ± 262.90^{a***}
range	1.18 – 238.43	70.74 – 2526.17	29.28 – 1297.95

BM – body mass; SD – standard deviation. UN – undernourished group; a – vs Obese group; b – vs UN group; c – vs Control group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Leptin/BM ratio was highest in the group of obese children (0.62 ± 0.86 pg/mL/kg) – statistically significantly higher compared to that in the UN children ($p < 0.001$). Furthermore, the value of this ratio was statistically significantly higher in the group of normally nourished children compared to the UN children ($p < 0.01$) (Table 2).

Leptin/ghrelin ratio was highest in the group of obese children (0.0582 ± 0.1536), with a statistical significance compared to that in the UN children ($p < 0.001$) and the controls ($p < 0.001$). The value of leptin/ghrelin ratio was statistically significantly higher in the controls, compared to that in the UN children ($p < 0.001$). Ghrelin/leptin ratio was highest in the group of lean children (570.15 ± 507.42 pg/mL/kg), with a statistically significant difference when compared with the controls ($p < 0.001$) and the obese children ($p < 0.001$). The value of ghrelin/leptin ratio in the group of controls (194.36 ± 262.90 pg/mL/kg) was statistically significantly higher than that in the obese children ($p < 0.001$) (Table 2).

Leptin and ghrelin levels calculated per kilogram of BM showed different profiles in obese, lean and UN children (Figures 1a and 1b). An inverse relationship in leptin/ghrelin was found in the obese and UN children ghrelin/leptin ratios (Figures 2a and 2b).

Leptin levels significantly positively correlated with BM, BMI and high SDS ($p < 0.001$) (Figure 3), while ghrelin levels correlated significantly negatively with BM and BMI ($p < 0.01$), and nonsignificantly with high SDS (Figure 4).

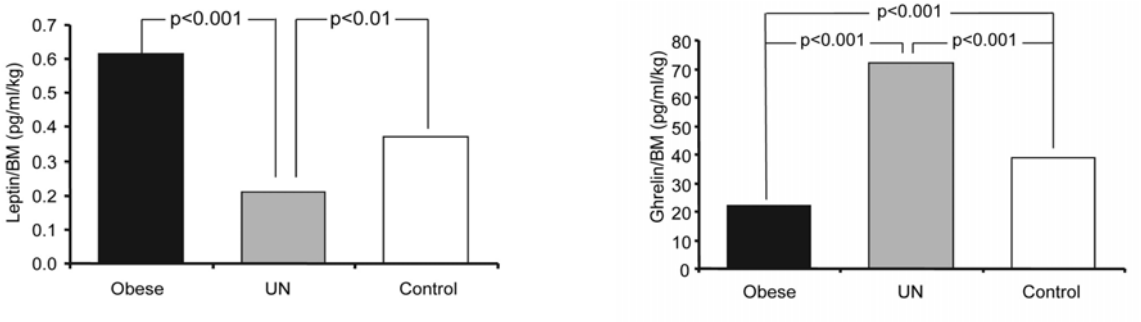

Fig. 1 – a) Leptin/BM and b) Ghrelin/BM ratios in children with different nutritional status. BM – body mass; UN – undernourished group.

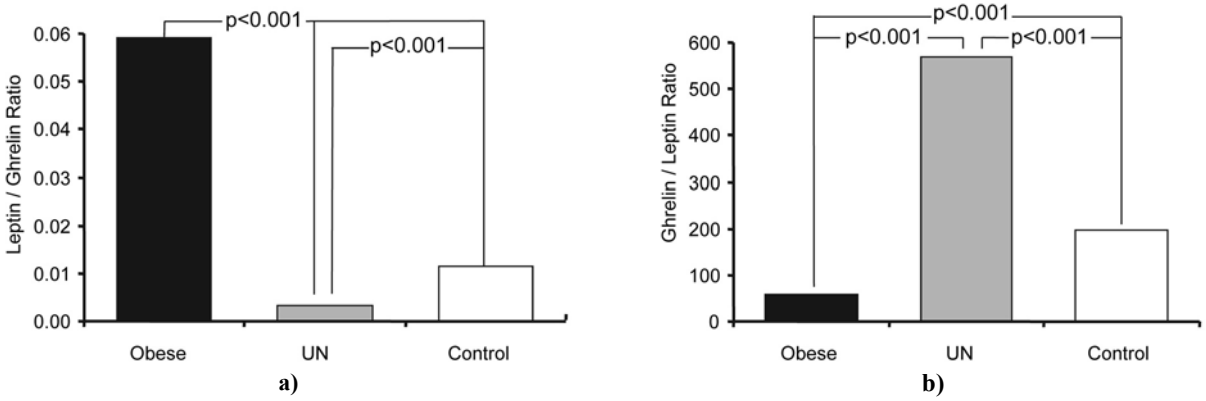

Fig. 2 – a) Leptin/ghrelin and b) Ghrelin/leptin ratios in children with different nutritional status. UN – undernourished group.

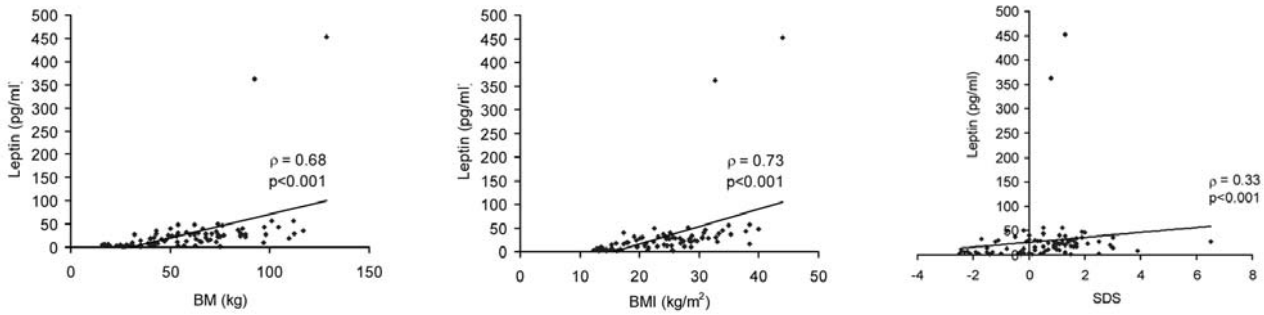

Fig. 3 – Leptin correlations with BM, BMI and SDS in children with different nutritional status. BM – body mass; BMI – body mass index; SDS – standard deviation score.

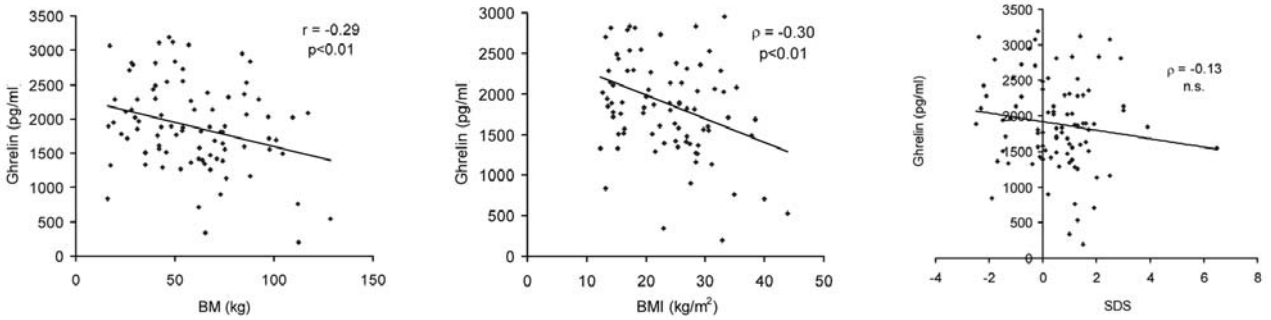

Fig. 4 – Ghrelin correlations with BM, BMI and SDS in children with different nutritional status. BM – body mass; BMI – body mass index; SDS – standard deviation score; n.s. – non significant.

Discussion

We intended to compare serum ghrelin and leptin levels in children with different nutritional status. These two hormones were chosen as potent appetite influencers in the opposite way. It was not surprising that we found the statistically significant difference among studied groups. In our study, the values of ghrelin were highest in the group of UN children. In the controls and in the UN children the values of ghrelin were statistically significantly higher compared to the group of obese children. The value of ghrelin/BM ratio was highest in the group of UN children, with a statistically significant difference compared to those of the obese children and controls, since ghrelin values were highest in the group of UN children with, at the same time, lowest BM, which suggested that in addition to the hunger/satiety status, the concentration of this hormone was also affected by BM, in agreement with the above-mentioned studies. The value of this ratio in the control group was statistically significantly higher compared to that in the obese children.

Mean ghrelin/BM level was more than tripled in the UN children in comparison with the same parameter in the obese ones. Modulatory effect of both forms of ghrelin, acylated and nonacylated on adipogenesis is well documented. It has been shown that both forms of ghrelin directly promote adipogenesis in rat bone marrow adipocytes [22, 23]. Although the orexigenic action of ghrelin itself predicts the impact on weight gain, ghrelin has also been shown to be able to directly act at the level of endocrine pancreas, liver and adipose tissue, thus modulating glucose and lipid metabolism [4, 5, 7]. In humans, ghrelin concentrations progressively decrease during childhood and adolescence, as well as with advancing puberty. In adolescents, similar to adults, ghrelin concentrations are inversely related to BMI and to circulating insulin [24]. One notable exception is the presence of elevated ghrelin concentrations in subjects with Prader-Willi syndrome, raising the possibility that ghrelin could be part of the etiology of excess food intake in this condition. The present study also shows that levels of ghrelin inversely correlated with BM and BMI.

In children with obesity, the decreased ghrelin and increased leptin levels suggest a possible adaptive process to positive energy balance. However, studies of ghrelin in children are scarce. Recently published study of Wali et al. [25] on ghrelin and obestatin level in obese children and children with failure to thrive showed significantly lower total ghrelin levels in children with obesity. In another study Shen et al. [26] investigated changes in ghrelin and obestatin levels before and after a meal in children with simple obesity (15 children) and anorexia (25 children). Their results confirmed the negative correlation between BMI and ghrelin, while the anorexia group had the highest values of obestatin and ghrelin. Recently Arrigo et al. [27] found that weight loss in prepubertal children was associated with a significant change in leptin, ghrelin and obestatin concentrations. They concluded that levels of these hormones are closely associated with obesity in childhood and might take part, as consequence but not as a cause, of weight changes.

Although well known as GHs (growth hormone secretagogue), ghrelin level was low in our group of obese children exerting optimal growth. We can conclude indirectly that our obese children growth because of insulin and/or *via* leptin stimulated conversion of T4 to T3 what is already documented in obese children [28, 29].

Plasma leptin concentrations correlate with the amount of energy stored as fat, and obese individuals express higher levels of leptin than lean individuals [14, 17]. Our study confirmed this positive correlation between leptin concentrations and BM and BMI. Also, our results demonstrated that leptin values were highest in the group of obese examinees, compared to the UN children and the controls. Moreover, leptin/BM ratio was lowest in the UN children in whom individual values of leptin and BM were lowest, while it was highest in the group of obese children, in whom individual values of leptin and BM were highest. Leptin/BM ratio was statistically significantly higher in the obese and control children, compared to the UN ones. This suggested a direct dependence of leptin values on BM, as shown by previously mentioned studies as well.

The height of investigated obese children was optimal (mean percentile being P78.30). High caloric and protein intake in overfed children provide full energy stores, high leptin values, and stimulation of growth hormone secretion and T4 to T3 conversion. This could explain the excellent growth in our study group. In addition, leptin *per se* stimulates T4 to T3 conversion and leptin receptors are identified in the thyroid gland [30]. Leptin, GH, insulin and thyroid hormones, acting synergistically, may be responsible for stimulation of growth in children with exogenous obesity [14, 17, 31].

The study was limited by small sample sizes and wide age range of investigated children. We avoided further dividing of study groups according to pubertal development, due to relatively small number of pubertal participants. The majority of studied children were in mid-puberty. We also recognize that boys dominated in the obese group and girls in the UN one, but the data about gender difference in orexitropic signals are still biased [28, 29].

Suppressed levels of ghrelin in our group of obese children and more than tripled in the UN children are in favor of the significant influence of this hormone on adaptation in conditions of overfeeding and starvation. Leptin levels were inversely regulated among study groups when compared to ghrelin. It is important to establish a healthy balance between these two hormones. In this interplay, genetic and environmental influencers are certainly of great importance, but this must be further investigated.

Leptin/ghrelin ratio was highest in the group of obese children, with statistical significance compared to the UN children and the controls. The value of leptin/ghrelin ratio was statistically significantly higher in the control group compared to that in the UN children. Leptin values were highest in the group of obese children, with the simultaneous presence of lowest ghrelin values, which could be possibly interpreted as the process of adaptation to a positive energy balance. Children with higher BMI had more adipose tissue, i.e. more leptin per kg of BM, as shown in the study. At the same time, leptin/ghrelin ratio was able to identify distinctly the obese children, and it can thus be used as an indicator of the status of obesity, supplementing BM and BMI.

Ghrelin/leptin ratio was highest in the group of UN children, with a statistically significant difference compared to that in the control group and the obese children. The value of ghrelin/leptin in the controls was statistically significantly higher than that in the obese children. The values of ghrelin were highest in the group of UN children, with simultaneous lowest leptin values; this association demonstrated that there was a mechanism of adaptation in the UN children to a negative energy balance. Ghrelin/leptin ratio clearly identified the UN children, and this relationship could be used as a supplemental indicant of undernutrition, in addition to the already known indicants (BM, BMI).

Such data are scarce for the children aged up to 12 years [27, 32], as those enrolled in this study. Moreover, there have been no studies involving all three groups of children (UN, normal weight and obese). This shows that in children of different ages a characteristic profile of secretion of these hormones is kept, as well as its association with BM, BMI SDS, and BMI-P.

Conclusion

The obese children, compared to other children, demonstrate significantly higher values of leptin, and the UN children demonstrate significantly higher values of ghrelin per kilogram of body weight. The results also illustrate the inverse ratio of ghrelin and leptin, which has been demonstrated as a clinically reliable indicator of the status of obesity or undernutrition in children, with significant implications concerning rather large variations in the concentration of these hormones not only with body mass but also with children age. The results provide a better understanding of hormonal regulation in different nutritional conditions. The important difference in appetite targeting hormones and their ratios (ghrelin/leptin and leptin/ghrelin profile) between the obese and the UN children was found.

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SHORT COMMUNICATION

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Association between C-reactive protein and normal tension glaucoma

Povezanost između vrednosti C-reaktivnog proteina i normotenzivnog glaukoma

Milan Stojčić*, Paraskeva Hentova-Senćanić†‡, Dragan Babić§, Biljana Stojčić‖, Milenko Stojković†‡, Lepša Žorić¶

Railway Health Institute, *Ophthalmology Department, Belgrade, Serbia; University of Belgrade, †Faculty of Medicine, Belgrade, Serbia; Clinical Centre of Serbia, ‡Clinic for Eye Diseases, Belgrade, Serbia; University of Belgrade, Faculty of Medicine, §Institute for Medical Statistics and Informatics, Belgrade, Serbia; ‖Primary Health Centre "Euromedic", Belgrade, Serbia; ¶Health Center Kosovska Mitrovica, Kosovska Mitrovica, Serbia; University of Priština/Kosovska Mitrovica, **Faculty of Medicine, Kosovska Mitrovica, Serbia

Abstract

Background/Aim. C-reactive protein (CRP) is a systemic inflammatory marker associated with risk for cardiovascular disease (CVD). Some risk factors for CVD are associated with normal tension glaucoma (NTG), but the association between CRP and NTG has not been well defined yet. The aim of our study was to compare high-sensitivity CRP (hs-CRP) levels in plasma between patients with NTG and normal controls. **Methods.** We studied 20 patients (4 males and 16 females) with the NTG diagnosis and compared their CRP values to those obtained in 25 controls (5 males and 20 females) with no ocular disease. Both groups had similar demographic parameters (age, sex, body mass index – BMI) and similar vascular risk profile. **Results.** Plasma CRP levels were comparable between patients with NTG and controls (mean values 4.99 ± 0.77 mg/L, median 4.50 mg/L, range 2.50–18.90 mg/L and mean value 4.19 ± 0.30 mg/L, median 3.50 mg/L range 2.20–8.50 mg/L, respectively, $p > 0.5$. **Conclusion.** The results obtained in this study suggest that CRP levels are not altered in NTG patients.

Key words:
low tension glaucoma; c-reactive protein; atherosclerosis.

Apstrakt

Uvod/Cilj. C-reaktivni protein (CRP) je sistemski inflamatorni marker koji je povezan sa rizikom od nastanka kardiovaskularnih bolesti (KVB). Određeni faktori rizika od KVB su ujedno i faktori rizika od razvoja normotenzivnog glaukoma (NTG), ali povezanost između CRP I NTG još uvek nije jasno definisana. Cilj ove studije bio je da se ispita povezanost između vrednosti visokosenzitivnog CRP u plazmi kod bolesnika sa NTG i u kontrolnoj grupi. **Metode**. Ispitano je ukupno 20 bolesnika sa NTG (4 muškaraca i 16 žena) i 25 osoba iz kontrolne grupe (5 muškaraca i 20 žena). Obe grupe ispitanika imale su približno iste demografske karakteristike (godine, pol i indeks telesne mase) i kod obe grupe bili su prisutni vaskularni faktori rizika u istom procentu. **Rezultati**. Vrednosti CRP u plazmi bolesnika sa NTG i u kontrolnoj grupi bile su poredive: srednja vrednost iznosila je 4.99 ± 0.77 mg/L, mediana 4.50 mg/L, opseg 2.50–18.90 mg/L kod bolesnika sa NTG, dok su te vrednosti u kontrolnoj grupi bile: 4.19 ± 0.30 mg/L, 3.50 mg/L, odnosno 2.20–8.50. **Zaključak.** Rezultati dobijeni u ovoj studiji pokazuju da vrednosti CRP nisu povećene kod bolesnika sa NTG.

Ključne reči:
glaukom, normotenzivni; c-reaktivni protein; ateroskleroza.

Introduction

Glaucoma is a group of diseases characterized by progressive optic neuropathy and loss of retinal nerve fiber layer with corresponding visual field defects. Elevated intraocular pressure (IOP) has been identified as the most important risk factor, but not the only one [1].

Normal tension glaucoma (NTG) is a special type of primary open angle glaucoma characterized by intraocular pressure levels in normal statistical range, but resulting in a progressive optic neuropathy [2, 3]. This type of glaucoma is associated with vascular risk factors such as vascular dysregulation with systemic hypotension and vasospasm [4, 5]. In the optic nerve head (ONH) in these patients, disk hemo-

Correspondence to: Milan Stojčić, Railway Health Institute, Savska 23, 11 000 Belgrade, Serbia. E-mail: milanstojcic@gmail.com

rrhages, notching of the neuroretinal rim and peripapillary atrophy are often encountered [6]. Patients with NTG have an increased endothelin-1 plasma level [7, 8].

C-reactive protein (CRP), an important serum marker of inflammation, the levels of which increase in response to acute inflammatory processes, is synthesized in the liver and regulated by cytokines [9]. CRP has recently been proposed as a marker of chronic inflammatory processes such as atherosclerosis [10]. Epidemiological studies show that an elevated CRP level is a strong predictor of cardiovascular risk [11].

Some risk factors for cardiovascular disease are associated with NTG, but the association between CRP and NTG has not been well defined yet. While some studies found elevated levels of CRP in patients with NTG [12, 13], other studies suggest that CRP levels are not altered in NTG patients [14–17]. The purpose of this study was to investigate CRP levels by using a highly sensitive CRP kit in NTG patients and to compare them to normal controls.

Methods

This study included 20 consecutive NTG patients who presented in our glaucoma consultations. The diagnosis of NTG was based on a clinical examination, including intraocular pressure measurements at 2-hour intervals from 8 am to 6 pm with the values consistently below 22 mmH, open chamber angle, optic nerve head damage typical of glaucoma and glaucomatous visual field defects. There was no history or signs of other eye disease or steroid use. Control subjects also underwent clinical eye examination including measurements of intraocular pressure at two hour intervals from 8 am to 6 pm, fundoscopic and optic nerve head examination and OCTOPUS visual field analysis, program full threshold (Octopus 900, Haag Streit AG, Koeniz, Switzerland). Based on the value of the mean defect (MD), all visual fields were divided into three groups. The first group consisted of patients with initial changes in the visual field (MD ≤ 6 dB). The second group consisted of patients with moderate changes in the visual field (6 dB < MD < 12 dB), while the third group consisted of patients with advanced changes in the visual field (MD ≥ 12 dB).

In both NTG and control groups, the detailed medical history was obtained. The patients and control subjects with the known systemic inflammatory disease, malignancy and/or steroid use were excluded from the study. Hypertension, hypotension, migraine, ischemic heart disease, vasospastic diathesis (positive history of cold hand and feet), cerebrovascular disease and diabetes mellitus were identified from the medical charts and history taking in each patient and subject. The control group consisted of the subjects of similar gender and age, with no ocular disease.

The quantitative determination of plasma CRP levels in all study participants was done using a highly sensitive CRP kit (Abbot AEROSET, Illinois, USA).

The study was approved by the relevant ethics committee and all the participants gave written informed consent according to the Declaration of Helsinki.

The results were analyzed by *t*-test, χ^2 test, ANOVA and Mann-Whitney *U* test. A value of $p < 0.05$ was considered significant.

Results

A total of 20 NTG cases (4 males, 16 females, mean age 65 ± 9 years) were compared with 25 controls (5 males, 20 females, mean age 63 ± 6 years) (Table 1). Both groups had similar demographic parameters (age, sex, BMI; all *p*-values > 0.05). Systemic vascular disorders identified from charts and medical history in the NTG patients were: 3 (15%) patients had hypotension, 10 (50%) had hypertension, 2 (10%) had a migraine, 2 (10%) had positive history of cold hands and feet, 2 (10%) had an ischemic heart disease and 2 (10%) had diabetes mellitus. Systemic vascular disorders that were identified in the control group were: 4 (16%) patients had hypotension, 12 (48%) had hypertension, 2 (8%) had a migraine, 2 (8%) had positive history of cold hands and feet, 3 (12%) had an ischemic heart disease and 2 (8%) had diabetes mellitus. No patient from both groups had the history of cerebrovascular disease.

Plasma CRP levels were comparable in NTG patients and controls [median (range) 4.50 2.5–18.9] mg/L compared with 3.50 (2.2–8.5) mg/L; Mann-Whitney ($p = 0.233$)]. Also,

Table 1

Characteristics of normal tension glaucoma (NTG) cases and controls

Characteristics	NTG cases (n = 20)	Control group (n = 25)
Age (years), mean ± SD	64 ± 8.9	62.8 ± 5.9
Male/Female, n	4/16	5/20
BMI (kg/m^2), mean ± SD	25.9 ± 4.9	26.1 ± 3.4
Hypotension, n (%)	3 (15)	4 (16)
Hypertension, n (%)	10 (50)	12 (48)
Migraine, n (%)	2 (10)	2 (8)
Vasospastic diathesis, n (%)	2 (10)	2 (8)
Coronary artery disease, n (%)	2 (10)	3 (12)
Diabetes mellitus, n (%)	2 (10)	2 (8)
CRP (mg/L), median (range)	4.50 (2.50–18.90)	3.50 (2.20–8.50)
CRP (mg/L), mean ± SEM	4.99 ± 0.77	4.19 ± 0.30

SD – standard deviation; BMI – body mass index; SEM – standard error of the mean; CRP – C-reactive protein.

the mean values (± standard error) were not significantly different between the patients with NTG and the controls (4.99 ± 0.77 mg/L compared with 4.19 ± 0.3 mg/L), *t*-test $p = 0.302$.

The values of mean defect of sensitivity were significantly higher in the patients with NTG than in the control group ($p < 0.01$). Most of the patients, 18 out of 20 (90%), belonged to the group with early and moderate changes in visual field (Table 2). The number of patients with initial changes in the visual field was 7 (35%), MD = 4.06 ± 1.02: Moderate changes in the visual field were noted in 11 (55%) patients, MD = 8.64 ± 1.65, while only 2 (10%) patients had advanced changes in visual field, MD = 15 ± 2.54. Also, CRP values were not significantly different among the 3 groups of the NTG patients and the controls, ANOVA (oneway) $p = 0.347$ (Table 2).

Table 2

Mean defect (MD) and C-reactive protein (CRP) values among the 3 groups of normal tension glaucoma (NTG) patients and controls

Parameters	Group I (MD < 6 dB)	Group II (6 dB < MD < 12 dB)	Group III (MD > 12 dB)	Controls
Number of patients, n (%)	7 (35)	11 (55)	2 (10)	25 (100)
MD ± SD (dB)	4.06 ± 1.02	8.64 ± 1.65	15 ± 2.54	2.72 ± 0.60
CRP (mg/L), mean ± SEM	4.10 ± 0.34	4.13 ± 0.32	5.30 ± 0.96	4.19 ± 0.32

NTG – normal tension glaucoma; SD – standard deviation; SEM – standard error of the mean.

Discussion

The results of this study show that CRP levels are not altered in patients with NTG as compared with the control group with no ocular disease, but with similar demographic parameters and similar vascular risk profile.

Previous researches on the connection between CRP and NTG were controversial. Our results are consistent with the findings obtained by Su et al. [15] and Choi et al. [16], even though they excluded patients with cardiovascular disease. That CRP is not elevated in patients with NTG when compared with normal controls, after exclusion of patients with cardiovascular and other systemic diseases has also been found by Lee et al. [17] in their study. Since NTG is a multifactorial disease in which the vascular risk factors independent of IOP take increasing importance, we did not exclude from our study patients with cardiovascular disease. Taking that into consideration we tried to match NTG patients and controls well. However, our results are contrary to the prior report by Leibovitch et al. [12] who showed significantly higher levels of CRP in 20 NTG patients, when compared to the control group (mean 3.21 ± 0.6 to 0.85 ± 0.17 mg/dL, $p > 0.001$). Leibovitch et al. [12] also did not exclude patients with cardiovascular disease, but, obviously, characteristics of the patients in the control group differed from those in our control group (CRP level in their control group was lower than in our controls). A possible explanation for the different results in our and in Leibovitch's study may be the fact that patients with NTG in our study belong to the same or a similar disease stages (initial and moderate loss of the visual field). It is possible that CRP values vary in the different stages of the disease. Further research is needed so that this factor could be taken into account, and that should include a larger number of subjects.

C-reactive protein is not only an important serum marker of inflammation but may have a direct role in the pathogenesis of atherosclerosis. CRP has been found in atherosclerotic plaque [18], and has an important role in cell adhesion molecular expression in human endothelial cells [19]. There are strong associations between the levels of CRP and the incidence of vascular atherosclerotic events such as myocardial infarction and stroke [11].

A number of studies dealing with the connection of glaucoma and systemic cardiovascular disease, despite some positive findings, did not find a strong link between atherosclerotic changes in blood vessels and the development of glaucoma [20–24]. Neither the Rotterdam study on a sample of 3,842 subjects after 6.5 years of follow-up found an association between CRP and atherosclerosis, with the prevalence of glaucoma in a relatively healthy population [14].

To the contrary, vascular dysregulation rather than atherosclerotic changes in blood vessels appears to cause reduced perfusion in the optic nerve head [25].

In research on NTG, vascular risk factors, in general, were considered as especially important. Interestingly, however, atherosclerosis itself and its risk factors are of minor importance. Systemic hypertension, dyslipidemia and diabetes mellitus are weak risk factors, if they are risk factors at all. Vascular dysregulation causing abnormal blood flow to the optic nerve seems to be a major risk factor. Such dysregulation may lead to systemic hypotension and to local vasospasms, but also to a disturbed autoregulation of blood flow in the optic nerve head [26–28]. Potential limitations of our study are relatively small group sizes of NTG subjects and normal controls. The selection process of patients must be strictly controlled to avoid all of those factors and systemic inflammatory disease that may affect the level of CRP. Patients with different types of open-angle glaucoma but at the same stage of the disease should be involved in future research.

Conclusion

Our findings suggest that CRP levels are not associated with NTG, in line with an assumption that the risk profile of atherosclerotic patients, which includes an increase in CRP, is not identical to the risk profile of NTG patients.

Declaration of interest

The authors declare no conflict of interest.

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CURRENT TOPIC

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Ultrasound accelerated thrombolysis for therapy of arterial and venous thrombosis – initial experience in the Military Medical Academy in Belgrade

Tromboliza ubrzana ultrazvukom u terapiji arterijske i venske tromboze – početno iskustvo u Vojnomedicinskoj akademiji u Beogradu

Jelena Kostić*, Siniša Rusović[†], Zoran Trifunović[‡§], Miodrag Mihajlović[†], Vlastimir Marinković[†], Milan Šćepanović[†], Milan Dobrić*, Slobodan Obradović[§‖]

University Clinical Center of Serbia, *Clinic for Cardiology, Belgrade, Serbia; Military Medical Academy, [†]Institute of Radiology, [‡]Clinic for Cardiac Surgery, [‖]Clinic for Emergency and Internal Medicine, Belgrade, Serbia; University of Defence, [§]Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Key words:
venous thrombosis; ultrasonic therapy; thrombolytic therapy; treatment outcome.

Ključne reči:
tromboza, venska; lečenje ultrazvukom; fibrinolitici; lečenje, ishod.

Introduction

Hemostatic system has been developed to preserve the integrity of circulation. Main components of hemostasis are: blood vessel wall, plasma proteins (coagulation and fibrinolytic factors), platelets and other blood elements such as monocytes, red blood cells, etc. [1]. All system components function interconnected. Coagulation plasma proteins normally circulate in its biologically inactive forms as proenzymes. Coagulation is the process which leads to formation of blood clots and turning from liquid to solid. It is very important, since if it does not work, it may cause bleeding disorders and if it works in excess it can lead to thrombosis. The whole process of clotting is based on many parts, each component performing a small but vital role. The clotting cascade details this (Figure 1), but in essence, damaged vessel walls cause platelets to get joined together by fibrin, forming a tight clot. Thus, the three main components of the coagulation process are: platelets, fibrin, and the damaged endothelium. Whilst platelets are naturally present in blood, fibrin is formed through the clotting cascade. There are two pathways: the contact activation pathway (also known as the intrinsic pathway) and the tissue factor pathway (known as the extrinsic pathway). The process of activation of platelets and fibrinogen in inappropriate blood vessel can end in thrombus formation, so that thrombosis may be considered excessive hemostasis in the wrong place [2-4]. Thrombi can occur in any part of the vascular system as a result of imbalance between the hemostatic system. The sequence of events in thrombus formation depends on the velocity of circulation: arterial (fast) and venous (slow).

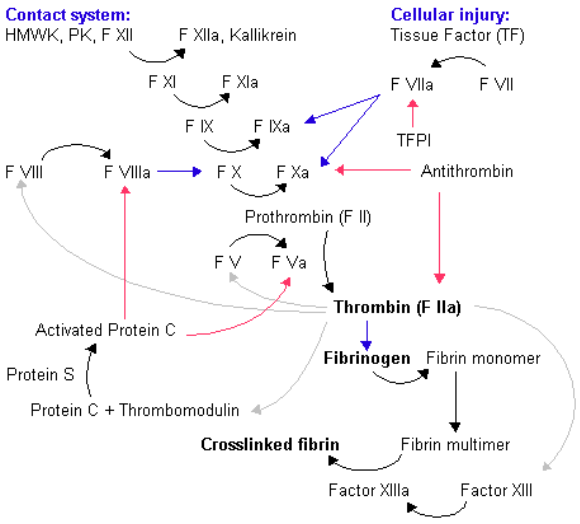

Fig. 1 – Coagulation cascade.
HMWK – high molecular weight kininogen; PK – prekallikrein; F – factor; TFPI – tissue factor pathway inhibitor (https://sh.wikipedia.org/wiki/Zgru%C5%A1avanje_krvi)

Correspondence to: Jelena Kostić, Clinic for Cardiology, Clinical Center of Serbia, Višegradska 26, 11 000 Belgrade, Serbia.
Fax: +381 11 3663 291. E-mail: jesikakostic@yahoo.com

The causes of arterial thrombosis can be atherosclerosis, coagulation disorders such as antiphospholipid antibodies syndrome, protein C resistance (factor V Leiden), the lack of protein C or S, heparin induced thrombocytopenia, essential thrombocytopenia and hyperhomocysteinemia [5–7].

Pulmonary thromboembolism (PTE) and deep vein thrombosis (DVT) is almost impossible considered separately, and is often together referred as venous thromboembolism (VTE). One or more factors of Virchow's triad (hemodynamic changes, endothelial injury/dysfunction and hypercoagulability of blood) are almost always the cause of VTE. Thrombotic process begins in the venous valve cusps, spreads and can lead to complete occlusion of the lumen and venous tributaries and embolization [8, 9]. The incidence of DVT is common in surgical and other disease in which there is inactivation of the muscle venous pump. Other causes may include: external obstruction, strictures, scars, pressures from surrounding structures, often tumor mass. PTE is a common, serious and potentially fatal complication that occurs when a clot from the deep vein, usually the lower limb or pelvic, cause embolisation of pulmonary arterial circulation. Rarely the source of emboli can be of the vena cava thrombus, deep veins of the upper extremities and the right heart.

New thrombolytic technique

EkoSonicTM Mach4 (EKOS® Corporation, Bothell, WA) endovascular system with rapid pulse modulation (RPM) is designed for the dissolution of vascular blood clots. EkoSonicTM is the only endovascular system that can deliver microsonic energy and thrombolytic drug simultaneously, providing safer, faster and more complete thrombus removal by accelerating dissolution. It is intended for controlled and selective infusion of thrombolytic drugs into the peripheral vasculature and pulmonary arteries. EkoSonicTM device obtain access for standard percutaneous endovascular procedures, 6F sheath is preferred. The system consists of a reusable and single use parts. The reusable parts are the control unit which provides ultrasound energy and the user interface, with his own connector interface cable (CIC). Single use parts of this device are: intelligent drug delivery catheter (IDDC) and microsonic device (MSD) (Figure 2).

Ekosonic control system generates ultrasonic energy waves at the treatment zone through piezoelectric transduction of radiofrequency (RF) energy generated by the control unit. The device frequency ranges from 2.05 to 2.35 MHz pulse modulated. The maximum output power is 50 W (30 W average), maximum temperature is 43°C. The control unit monitors output power and temperature of the endovascular unit. It has the safeguard circuits to prevent deviation of these parameters from preset range. If there is a disorder, the system automatically turns off.

Infusion catheter (IDDC) has a unique design (Figure 3) with two separate lumens, one for the drug delivery through the side holes, and the central lumen for the exchange of the guide wire and MSD, as well the delivery of saline for cooling the ultrasound probes. The IDDC catheter has two markers on the distal end between which are located side holes – treatment zone of the catheter. Infusion of the drug and saline are administered *via* a standard infusion clinical pump. The MSD device has 30 fully isolated, radiopaque marked probes in its distal part. Both catheters exist in different length for different vessel localization and thrombus length. The available lengths are: 106 cm working length with the treatment zone 6, 12, 18, 24, 30, 40, 50 cm and 135 cm working length with the treatment zone 12, 30, 40, 50 cm.

How it works?

When it comes to the formation of thrombus in peripheral blood vessels that obstruct the lumen and prevent normal blood flow or in PTE as the clot forms, plasminogen is incorporated into the fibrin [11]. In order to resolve the clot thrombolytic agent must reach the receptor sites on the plasminogen. The EkoSonicTM endovascular system facilitates this process. It simultaneously releases thrombolytics and ultrasound energy within the thrombus [12, 13]. Delivering drug without ultrasound may limit its dispersion, as the drug will follow the path of least resistance along the side holes of non ultrasound catheter. By contrast activating the EkoSonicTM endovascular system it generates a radial pressure within the treatment zone around the catheter [14]. The pressure effectively drives the drug into the thrombus and

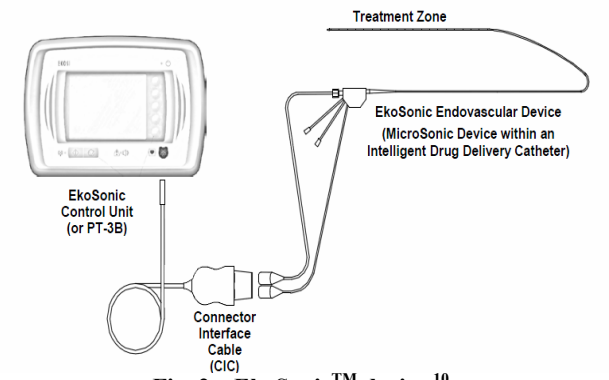

Fig. 2 – EkoSonic™ device [10].

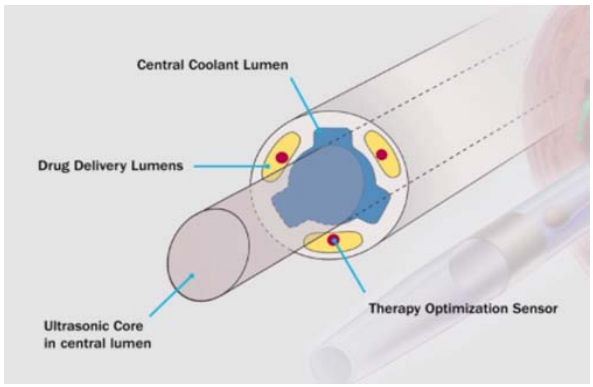

Fig. 3 – Cross section of the intelligent drug delivery catheter [10].

immediately accelerates the dispersion of the drug. At the same time acoustic energy conditions the clot by thinning fibrin into individual strands. This increases the pore size and allows more effectively delivery of the drug deep inside the thrombus. As the drug moves further in the fibrin mesh, it surrounds each fibrin strand and bounds with newly exposed plasminogen activator receptor sites. The fibrin strands begin to dissolve releasing red blood cells and other blood components trapped into the fibrin matrix.

Our initial experiences

Using this method patients have been treated in Radiology Institute in Military Medical Academy in Belgrade since 2013 untill February 2017, co working with clinicians from Cardiac Surgery Clinic, Vascular Surgical Clinic and Clinic for Emergency and Internal Medicine. Purpose of the report is to show efficiency of method for dissolving thrombus in targeted blood vessel. All patients had signs of arterial or venous system thrombosis. The diagnosis of thrombosis of the vessel was established on the basis of clinical examination, laboratory findings, echocardiography, multidetector computed tomography (MDCT) angiography or conventional angiography. Following the established diagnosis all the patients were subjected to treatment with ultrasound accelerated thrombolysis by the assigned protocol.

Four patients had thrombosis of femoropopliteal bypass graft, one had thrombosis of the superior mesenteric artery, one with thrombosis of vena cava and twenty patients with PTE. Depending on the location of thrombosis and its duration clinical symptoms were different. The duration of symptoms in patients varied from rapid onset up to six months. There had been treated acute (< 14 days), subacute (15–28 days) and chronic (> 28 days) old thrombosis. After abovementioned examination all the patients were presented to the Catheterization Laboratory.

Device and procedure description

Percutaneous transfemoral or transjugular approach was used. After the angiography of the occluded vessel, through the 6F sheath guide wire was introduced into the blood vessel that was necessary to treat passing through the entire length of the clot under the control of fluoroscopy. Hydrophilic guide wire of 0.035 inch was used. Then the IDDC catheter was inserted over the guide wire until the tip of the catheter passes entirely through the clot with the tip in healthy part of the vessel. The guide wire was then pulled out and replaced by the MSD with positioning of the radiopaque probes within the proximal and distal marker on the IDDC. After connecting drug solution and saline with the adequate ports and attaching the CIC with IDDC and MSD, treatment procedure was started. The duration of the therapeutic procedure depended on thrombus maturity and its size. The time ranged from 6 to 24 hours. Thrombolytic therapy [recombinant tissue plasminogen activator-tPa (rtPA) – alteplase] was given at the dose of 1–3 mg per hour. The solution was made as follows: 50 mg alteplase inserted into 500 mL of saline. The drug was running through the standard infusion pump 5–15 mL per hour (depending on the required dose), saline solution for cooling the probe went 35 mL per hour. Upon completion of the therapy procedure the device was pulled out. Final angiography was made and MDCT angiography was made next day. Further monitoring of the patient's was clinical.

Views of individual patients performed

There were 4 patients 1–2 years after surgical revascularization of the lower limbs, femoropopliteal bypass grafts (due to occlusion of superficial femoral artery), with the presentation of symptoms that indicated graft occlusion. In 3 patients according to the medical history graft occlusion was subacute (up to one month), while one was chronic (up to 6 months). They all had clinical symptoms of chronic lower limb ischemia as they already had ischemic arterial disease, but they all complained on intermittent claudications, distance shortening. Claudication is defined as fatigue, discomfort or pain that occurs during exercise in a particular group of legs muscles. People with claudication have enough blood supply so in rest symptoms disappear [15, 16]. Patients with typical claudication described: pain, itching, pressure, cram ping or fatigue in one or more muscle groups of the lower extremities. The symptoms are usually caused by exertion and subside during resting. Localization of claudications occurrence was distal to the diseased segment of the artery. Anatomic site of the lesion is usually associated with pain in the leg at a certain point. Patients complained of pain in the region below the knee, which is typical for the femoropopliteal or tibial artery disease. After confirmed diagnosis of thrombosis of femoropopliteal graft they received the therapy (ultrasound accelerated thrombolysis) which lasted for 6 hours at a dose of 1 mg per hour. After reestablishing perfusion in all four patients were detected stenosis at proximal or distal anastomosis which was solved by stent implantation (Figure 4). All given results refer to immediate founding after the intervention. The prognostic outcome of patients limb salvage in spite of complete resolution of thrombus depends on the condition of distal microvascular circulation.

Acute occlusion of the superior mesenteric artery appear in the patient on the third postoperative day after coronary artery bypass grafting (CABG) for myocardial revascularisation. The patient had strong, tearing pain in the abdomen, bowel peristaltic was not audible. After MDCT examination the occlusion of the superior mesenteric artery was found. First thrombus aspiration was done, by using the right coronary guiding catheter 7F (due to the larger lumen width) with no results. Then the ultrasound accelerated thrombolysis was applied for 6 hours at the dose rtPA 1 mg/hour. Near complete dissolution of the thrombus was achieved, soon after peristalsis was restored and the patient fully recovered (Figure 5).

Acute vena cava inferior thrombosis appeared after resection of the right liver lobe due to hepatocellular carcinoma. During the surgery, there was an iatrogenic lesion of the

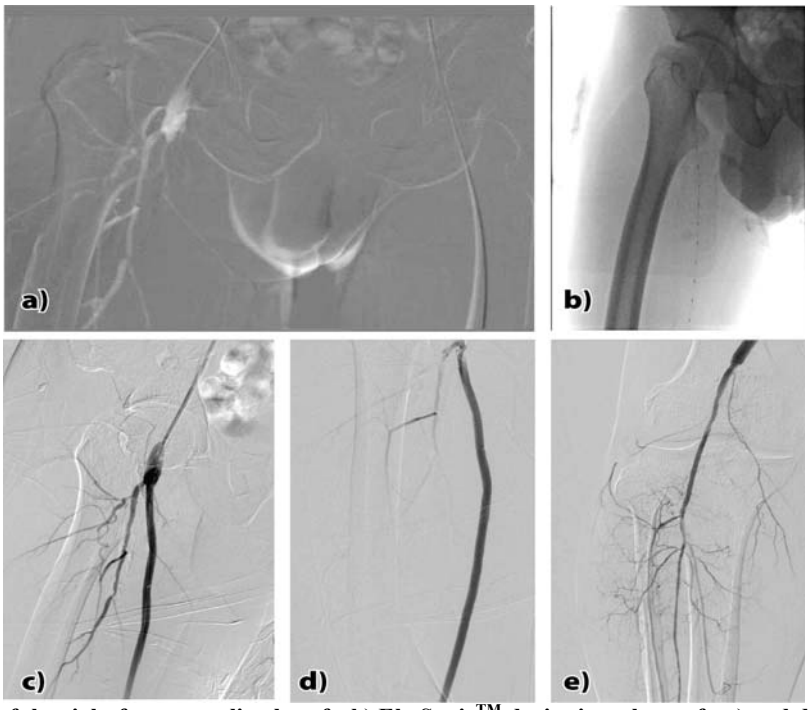

Fig. 4 – a) Occlusion of the right femoropopliteal graft; b) EkoSonic^{TM} device into the graft; c) and d) Control angiograph by findings; e) Stent placement at the site of distal anastomosis.

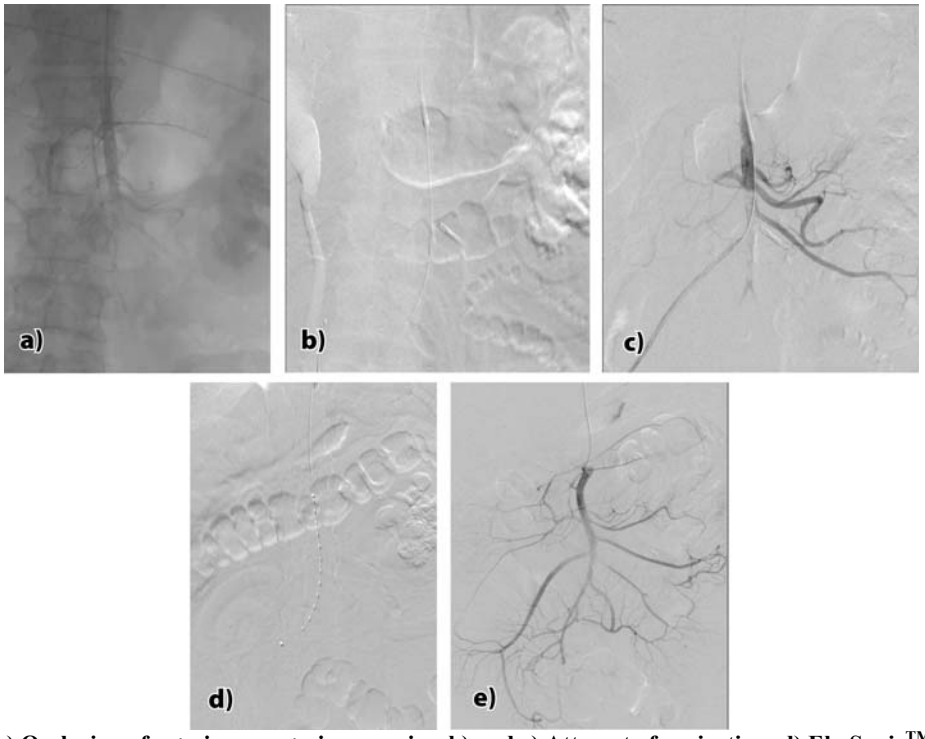

Fig. 5 – a) Occlusion of *arteria mesenterica superior*; b) and c) Attempt of aspiration; d) EkoSonic^{TM} device; e) Final angiogragram.

inferior vena cava that was resolved surgically. On the third postoperative day the patient developed symptoms of acute renal failure. MDCT examination showed thrombosis of the inferior vena cava, which starts from the hepatic vein stretching down and capturing the renal veins. Ultrasound accelerated thrombolytic therapy was applied for 24 hours with the dose of thrombolytic 1 mg/hour. Control angiography revealed reestablished venous drainage. The patient recovered uneventfully with no further renal insufficiency.

There were twenty patients with PTE, treated with this method. Most of them had high risk for bleeding or were resistant to conventional therapy. In all patients were registered significant improvement of symptoms, decrease of systolic right ventricular pressure and decrease of thrombus burden on

control MDCT pulmoangiography. There were no intrahospital deaths in PTE patients treated with EKOS (Figure 6).

Comparison to other techniques

EkoSonic™ Mach4 (EKOS® Corporation, Bothell, WA) infusion system with an ultrasound probe was designed in 1996. The Food and Drugs Administration (FDA) and European Community mark (CE mark) for therapeutic use in peripheral blood vessels was received in 2004.

For systemic administration of rtPA doses are 0.6–0.9 mg/kg for 2 hours in infusion, with a starting bolus of 10 mg previously, but not exceeding 100 mg in total. This way of giving carries a high risk of bleeding and contraindications for administration are hemorrhagic stroke or stroke of unknown etiology anytime, ischemic stroke in the previous 6 months, central nervous system damage or neoplasm, recent trauma and/or surgical intervention, gastrointestinal bleeding in preceding month and positive hemorrhagic diathesis. Relative contraindications are: transitory ischemic attack in the preceding 6 months, oral anticoagulant therapy, pregnancy or the first week postpartum, advanced liver disease, infective endocarditis, active peptic ulcer, refractory hypertension (systolic pressure greater than 180 mmHg).

The total dose of the given thrombolytic therapy using the ultrasound accelerated thrombolytic device is 50–70% smaller comparing to the systemically given.

Classic transcatheter delivering of the drug entails the necessary long time, leaving intraluminal catheter 48 hours or more, which carries more than 10% higher risk of bleeding [17]. This method increases the length of time of therapy, which brings along delivering greater total dose and thus a higher risk of bleeding. According to the recommendations of the American College of Chest Physicians (ACCP) from 2008, in order to reduce the period of drug administration through a catheter, proposed was combining with mechanical method for faster removal of thrombus and thereby shortening the duration of the procedure and the total given dose [18]. Pharmacomechanical thrombolysis involves a longer time of exposal to radiation to patients and staff, the risk of residual thrombus. Those methods mechanically remove a clot only from the place where the aspiration is made or clot is removed in another manner, therapy is usually continued with thrombolytic. There is always hemolysis, high risk of distal remobilization [19] as well as mechanical injury of the vessel wall and vein valves [20, 21]. The difference in protocols and duration of procedures and last but not the least important is that the success rate of the three most common methods that are in use are very different. The protocol for the use of the EKOS® system is as follows: image occlusion, passing a guide wire through the thrombus, catheter insertion IDDC, replacement guide wire catheter MSD, release therapy (thrombolysis plus ultrasound) (Figure 7). The protocol for the use of the trellis system is quite complicated and time-

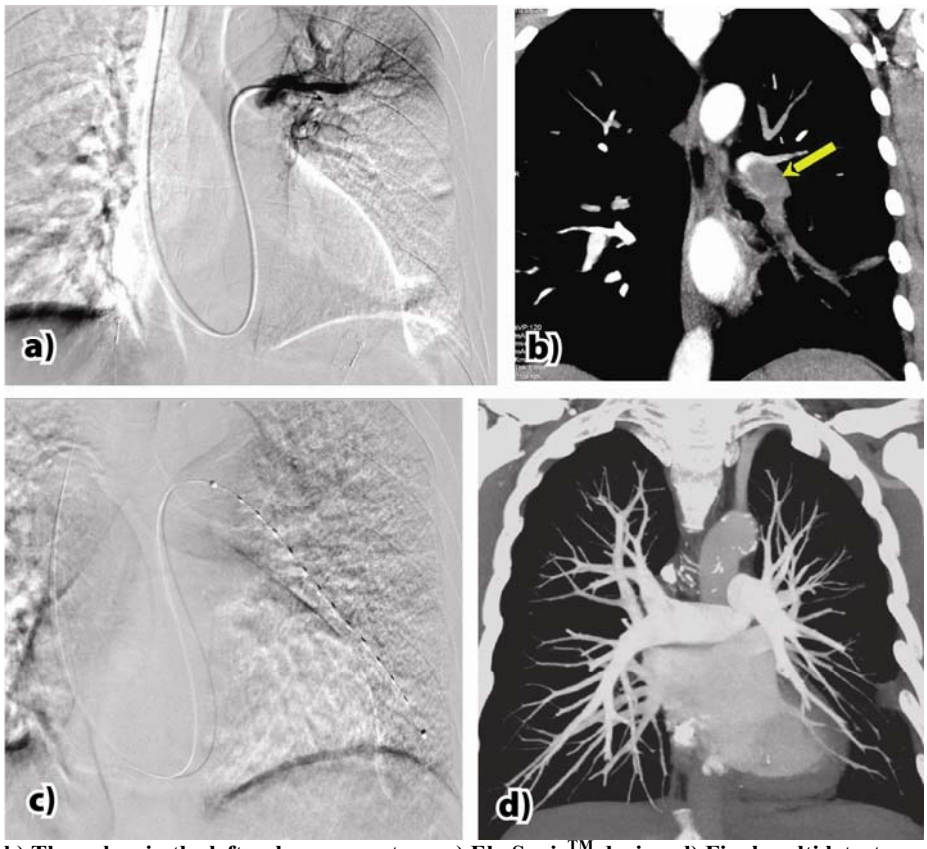

Fig. 6 – a) and b) Thrombus in the left pulmonary artery; c) EkoSonic™ device; d) Final multidetector computed tomography with pulmonary angiography.

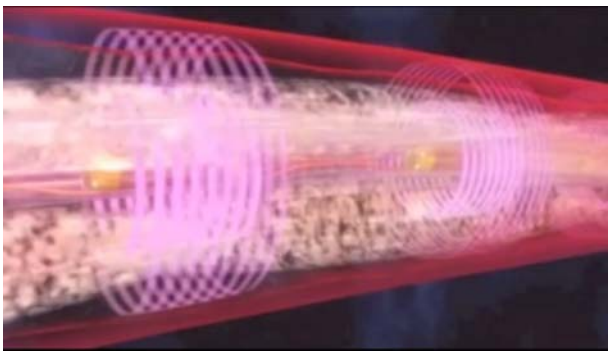

Fig. 7 – Ultrasonic pressure waves encompass entire vessel.

consuming and involves: image occlusion, passing a guide wire through the thrombus, place trellis device into clot, inflate the first balloon, infuse contrast to confirm flow is occluded, inflate second balloon, infuse treatment dose of thrombolytic, insert dispersion wire, attach trellis device unit and activate, manually control drive speed (maximum balloon inflation time: 15 minutes per position), deflate proximal balloon, advance introducer sheat to near distal balloon, aspirate residual thrombus, the distal balloon deflation, image vessel and assess progress. If there is a residual thrombus, the entire procedure is repeated (Figure 8). The entire procedure should not take longer than 60 minutes. If it still persists thrombus applies to any other method [22].

The protocol for the AngioJet® system: occlusion seen, cross clot with guide wire, system setup (8 step the old system, the new ultra 3 steps AngioJet®), set up system for power-pulse mode (unspike saline and exange for thrombolytic), place AngioJet® at proximal end of thrombus formation, activation of the release of thrombolytic in the dose of about 10–20 mg tPA while AngioJet® moves forward/backward through the thrombus, waiting for about 30 min., excludes the thrombolytic, includes a saline solution, cross clot with AngioJet® while aspirating residual material, image vessel and assess progress, if there is residual thrombus entire procedure is repeated (maximum run time is 8–10 minutes depending on the model) (Figure 9). If it still persists thrombus applies to any other method. The advantage of ultrasound accelerated resolution of a thrombus over classical catheter for drug release is: reduced recanalization time, significantly lower risk of bleeding, shortness of in-hospital time. The advantage of ultrasound accelerated resolution of thrombus, over pharmacomechanical thrombolysis is using just one system, no hemolysis, minimal risk of distal embolization, no injuries of blood vessel wall and vein valves, complete resolution of the thrombus, significantly lower exposed dose to radiation of patients and staff.

Considering all the abovementioned EkoSonic® endovascular device imposes as the simplest, safest and most comfortable.

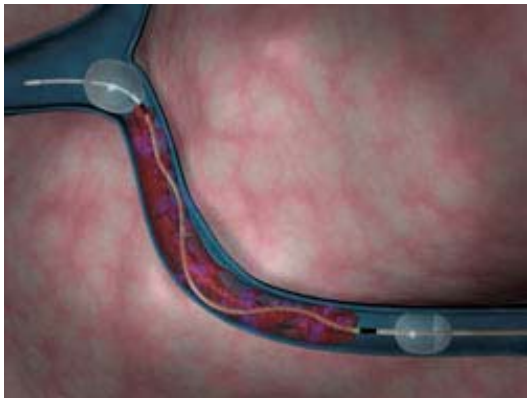

Fig. 8 – Trellis system.

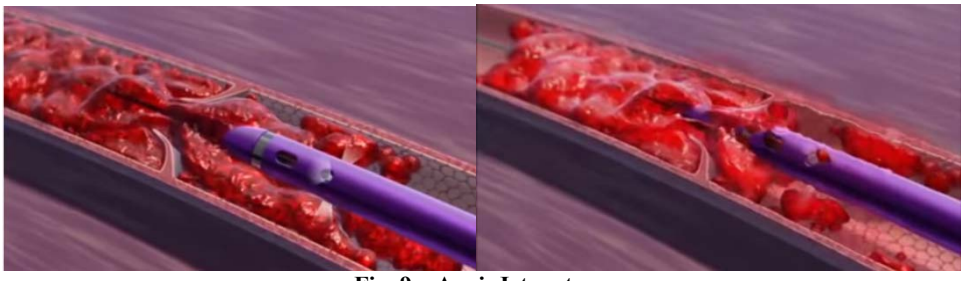

Fig. 9 – AngioJet system.

We may ask why is so difficult to dissolve the thrombus? The fibrin network that is formed (the matrix) does not permit thrombolytic agents to penetrate inside of the thrombus, and thus is limited to access to plasminogen receptors sites. The reason is that they are embedded deep into thrombus during formation [23]. Speed of thrombolysis depends on ability to access the abovementioned receptor sites. EkoSonic™ Mach4 ultrasound energy leads to the dissolution of fibrin network, exposing sites for plasminogen receptors, leading to increased permeability of the thrombolytic and ultrasound waves reaching all the way to the vessel wall and the venous valves, not allowing thrombolytic to migrate into the circulation, thus the dissolution process is accelerated with a lower risk of unwanted bleeding [12] (Figure 10). A study by Francis et al. [12] shows that in relation to ultrasound accelerated thrombolysis, the dosage of given tPA is 48% greater after hour, 84% after 2 hours and 89% after 4 hours.

The difference in penetration of thrombolytic using an ordinary infusion catheter and EkoSonic™ system with the identical amounts of tPA for 15 minutes is as follows: 38% more penetrated with thrombus EkoSonic™ system compared to 17% with regular infusion catheter (Figure 11). Comparative overview of the thrombus dissolution with recombinant urokinase through a standard drug delivery catheter [23] and Ekosonic™ system [10, 24] with the percentage of bleeding demonstrated that more than 26% of patients treated with EkoSonic™ system had a complete dissolution of the thrombus, more than 82% reduction in bleeding and more than 75% of the dissolution of thrombus formation in patients resistant to conventional therapy in a shorter period of time vs drug delivery catheter.

Conclusion

Our initial experience shows that all the patients achieved complete dissolution of the thrombus. There were no complications as bleeding, distal embolization, mechanical injury of the vessel wall, including the vein valves. The total dose of the given rtPA treatment in ultrasound accelerated thrombolysis ranged from 6 to 24 mg in the infusion that lasted from 6 to 24 hours. All the patients successfully achieved complete thrombus dissolution and established normal circulation immediately after the intervention. The prognostic outcome of patients limb salvage in spite of complete resolution of thrombus depends on the condition of distal microvascular circulation.

EkoSonic™ Mach4 is a minimally invasive method that carefully and completely dissolve thrombus in the arterial and venous system leading to reestablishment of the circulation (blood flow), with low risk of complications. The dose of tPA is 50–70% less than the implementation in protocols for systemic administration or other application of the above methods, which gives minimal risk of bleeding, shortens the time of infusion and exposing time to radiation and length of in-hospital stay also. In patients who had recent surgery or trauma, thrombocytopenia, or other contraindications to systemic administration of thrombolytic therapy, or are refractory to conventional therapy, EkoSonic™ system has no alternative.

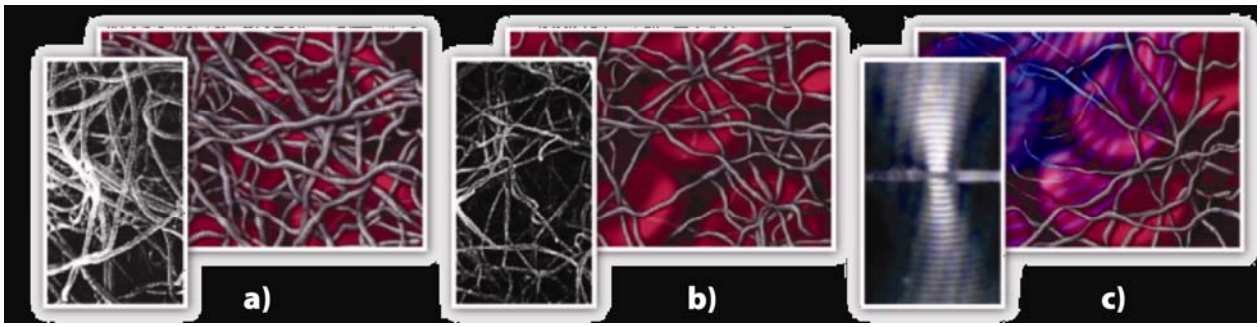

Fig. 10 – Fibrin network dissolution by: a) Only thrombolytic therapy; b) Only ultrasound; c) Ultrasound and thrombolytic therapy.

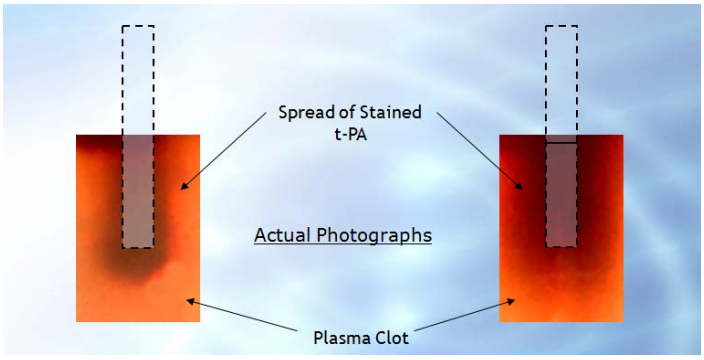

Fig. 11 – Dispersion of thrombolytic with a standard infusion catheter *vs* EkoSonic™ system.

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CASE REPORTS

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Reninoma as a cause of severe hypertension and poor pregnancy outcome in young woman

Reninom kao uzrok teške hipertenzije i lošeg ishoda trudnoće mlade žene

Danica Stamenković-Pejković*, Mirjana Šumarac-Dumanović*†, Nebojša Bojanić†‡, Jasmina Marković-Lipkovski†§, Jelena Vještica†§, Aleksandar Ivanović†‖, Goran Cvijović*†, Ana Gligić*, Uroš Bumbaširević‡, Svetlana Jelić†¶, Snežana Polovina*, Dragan Micić*†

Clinical Center of Serbia, *Clinic for Endocrinology, Diabetes and Diseases of Metabolism, ‡Clinic for Urology, §Institute for Pathology, ‖Center for Radiology and Magnetic Resonance, Belgrade, Serbia; University of Belgrade, †Faculty of Medicine, Serbia; ¶Clinical Hospital Center "Bežanijska kosa", Belgrade, Serbia

Abstract

Introduction. Juxtaglomerular cell tumor (JGCT) or reninoma is a very rare cause of curable hypertension among young people. The early diagnosis is the most important based on the clinical presentation, hormonal and radiological findings observed on computed tomography (CT) and/or magnetic resonance imaging (MRI). The final confirmation of the JGCT is the lateralization of the plasma renin activity (PRA) during the selective renal venous sampling. **Case report.** This report presents a typical case of young women with JGCT which was manifested for the first time with severe hypertension during the pregnancy and was the reason of fetal death. After the miscarriage, the diagnosis of JGCT was made by the CT scanning and confirmed by the selective renal venous sampling. After the partial nephrectomy, the blood pressure and serum potassium normalized without the medications. **Conclusion**. Reninoma should be considered in the differential diagnosis as a cause of severe hypertension in pregnancy and also should be suspected in young hipertensives (especially females) with hypokalemia and secondary hyperaldosteronism after the exclusion of other causes particularly renal artery stenosis. A dynamic contrast-enhanced CT, MRI and selective renal venous sampling are the most important tools in the diagnosis of JGCT.

Key words:
juxtaglomerular apparatus; kidney neoplasms; hypertension; fetal death; diagnostic techniques and procedures; diagnosis, differential; urologic surgical procedures.

Apstrakt

Uvod. Jukstaglomerularni ćelijski tumor (JGĆT) ili reninom je veoma redak uzrok hipertenzije kod mladih ljudi. Najvažnija je rana dijagnoza koja se postavlja na osnovu kliničke prezentacije, hormonskih i radioloških nalaza, pre svega kompjuterizovane tomografije (KT) i magnetne rezonance (MR). Finalna potvrda dijagnoze JGĆT je prisustvo lateralizacije plazma reninske aktivnosti (PRA) tokom selektivnog uzorkovanja krvi iz renalne vene. **Prikaz slučaja.** Prikazan je tipičan slučaj mlade žene sa JGĆT koji se prvi put manifestovao ozbiljnom hipertenzijom tokom trudnoće i bio uzrok smrti ploda. Nakon pobačaja dijagnoza JGĆT postavljena je na osnovu CT pregleda i potvrđena je selektivnim uzorkovanjem krvi iz renalne vene. Nakon parcijalne nefrektomije došlo je do normalizacije vrednosti krvnog pritiska i vrednosti kalijuma u serumu. **Zaključak.** Na JGĆT treba misliti u diferencijalnoj dijagnozi ozbiljne hipertenzije tokom trudnoće kao i kod mladih ljudi (naročito žena) sa hipokalijemijom i sekundarnim hiperaldosteronizmom nakon isključenja drugih uzroka kao što je renalna arterijska stenoza. Dinamski kontrastni KT, MR i selektivno uzorkovanje krvi iz renalne vene najvažnije su procedure u postavljanju dijagnoze JGĆT.

Ključne reči:
jukstaglomerularni aparat; bubreg, neoplazme; hipertenzija; fetus, smrt; dijagnostičke tehnike i procedure; dijagnoza, diferencijalna; hirurgija, urološka, procedure.

Correspondence to: Mirjana Šumarac-Dumanović, Clinical Center of Serbia, Clinic for Endocrinology, Diabetes and Diseases of Metabolism, Dr Subotica 13, 11 000 Belgrade, Serbia. E-mail: drdanicastamenkovic@gmail.com

Introduction

Juxtaglomerular cell tumor (JGCT) or reninoma is a very rare cause of curable hypertension among young people. It is typically presented with hypertension, hypokalemia, and hyperaldosteronism secondary to excessive renin secretion by tumor cells [1]. Approximately 119 cases were published and the majority of the reported cases were benign tumors, except in four cases [2–5]. However, its clinical behavior can be malignant as a result of severe systemic complications of hypertension. The early diagnosis is the most important based on clinical presentation, hormonal and radiological findings observed on computed tomography (CT) and/or magnetic resonance imaging (MRI). The final confirmation of the JGCT is the lateralization of the plasma renin activity (PRA) during the selective renal venous sampling [6, 7]. However, patients with JGCT can be misdiagnosed due to the small size of the tumor which can not be visualized and/or the lack of the lateralization of PRA during the selective renal venous sampling. The usual treatment consists of partial or complete nephrectomy which results in normalization of blood pressure.

This report presents a typical case of young women with JGCT WHO manifested for the first time severe hypertension during the pregnancy, which was the reason of fetal death. After the miscarriage, the diagnosis of JGCT was made by using CT scanning and confirmed by the selective renal venous sampling.

Case report

A 20-year old female patient was referred to our hospital for a further examination of persistent and severe hypertension and hypokalemia lasting for over one year. The diagnosis of severe hypertension was established at 20th gestational weeks at 19 years of age. Antihypertensive therapy was started with methyldopa (500 mg two times daily). Except for occasional headache, the patient denied any other symptoms. PRA and plasma aldosterone concentration (PAC) were elevated (PRA 23.6 ng/mL/h, normal range 0.2–2.8 ng/mL/h; PAC 949 ng/L, normal range 42–201.5 ng/L). The pregnancy was discontinued at 24th gestational week when fetal death was diagnosed. Pathoanatomical diagnosis of the fetus and pathohistological diagnosis of the placenta showed the fetal mass of 425 g which was adequate for the 22 weeks of gestation. In the placental bed and in the intervillous space recent hemorrhage and fibrin deposits were seen. Significant syncytial nodules and villous fibrosis were present. Thickened walls of fetal blood vessels were also present.

Few months after the miscarriage she was still hypertensive and because of the hypertensive crisis (blood pressure – BP 240/120 mmHg) she was hospitalized in the regional clinical center. The renal vascular stenosis and aortic coarctation were excluded using renal angiography and cardiac ultrasound, but severe hypokalemia (2.6 mmol/L) was noticed. The antihypertensive therapy was changed to captopril (50 mg three times a day), bisoprolol (5 mg twice a day), amlodipine (10 mg once a day) and potassium chloride (1 g twice a day) in the local hospital. After that, she was referred to our hospital for further investigation of hypertension and hypokalemia. On admission, her blood pressure was normal (115/70 mmHg) and the physical examination showed no significant findings. The serum potassium level was normal on the substitution therapy and the results of other routine laboratory tests were within the normal ranges. The endocrine examination was performed after washout period of two weeks (taking amlodipine 10 mg only) and it revealed elevated PRA (27.6 ng/mL/h, normal range 0.2–2.8 ng/mL/h) and PAC levels (1,633.7 ng/L, normal range 42–201.5 ng/L) indicating secondary hyperaldosteronism. Adrenocorticotropin (ACTH), cortisol, dehydroepi-androsterone-sulfate (DHEA-S), thyroid-stimulating hormone (TSH), thyroxine (T4), catecholamines and chromogranin were normal. Fundoscopy demonstrated hypertensive retinopathy grade II.

A dynamic contrast-enhanced CT image revealed a small renal tumor (10 mm in diameter) (Figure 1, A and B). On the other hand, there was no evidence of renal artery stenosis and the adrenal glands were normal. However, considering the findings of dynamic contrast-enhanced CT we could not completely exclude the possibility that the renal tumor was not JGCT, but rather some other tumor of the kidney and the possibility that renin was being secreted by an ectopic extrarenal tumor. Therefore, we performed selective renal venous sampling to assess the level of PRA and direct renin secretion by the tumor of the left kidney described on CT scan. We did not perform a strict low-sodium diet four days before the test nor did apply the intravenous fast acting angiotensin converting enzyme (ACE) inhibitors during the venous sampling. The only preparation for the test was discontinuation of potentially interfering medications 4 weeks before the test (the patient was on amlodipine therapy 10 mg once daily). The renal venous sampling was done in the early morning after overnight recumbency. Consequently, the PRA level was 37.9 ng/mL/h in the left renal vein, 3.1 ng/mL/h in the right renal vein and 31.7 ng/mL/h in the low inferior vena cava. This indicated the clear lateralization of PRA on the left side as the lateralization rate was 12.2 (the accepted rate of lateralization is > 1.5). At the same time, the renin concentration was measured. At first, we got the same high values in both veins (> 500.0 μIU/mL, normal range 2.8–39.9 μIU/mL, CLIA). After dilution, the renin concentration in left vein was 2,796.0 μIU/mL and in the right vein 520 μIU/mL. Taken together, we strongly suspected that the tumor in the left kidney was JGCT and the patient was prepared for the operation with the spironolactone (50 mg twice a day) and fosinopril (20 mg once daily) having achieved excellent control of blood pressure and potassium level. The open partial nephrectomy with intraoperative ultrasound was done and no complications were observed (Figure 1, C and D). The histological examination and immunohistochemistry confirmed the diagnosis of JGCT (Figure 2). After the operation, the measured levels of PRA and PAC were in the normal ranges (PRA 0.74 ng/mL/h, PCA 74.2 ng/L). The blood pressure and serum potassium normalized without the medications.

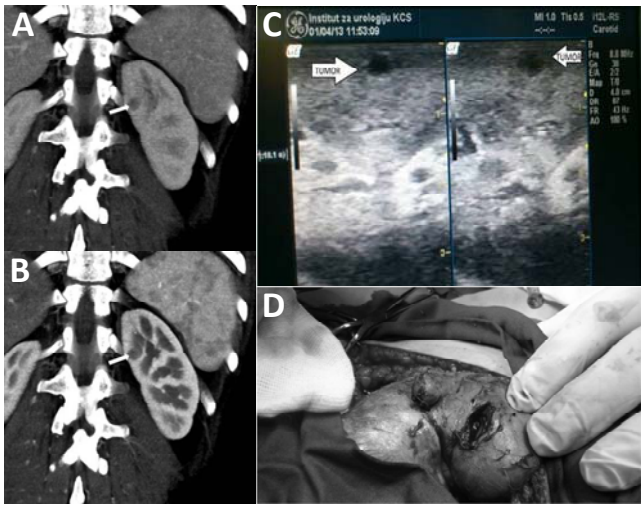

Fig. 1 – A) Contrast enhanced multidetector computed tomography (MDCT) of the left kidney, cortical phase. Frontal multiplanar reformation. Picture shows discrete rounded 10 mm lesion, that appears izodense with surrounded medulla of the upper third of the left kidney (arrow). Renal cortex is normal; B) Contrast enhanced MDCT of the left kidney, nephrographic phase. The lesion is well bordered with kidney during nephrographic phase, nonenhanced, low attenuated and hypovascular (hypodense), typical for reninoma (arrow). Contour of the kidney is not altered, no distortion of the renal hilum; C) Intraoperative ultrasound of small renal tumor – reninoma (arrow); D) Operative finding of small tumor imunohistochemically confirmed as reninoma.

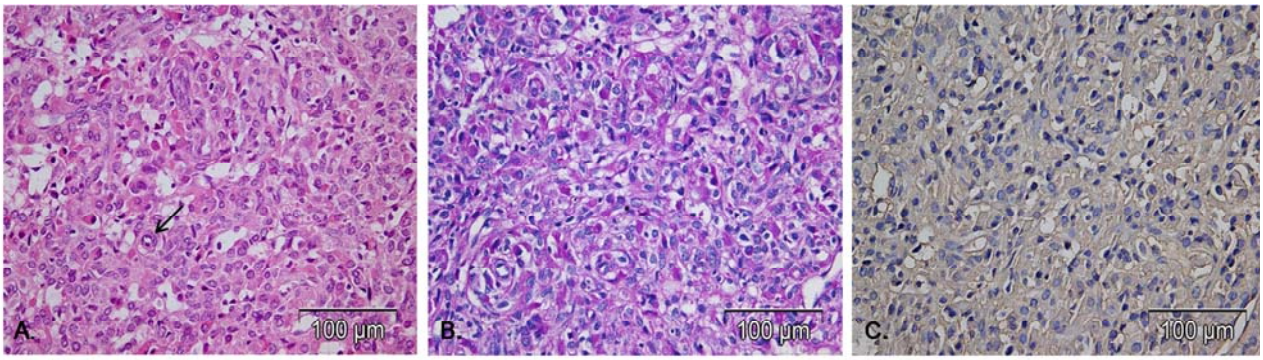

Fig. 2 – A) Reninoma: the tumor is highly cellular, composed of round, polygonal or spindle cells with granular eosinophilic cytoplasm and distinct cell borders, in the background of minimal mixomatous stroma, some of them forming the walls of small vessel (arrow) hematoxylin and eosin staining; B) the cytoplasmic granules react with periodic acid-schiff (PAS); C) Immunohistochemical positivity of the tumor to CD34. Original magnification ×400. Scale bar = 100 µm.

Discussion

Herein we presented the case of a typical JGCT in a young woman with severe hypertension in pregnancy, hypokalemia and secondary hyperaldosteronism diagnosed with JGCT after poor fetal outcome by using the CT and selective renal venous sampling.

Diagnosis of hypertension was made during the pregnancy which was terminated at 24th gestational week due to fetal death. To the best of our knowledge this is the fourth case of JGCT complicating pregnancy [8–10]. Secondary aldosteronism in pregnancy is a normal physiologic response to estrogen-induced increases in circulating levels of renin substrate and PRA and to the anti-aldosterone actions of progestagens. The pregnancy might be the trigger for aggravation and manifestation of existing hypertension and secondary aldosteronism in JGCT. As it was mentioned above the patient has high PRA and aldosterone levels measured at 20th gestational weeks.

JGCT primarily affects adolescents and young adults, with peak prevalence in the second and third decades of life, and it is twice as common in women. Haab et al. [11] described eight JGCT among 30,000 hypertensive patients, the largest series in the literature. Average age at diagnosis was 22 years (range: 7 to 58 years). Our patient was 19 years old women when the diagnosis of hypertension was made, and she was in the typical age group for the diagnosis of JGCT. According to the clinical presentations, laboratory and imaging examinations and pathologic results the JGCT can be classified into 3 types: typical, atypical and non-functioning type [12]. The typical JGCT is characterized by marked hypertension, severe hypokalemia and hyperaldosteronism secondary to tumor renin secretion. The typical variant is the most common type of JGCT. Our patient is the example of

the typical JGCT with all the mentioned features and with the excellent control of severe hypertension on the ACE inhibitor and spironolactone therapy using small doses. The clinical presentation of atypical JGCT includes marked hypertension with normal serum potassium and renin secretion. The clinical presentation of the non-functioning JGCT includes renal tumor with normal blood pressure and potassium [12, 13]. A non-functioning variant is the rarest and is thought to produce inactive renin [13].

In our case, the suspicion of JGCT was made after the exclusion of the other causes of hypertension in young adults such as renal artery stenosis, aortic coarctation, pheochromocytoma and causes of primary aldosteronism. MRI and CT are generally able to identify renal tumors accurately and can be equally effective in the detection of JGCT with rates of detection approaching 100% in some series [1, 14]. However, JGCT tumors smaller than 5 mm can cause severe hypertension and can not be seen using standard imaging techniques [15]. JGCT usually appears isodense or hypodense to the renal medulla. If the tumor is small and isodense to the renal medulla on non-enhanced CT, it may not be detected. Taken together, the use of enhanced CT should be considered in all cases of suspected JGCT [6]. In our case, CT revealed small tumor and in combination with secondary aldosteronism, it was obvious that JGCT can be the cause of hypertension. However, considering the findings of CT we could not completely exclude the possibility that the renal tumor was not JGCT but rather another type of tumors such as angiomyolipoma or renal cell carcinoma and that renin was being secreted by an extrarenal tumor (lung carcinomas, pancreatic adenocarcinomas, fallopian tube adenocarcinomas, ovarian leiomyosarcoma) [16, 17]. Therefore, we performed selective renal venous sampling to evaluate direct PRA and renin secretion from the tumor and we got the clear lateralization rate of 12.2. A previous study of 50 cases of renal venous sampling reported that the sensitivity and specificity were 56% and 94%, respectively for the lateralization rate of 1.5 [1]. However, the variable success of this procedure in achieving accurate lateralisation of the JGCT has been published in the literature. Haab et al. [11] reported that 3 of 8 patients with JGCT were unable to be diagnosed by the selective renal venous sampling despite repeated attempts and its visualization on CT. Although the detailed reasons for the failure of previously reported cases of renal venous sampling are unclear, one proposed that the tumors are primarily located on the surface of the kidneys and most of the venous supply of the tumors is collected into the perivascular veins instead of the main renal vein [11, 18]. Precise details for preparing patients for the selective renal venous sampling still do not exist but there are some recommendations as the administration of dietary salt restriction (40 mmoL/day) for 4 days before the sampling. In addition, cessation of potentially interfering medications is recommended (diuretics, beta blockers, ACE inhibitors, angiotensin II receptor blockers, spironolactone) where it is possible for at least 4 weeks before the test. The overnight recumbency is also proposed [7]. The administration of a rapidly acting ACE inhibitors during the sampling can be beneficial improving the sensitivity of the test [19]. In our case, we did not perform dietary salt restriction before the sampling and we did not use ACE inhibitors during the procedure but we got the clear lateralization of the PRA and renin. As there are many cases of unsuccessful renal venous sampling we suggest careful patient preparation.

Clinical behavior of JGCT can be malignant due to severe systemic complications of hypertension, especially in cases with a delayed diagnosis of the tumor. Retinopathy, renal insufficiency and left ventricular hypertrophy have been reported in 24%, 3% and 7% of cases, respectively [1]. Cerebrovascular accident and intestinal ischemia have been also reported [20, 21]. Our patient had hypertensive retinopathy grade II and it significantly resolved few months after the operation.

Because JGCT is mostly benign, partial nephrectomy is the proposed treatment with successful outcomes reported. Laparoscopic partial nephrectomy is particularly recommended as the tumor is usually small [1, 11].

Conclusion

This case of JGCT was diagnosed during the pregnancy and was the reason of poor fetal outcome. This tumor should be considered in the differential diagnosis as a cause of severe hypertension in pregnancy. The tumor had the typical presentation and after the exclusion of the other causes of hypertension in young adults, particularly renal artery stenosis, the investigation was directed to the JGCT. A dynamic contrast-enhanced CT, MRI and selective renal venous sampling are the most important tools in the diagnosis of JGCT.

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CASE REPORT

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Alveolar bone defect regeneration after bilateral periapical cyst removal with and without use of platelet rich fibrin – A case report

Zarastanje koštanog defekta nastalog enukleacijom bilateralnih periapikalnih cista sa i bez upotrebe fibrina obogaćenog trombocitima

Nemanja Vuković*, Marjan Marjanović*, Bojan Jovičić[†‡], Ema Aleksić[§], Katarina Kalevski[‖], Nenad Borotić[¶], Djordje Pejanović*, Marko Andjelković**

University of Business Academy in Novi Sad, Faculty of Dentistry, *Clinic for Oral Surgery and Implantology, [†]Clinic for Mouth Diseases, [§]Clinic for Orthodontics, [‖]Clinic for Child and Preventive Stomatology, [¶]Clinic for Prosthodontics, Pančevo, Serbia; Military Medical Academy, [‡]Clinic of Dentistry, Belgrade, Serbia; University of Belgrade, Faculty of Dental Medicine, **Department of Prosthodontics, Belgrade, Serbia

Abstract

Introduction. Periapical inflammatory lesions are local bone responses around the apex of a tooth that occur after necrosis of the pulp tissue. The ultimate goal of reconstructive surgical techniques in the treatment of the intra-bone defects is a regeneration of lost bone tissue. The aim of this report was to evaluate clinical and radiographic outcome following the removal of two big, periapical lesions, approximately of the same size, located around maxillary lateral incisors, in the same person at the same time, using two different regenerative approaches. **Case report.** A healthy, 21-year-old female presented with two large periapical lesions around both upper lateral incisors, and a surgical treatment was indicated. One residual defect (tooth #12) was filled with the mixture of bovine-derived hydroxyapatite xenograft and platelet rich fibrin (PRF) gel and covered with PRF membrane, while the other (tooth #22) was filled with bovine-derived hydroxyapatite xenograft only and covered with a resorbable collagen membrane. Clinical and radiographic examinations were performed seven months after the surgery. All clinical and radiographic parameters were significantly improved after the treatment on both sites; however, a newly formed bone around the tooth 12 showed a higher bone density. **Conclusion.** The use of PRF significantly speeded up filling of the defect compared to bovine-derived hydroxyapatite xenograft.

Key words:
oral surgical procedures; platelet rich plasma; bone regeneration.

Apstrakt

Uvod. Inflamatorne periapikalne lezije su posledica širenja infekcije iz kanala korena zuba. Cilj moderne periapikalne hirurgije je uklanjanje lezija u celosti, kako bi se omogićila potpuna restitucija periapikalnog, sa težnjom da se povrati izgubljeno koštano tkivo. Cilj ovog prikaza bolesnika bio je da se klinički i radiografski proceni rezultat zarastanja koštanog defekta nakon uklanjanja dve velike periapikalne ciste kod iste osobe u isto vreme, koristeći dva različita regenerativna pristupa. **Prikaz bolesnika.** Kod zdrave, dvadesetjednogodišnje bolesnice dijagnostivane su dve periapikalne ciste gornjeg levog i desnog lateralnog sekutića i indikovano njihovo hirurško uklanjanje. Jedan koštani defekt je popunjen mešavinom serumskog eksudata dobijenog kompresijom plazme obogaćene fibrinom (PRF) ugruška i govedjeg koštanog ksenografta, a zatim prekriven membranom od PRF. Drugi defekt popunjen je korišćenjem samo hidratisanog govedjeg koštanog ksenografta i prekriven resorptivnom kolagenom membranom. Kliničko i radiografsko ispitivanje izvršeno je sedam meseci posle hirurškog tretmana. Svi klinički i radiografski parametri bili su značajno poboljšani posle tretmana na oba mesta. Međutim, novoformirana kost oko zuba br. 12 imala je veću koštanu gustinu. **Zaključak.** PRF je značajno ubrzao zarastanje koštanog defekta.

Ključne reči:
hirurgija, oralna, procedure; plazma bogata trombocitima; kost, regeneracija.

Correspondence to: Nemanja Vuković, Faculty of Dentistry Pančevo, Clinic for Oral Surgery and Implantology, Žarka Zrenjanina 179, 13 000 Pančevo, Serbia. E-mail: drnemanjavukovic@gmail.com

Introduction

Periapical inflammatory lesions are local bone responses around the apex of a tooth that occur after necrosis of the pulp tissue caused by dental caries, mechanical or thermal trauma and chemical agents. Modern periapical surgery aims to remove periapical lesions to achieve complete wound healing and aid regeneration of bone and periodontal tissue. Since natural healing takes a relatively long period of time for the bone to fill the residual cavity [1], regenerative approaches that help restore lost tissue and speed up regeneration have been introduced [2, 3]. Regeneration is the process of reproduction or reconstitution of a lost or injured part of the body in such a way that the architecture and function of the lost or injured tissues are completely restored, and it is a natural process of wound healing. The ultimate goal of reconstructive surgical techniques in the treatment of intra-bone defects is a regeneration of lost bone tissue.

Many regenerative techniques, utilizing bone grafts and barrier membranes, were introduced to help the optimal healing of the residual defects after the removal of intra-bone lesions, especially large cysts [4–6]. Lately, the use of platelet rich fibrin (PRF) showed very promising results in regenerative surgical procedures [7], although autologous platelet concentrates and their advantages are already very well-known [8]. The PRF technique was developed in 2001 by Joseph Choukroun and associates with the idea to combine characteristics of both platelets and growth factors into a fibrin clot in order to accelerate healing and remodeling of bone and soft tissue [9].

So far, there has been no consensus on whether or not alveolar bone defects left after large periapical cysts removal should be filled with bone grafts or other healing inducing derivate [10], but the positive effects these techniques have on alveolar bone formation are undisputed [11]. The aim of this report was to show clinical and radiographic outcome following the removal of two big periapical lesions, approximately of the same size, located around maxillary lateral incisors, in the same person at the same time, using different regenerative approaches.

Case report

A healthy, 21-year-old female patient complaining of the pain in the upper left anterior region presented to the Department of Conservative Dentistry and Endodontics, Faculty of Dentistry in Pančevo. During the intraoral examination, a slight discoloration of upper left lateral incisor was observed; tooth was mobile and sensitive to vertical percussion, with non-exudative swelling in the apical region. The patient was provided with the first aid dental treatment and sent to the Department of Radiology. The panoramic radiograph revealed the presence of periapical intrabony defects around apices of teeth #12 (approximately 13 mm in diameter) and #22 (11 mm in diameter) and inadequate endodontic treatment of the tooth #12 (Figure 1).

Root canal treatment of teeth #12 and #22 was performed just before surgery. The PRF was prepared in accordance with the standard protocol. Just prior to surgery, 30 mL

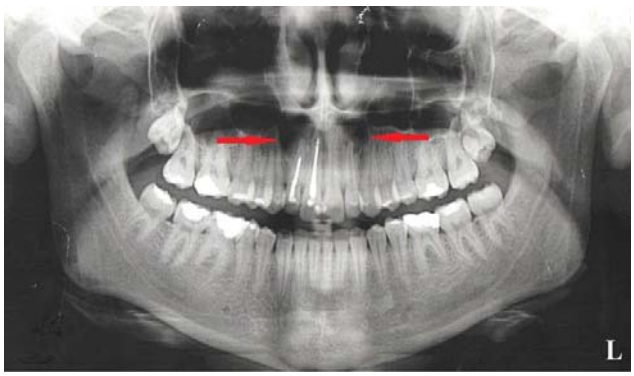

Fig. 1 – The panoramic radiograph before the treatment.

intravenous blood was collected in a 10-mL sterile tube and immediately centrifuged in centrifugation machine (A-PRF 12, APRF, Nice, France) at 3,000 revolutions per minute for 10 minutes. This protocol of blood centrifugation allows the forming of a 3D fibrin structured clot in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma (platelet-poor plasma) at the top. PRF was easily separated from red corpuscles base (retaining a small red blood cell layer) using sterile tweezers (Figure 2) and scissors just after the removal of the tube and then transferred to a sterile dish.

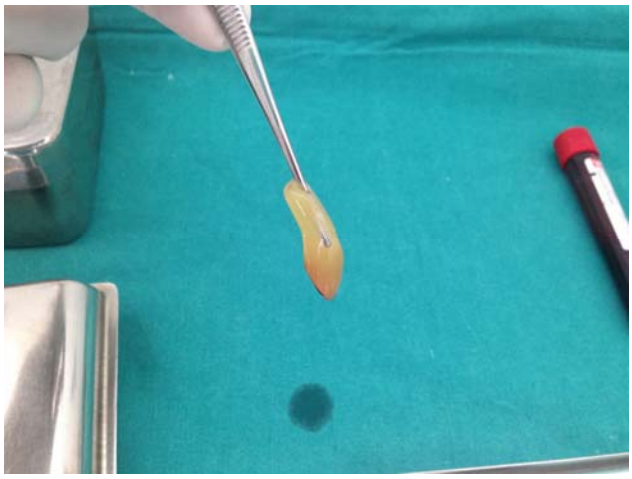

Fig. 2 – The platelet rich fibrin (PRF) clot.

After administration of local anesthetic, buccal angular incisions were made and full thickness mucoperiosteal flap was elevated. Labial cortical plate around the apex of the tooth #12 was removed, revealing the periapical intrabony defect in evident relation to the tooth. The cystic lining was enucleated and sent for a biopsy which identified the lesions as radicular cysts. Using tapered fissure bur, tooth root was resected and mineral trioxide aggregate (MTA) (ProRoot MTA; Dentsply, Tulsa, OK, USA) was used as the root end filling material. Bovine-derived hydroxyapatite xenograft (Bio-Oss®, GeistlichPharma AG, Switzerland) was sprinkled over the PRF gel and the whole mixture was placed into the defect (Figure 3). PRF membrane was prepared with compresses and placed in two layers over the edge of the defect (Figure 4). The mucoperiosteal flap was repositioned using 3-0 non-absorbable black silk surgical simple interrupted sutures. The same surgical technique was used for the removal

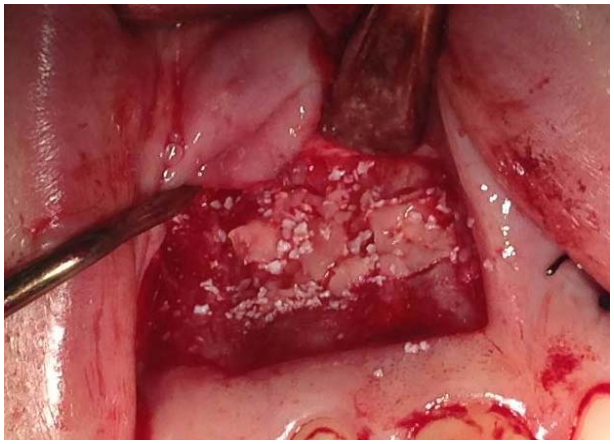

Fig. 3 – BioOss mixed with platelet rich fibrin (PRF) gel and placed into the defect.

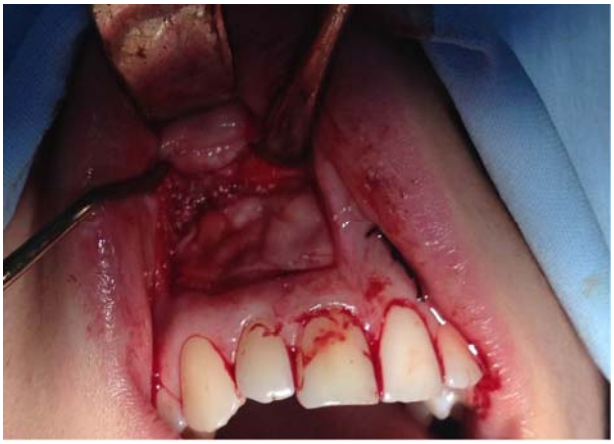

Fig. 4 – Platclet rich fibrin (PRF) membrane placed over the defect previously filled with the mixture of BioOss and PRF gel (upper right lateral incisor).

of the tooth #22 periapical lesion, but this time bovine-derived hydroxyapatite xenograft mixed with a physiological solution was placed into bone defect and resorbable collagen membrane (Bio-Gide® COLLAGEN – GeistlichPharma AG, Switzerland) was placed to cover the edge of the defect.

Clinical and radiographic examinations were performed seven months after the surgery. The following parameters were evaluated: bone density, alveolar ridge width at the place of residual defect and mobility of the teeth. All clinical and radiographic parameters were significantly improved after the treatment on both sites; however, a newly formed bone around the tooth 12 showed a higher bone density (Figures 5–8). No mobility of teeth was observed at both sites.

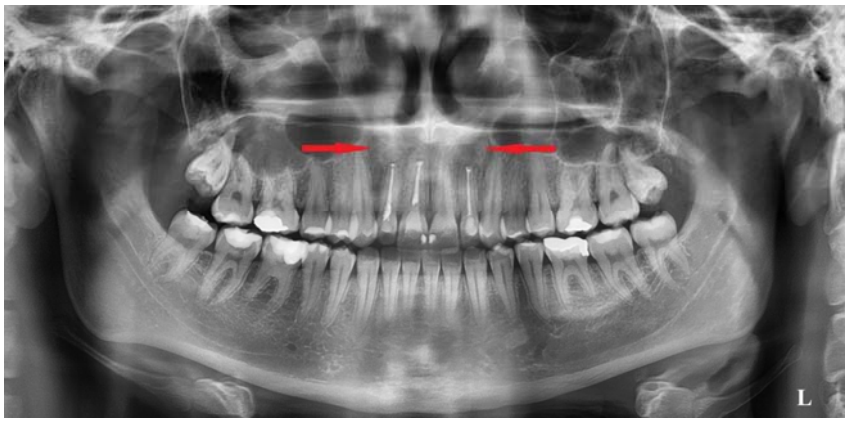

Fig. 5 – The panoramic radiograph after the treatment.

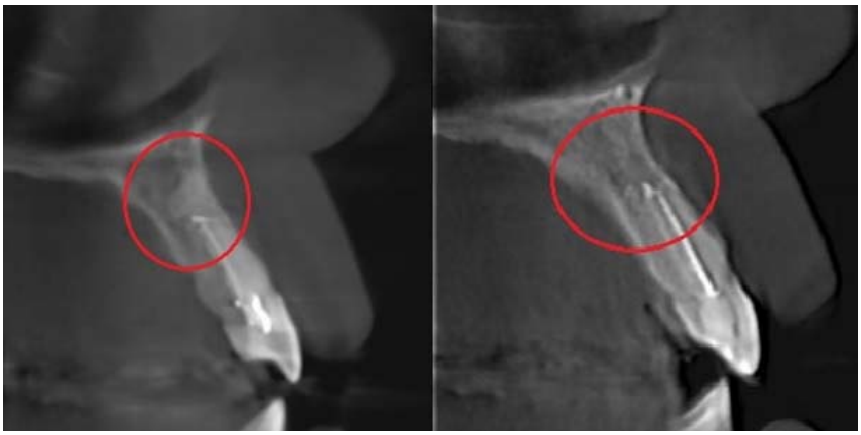

Fig. 6 – Region of the former defects filled up with newly formed bone above the root of the tooth #12 (left) and the tooth #22 (right) (Ez3D plus software, Vatech Global, Hwaseong-si, Gyeongii-do, Korea).

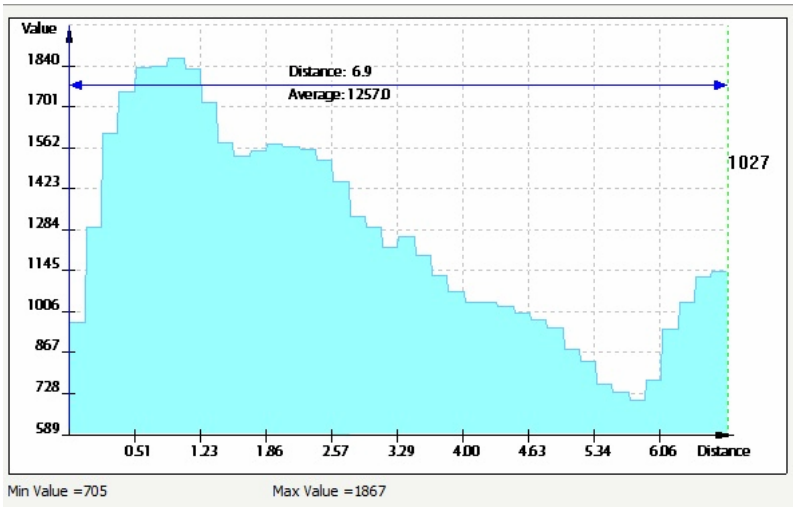

Fig. 7 – Bone density measured in Hounsfield units and alveolar ridge width 3 mm above resected root of the tooth #12 (measured in Ez3D plus software, Vatech Global, Hwaseong-si, Gyeonggi-do, Korea).

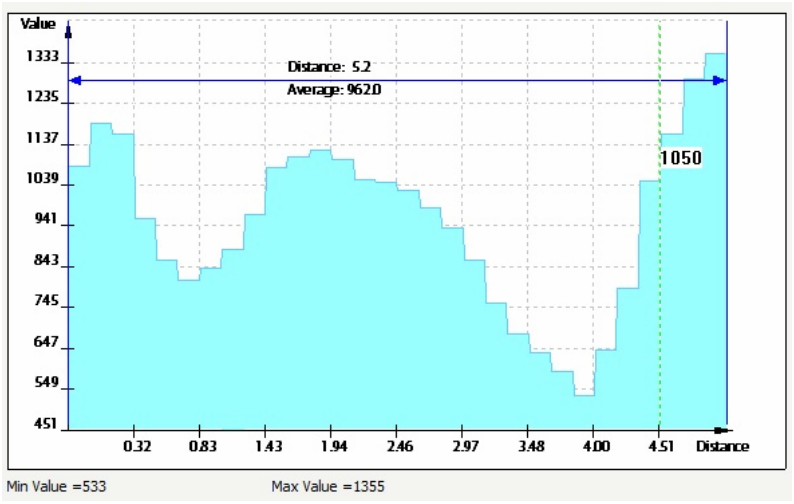

Fig. 8 – Bone density measured in Hounsfield units and alveolar ridge width 3 mm above resected root of the tooth #22 (measured in Ez3D plus software, Vatech Global, Hwaseong-si, Gyeonggi-do, Korea).

Discussion

After periapical surgery, large bone defects may be created, which sometimes cannot be adequately filled on their own, so the use of suitable graft material is required. PRF is a matrix of autologous fibrin which contains a lot of platelets and intrinsic cytokines within the fibrin mesh, allowing their gradual release over time (7–11 days), correlating with the resorption of fibrin network [9]. The use of PRF grafting offers several advantages: PRF clot and membrane play mechanical role by protecting and linking the grafted biomaterials with themselves and with bone tissue, fibrin network accelerates cellular migration (endothelial cells) necessary for the neo-angiogenesis, platelet cytokines (platelet-derival growth factor – PDGF, transforming growth factor alpha – TGF-α, insulin like growth factor – IGF-1) are released, helping the process of healing, and leukocytes and cytokines in the fibrin network play a significant role in the regulation of inflammatory and infectious processes during wound healing [12, 13].

PRF is a derivate of patient's own blood, and as such does not trigger an immune response and minimize chances of infectious disease transmission [14]. Preparation of PRF is easy and fast and is done in the dental office immediately before the intervention, which simplifies the procedure and saves the patient of blood harvesting in the hospital [14].

In this case report, we analyzed the clinical efficacy of PRF autologous graft comparing it with heterologous graft in the treatment of intrabony defects. It was shown that the use of PRF speeds up the filling of the defect compared to bovine-derived hydroxyapatite xenograft.

Conclusion

This case report indicates that the use of PRF should be considered as a promising solution for a successful augmentation of large bone defects in everyday dental surgical practice.

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CASE REPORT

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Sneezing – a symptom of respiratory or psychogenic superposition of illness in a teenager?

Kijanje – simptom respiratorne ili psihogene superpozicije bolesti u tinejdžera?

Andjelka Stojković*†, Slobodan Obradović*†, Dejan Jeremić*, Aleksandra Simović*†, Maja Vulović*

University of Kragujevac, *Faculty of Medical Sciences, Kragujevac, Serbia; Clinical Center of Kragujevac, †Pediatric Clinic, Kragujevac, Serbia

Abstract

Introduction. The coincidental combination of allergic respiratory diseases and psychogenic or psychiatric illness is possible but rarely associated in a female teenager. **Case report.** A girl aged 12.5 years was admitted to the Pediatrics Clinic in the Clinical Centre Kragujevac with the main difficulty of sneezing as 10 sneezes in each of the 500–600 series a day, only in the waking state. Working diagnoses were tic disorders associated with Allergic rhinitis, asthma and biochemically determined rickets. The patient was treated with chlorpromazine, desloratadine, montelukast, fluticasone propionate, vitamin D, azelastine hydrochloride along with the elimination diet. After a year and a half, the patient rarely sneezed, but periods without sneezing were not longer than 3 months, and she 'cleared her throat' occasionally. Although her clinical condition is less severe now and without additional motor tics or symptoms of Tourette's disorder, because of its length we suspect the tic turned into a chronic vocal tic disorder. **Conclusion.** We reported a case of rare association between allergic rhinitis and condition of tic-related conversion in a female teenager and emphasized the necessity of revising the systematisation for the tic disorders and protocol for the allergic rhinitis in terms of their association.

Key words:
adolescent; rhinitis, allergic; tic disorders.

Apstrakt

Uvod. Udruženost alergijskih respiratornih oboljenja i psihogene ili psihijatrijske bolesti je moguać, premda se javlja retko kod devojčica u tinejdžerskom uzrastu. **Prikaz bolesnika.** Devojčica uzrasta 12,5 godina primljena je u Pedijatrijsku kliniku Kliničkog centra Kragujevac sa glavnom tegobom kijanje, po 10 uzastopnih kijanja u serijama od 500–600 dnevno i to samo u budnom stanju. Radne dijagnoze bile su tik poremećaj udružen sa alergijskim rinitisom, astmom i biohemijski utvrđenim rahitisom. Tinejdžerka je lečena hlorpromazinom, desloratadinom, montelukastom, flutikazon propionatom, vitaminom D, azelastin-hidrohloridom, sa pratećom eliminacionom ishranom. Posle perioda od godinu i po dana, bolesnica je ređe kijala, periodi bez kijanja nisu bili duži od 3 meseca i povremeno je „čistila grlo". Mada je sada klinička slika bila blaža i bez dodatnog motornog tika ili simptoma Tourette poremećaja, zbog dužine trajanja tegoba postoji sumnja da se tik preobratio u hronični glasovni tik poremećaj. **Zaključak.** U radu je prikazana retka udruženost alergijskog rinitisa i stanja tik-srodne konverzije kod tinejdžerke i istaknuta je potreba za reviziju sistematizacije tik poremećaja i protokola za alergijski rinitis u smislu njihove udruženosti.

Ključne reči:
adolescenti; rinitis, alergijski; tik poremećaji.

Introduction

Sneezing in children is the most common symptom of allergic and infective rhinitis. Sneezing (or blowing) provides a high-velocity ejection from the mucosal surface [1]. Sneezing is an involuntary reaction, and a protective reflex that starts in the nasal mucosal lining travels to the medulla oblongata, from which many muscles are stimulated to take a deep breath [2]. After that the breathing stops for a short time, the soft palate rises and closes the inner nasal opening. At the same time, the respiratory muscles, participating in the exhalation, are strained, and the pressure in the lungs rises. The soft palate lowers, and the air penetrates into the nose under high pressure, goes through the nasal passages removing all foreign and harmful substances from the nose.

As a sudden, strong and uncontrollable expulsion of the air through the nose and mouth, sneezing is rarely a serious health issue in children. The infective rhinitis and allergic

Correspondence to: Andjelka Stojković, Clinical Centre "Kragujevac", Pediatric Clinic, Zmaj Jovina 30, 34 000 Kragujevac, Serbia.
Phone: +381 34 505 175; E-mail: andja410@mts.rs

rhinitis are, logically, first suspected in primary health care and the treatment begins without testing protocols [3], which turns out in this case as a reason for potentiating the health problem of a patient. Sneezing is, also, a common symptom of nonallergic-noninfective rhinitis, acute and chronic rhinosinusitis and the like.

It is rare that, among the multiple common triggers of sneezing in children, a strong emotion is associated with allergic rhinitis, resulting in frequent and exhausting sneezing prolonged over several days, weeks and even months. As it is presented in this case report, such hardships were put forward for the consideration as a particular disorder, among various psychiatric and psychological disorders in a child or teenager [4].

Tic, in medicine, does not imply any voluntary action of a body part when a person is excited, stressed, anxious, and after an excessive intake of caffeine or alcohol [5]. In that case, the human body shows that it is under stress or tired, although it was not always so easy to discern, especially during the teenage period. At this age, the frequent mood swings are part of growing up. During puberty, teenagers begin to change physically – most girls get their first period and a beard begins to grow in boys. For teenagers, these changes are very important and not easy to accept. While maturing, teenagers have to deal with a lot of things in their mind, which can contribute to their anxiety, irrational fear or somatization of an organic disease such as the allergic rhinitis. In this case report, the treatment of the allergic rhinitis symptoms in a teenager was difficult, time-consuming and on the edge of being unsuccessful due to the lack of information, about somatization of allergic rhinitis or possible association with tic in the ARIA (the Allergy Rhinitis and its Impact on Asthma) approaches [6].

Case report

We considered the unusual tic related to sneezing in a 12.5-year old female teenager associated with the allergic rhinitis, mild asthma [7] and rickets. The girl was hospitalized because of sneezing, headache, and constipation in the early winter period. She sneezed in approximately 500–600 daily series of ten sneezing each, and exclusively while being awake. The order of all clinical findings, related to this case, is shown in Table 1. After 3 days of the treatment with chlorpromazine, the teenager sneezed 42 series of 10 sneezing each. Chlorpromazine was taken during following 5 months with a gradual dose reduction. Currently, after a year and a half, the patient rarely sneezes, periods without sneezing have not been longer than 3 months, and she 'clears her throat' occasionally. She had no symptoms of conjunctivitis.

At the beginning, sneezing was accompanied with the fever up to 38.5°C during 10 days, and the therapy was applied in the school infirmary, including xylometazoline nose-drops, paracetamol and ascorbic acid, desloratadine and mometasone furoate nasal spray. Because of the protracted sneezing, pharyngeal and nasal swabs were taken, so that in accordance with the antibiogram, amoxycillin with clavulanic acid was administered over 7 days. Since the teenager kept on sneezing despite her good general health, she was sent to the General Hospital for examination.

On admission to the Department of Pediatrics, in the General Hospital in the countryside of Serbia, main reasons for the hospitalization were sneezing, headache and constipation, despite normal physical findings. Diagnostic findings are shown in Table 1. During a 7-day monitoring and examination, the girl sneezed in approximately 200 series of ten sneezing each, suffered from substernal chest pain and constipation. Electroencephalography (EEG) showed the following: irritant activity was noted bilaterally at well-expressed and regular OA 12Hz in the frontal-central-temporal region (FCT). Due to the EEG findings and performed analyses, the patient was empirically treated with diazepam 5 mg tablets for 5 days. However, sneezing continued with variable intensity and frequency, exclusively during the state of wakefulness. As a consequence of constipation, a fissure appeared in the anal region and it was treated with policresulen and cinchocaine ointment and benzydamine solution. She was discharged from the General Hospital with the recommended therapy: omeprazole 20 mg capsule once a day, metronidazole 750 mg daily, lactulosa syrup 30 mL daily and policresulen and cinchocaine ointment.

During 9 days at home, the girl continued to sneeze even more intensely, 10 sneezes in each of the 500–600 series a day, only while being awake, during the following month and a half.

Because of this, the girl was sent to the additional examination to the Pediatrics Clinic in the Clinical Centre Kragujevac. On admission, the girl was eutrophic, complained about fatigue and exhaustion; the pharynx was hyperemic; livid nasal mucosa was without edema; diffuse expiratory wheezing was noted, and other physical findings were normal. Diagnostic findings are shown in Table 1. Detected vitamin D deficiency with elevated alkaline phosphatase and a high total IgE level suggested the association of allergic disease and rickets. Skin prick test clearly showed characteristics, potential risk factors and triggers of allergic diseases. Pulmonary function tests showed moderately constricted small airways along with moderately elevated total and specific airway resistance, mild "air trapping", and a positive bronchodilator response. The female teenager did not have asthma symptoms (a cough, wheezing, shortness of breath, chest tightness) and asthma was diagnosed using functional assessment of airflow limitation and airflow reversibility [7]. The above mentioned pulmonary and other examinations confirmed the following diagnoses that were intertwined: asthma (organic), rhinitis (both allergic and, predominantly, psychogenic superpositioned), the condition of tic related conversion and biochemically confirmed rickets.

The female teenager was discharged from the Clinic with recommended therapy: chlorpromazine 25 mg at every 8 hours over a month and parents were requested to keep evidence of the number of tics (sneezing) during the day. At the same time, the patient received therapy for asthma, allergic rhinitis and rickets including vitamin D_3 2000 IJ daily, desloratadine 2.5 mg daily, montelukast 5 mg daily, fluticasone propionate 100 mcg inhaled daily, and she was given

Table 1

Discomforts, clinical features, diagnostic findings, and the choice of drugs during treatment of sneezing exclusively in the waking state

Place of treatment	Daily series of 10 sneezing each	Other discomforts, clinical signs, clinical findings	Diagnostic findings	Therapy
School infirmary /6 weeks before admission to the Pediatric Clinic/	500–600	Fatigue, exhaustion Fever up to 38.5oC over 10 days Hyperemic pharynx Livid nasal mucosa (without edema) RR 22/min, HR 92/min, SaO2 98%, Eutrophic	Pharyngeal swab: A beta-hemolytic *streptococcus* Nasal swab: *Staphylococcus aureus* 100%	Xylometazoline nosal-drops, paracetamol, ascorbic acid, amoxycillin with clavulanic acid (7 days)
General hospital /2 weeks before admission to the Pediatric Clinic/	200	Headache, substernal chest pain, constipation RR 20/min, HR 88/min, SaO2 98%	Physical findings were normal. CRP 0; Le 4.98 × 10/-9L; neu.0.48; mo.0.08.; Er 5.33 ×10/-12L, Hg 149 g/L, PLT 236 × 10/-9L) EEG: irritant activity was noted bilaterally at well-expressed and regular OA 12Hz over fronto-centro-temporal region.	Observation
	500–600		bMRI: normal Abdominal ultrasound: normal Ocular fundus review were normal. Pharyngeal and nasal swab: normal Perianal swab at intestinal parasites: normal	Diazepam 5 mg (5 days)
Admission to the Pediatric Clinic	560	Headache, substernal chest pain, constipation	Video-EEG monitoring: bilateral mild nonspecific changes in fronto-central region along with well-presented sleep phases that rated as being normal. Psychologist: suspected tics. Psychiatrist: tic disorder. Serum level of Vitamin D 18 ng/mL, Alkaline phosphatase 236U/L,Total IgE level 379IU/L. Skin Prick test: grass pollen-2 mm, Dermatophagoides pteronyssinus-3 mm, house dust-2 mm, freshwater fish-2 mm, cow's milk-2mm, tomato- 2mm, seafood-2mm. Lung function tests: FVC 81%, FEV1 82%, PEF 52%, FEF75/25 59%	Chlorpromazine 12.5 mg, every 6 hours
After 1 day of treatment	280			Chlorpromazine 12.5 mg, every 6 hours
After 2 days of treatment	160			Chlorpromazine 12.5 mg, every 6 hours
After 3 days of treatment	42			Chlorpromazine 12.5 mg, every 6 hours
The 4th days: Discharge from the clinic to the home treatment			Bronchodilator response +15% FEV1 Specific airway resistance (Sreff 179%, sRtot 204%), RV/TLC 138%, RV 147%, Fres 16.8 TLCO normal ENT examination: allergic rhinitis, excluded sinusitis. Normal results of: C-reactive protein, peripheral blood smear, ionogram with calcium, phosphorus, magnesium ion in serum, chlorine in sweat, the microbiological findings of aspirate samples and nose and throat swabs, radiological examinations of the lungs and left hand, perianal swab for intestinal parasites, abdominal ultrasound findings, and the ocular fundus.	- Chlorpromazine 12.5 mg, every 8 h over a 6 weeks - Desloratadine 2.5 mg daily - Montelukast 5 mg daily - Fluticasone propionate 100 μg inhaled daily with chamber with mask - Vitamin D3 2000IJ daily - Elimination diet (without freshwater fish, cow's milk, tomatoes, seafood).
After 6 weeks of treatment at the home	15		The lung function and total respiratory system resistance were normal; Serum level of vitamin D 26.64 ng/mL; Alkaline phosphatase 91 U/L	- Chlorpromazine 12.5 mg, every 8 h, still 4 months - Desloratadine 2.5 mg daily, still 4 months - Momethason furoat 100 μg daily, over a 4 months

Le – leukocytes, Neu – neutrophils; Mo – monocytes; Er – erythrocytes; PLT – platelets; RR – respiratory rate; HR – heart rate; SaO2 – percutaneous oxygen saturation on room air; bMRI – Brain magnetic resonance imaging; EEG – electroencephalography; FVC – Forced vital capacity; FEV1 – Forced expiratory volume at the end of the first second of forced expiration; PEF – Peak expiratory flow; FEF25 – Forced expiratory flow related to 25% portion of the FVC curve; RV/TLC – Residual volume expressed as percent of total lung capacity; sReff – Effective specific resistance; sRtot – Total specific resistance; Fres – resonant frequency; TLCO – carbon monoxide transfer factor; ENT – Ear, nose, throat.

advice on the elimination diet (without freshwater fish, cow's milk, tomatoes, seafood). After a month of the treatment, the girl still sneezed up to 15 series per day, the lung function was normal, with vitamin D insufficiency detected and normal alkaline phosphatase activity in the serum.

Chlorpromazine, as a phenothiazine neuroleptic, was taken during 5 months with a gradual reduction of the dose. In the same period allergic rhinitis and asthma were treated (desloratadine, montelukast, fluticasone propionate) and after that, in the following six months only prophylaxis of allergic rhinitis continued (desloratadine, mometasone furoate). After a year and a half period of time, the patient rarely sneezed, periods without sneezing were not longer than 3 months, and she 'cleared her throat' occasionally. Prophylaxis of allergic rhinitis continued using topical antihistamine azelastine hydrochloride along with the elimination diet. Given that the number of tics (sneezing and "throat clearing") was small, psychological treatment, in the form of a counseling and conversation, was also recommended before the next check up in 4 months. Then, we suspected that there was a "hidden anxiety" which with available psychological (projective techniques, Beck Hopelessness Scale, Children's Depression Inventory) and psychiatrist (katamnestic) tests was not established.

The data from the personal history should also be mentioned: 5 months prior to the hospitalization, the female teenager was admitted to the Pediatric Surgical Care because of the episodes of belching and pain in the stomach which were successfully cured with ranitidine. Personal medical history revealed that she was prone to sneezing during the spring and autumn since she was 9 years old, but she not been tested or treated for allergic rhinitis so far.

There was no information on the use of psychoactive substances and drugs or exposure to toxic and other substances or about the similar problems identified in family members.

Discussion

In this paper, we described the case of an unusual tic related to sneezing in a female teenager associated with the allergic rhinitis and asthma. We considered this as "the condition of tic related conversion", what was working diagnosis. Accordingly, we did a differential diagnostic consideration.

Tic disorder is, as suggested by the American Academy of Child and Adolescent Psychiatry, a neuropsychiatric disorder associated with other psychiatric disorders, including attention deficit disorder, hyperactivity and obsessive-compulsive disorder [5, 8, 9]. Simple vocal tics indicate personality development disorder, indeed; that usually disappears because of its transient nature and is more common in male children. It is most commonly manifested as a cough, grunting, throat clearing and sniffling. It is transient if it lasts between 4 weeks and 12 months in children under 18 years of age, but if not due to the effects of psychoactive substances or other drugs, and not meeting the criteria of Tourette's disorder or other chronic motor or vocal tic disorders [8, 9]. Tic disorder is a neuropsychiatric disorder associated with other psychiatric disorders, including attention deficit disorder, hyperactivity, and obsessive-compulsive disorder [5, 7, 10].

From patient's medical history we were not informed about her or her family suffering from Gilles de la Tourette's syndrome [9] and we excluded the influence of the various substances and drugs. However, tic-related sneezing in our teenager is not of transient nature (the simple vocal tics) [5, 8, 9] because it has lasted for year and a half, and although its clinical picture is less severe now and without additional motor tics, or symptoms of Tourette's disorder, because of its duration, we reflect the tic turns into a chronic vocal tic disorder.

The psychologist and psychiatrist did not find the reason for the teenager's emotional instability or excessive dependence on her parents and the fear of her parental deprivation, especially in receiving love and any exposure to punishment. There is a disorder marked as a psychogenic superposition of allergic rhinitis. Then, the "hidden anxiety" was not established with the available psychological and psychiatrist tests. The symptoms as a headache, constipation, retrosternal pain (with normal physical findings) were perceived as somatic symptoms because they have manifested at the same time with intense sneezing. This was the reason to think about "hidden psychological factors" that affected other medical condition but was not recognized by the Diagnostic and Atatistical Manual of Mental Disorders, Fifth Edition (DSM-5).

After this, a neurologist, as a treating physician, however, prescribed chlorpromazine. The low-potency, typical antipsychotic effect of chlorpromazine [10] was achieved in this patient. The treating physicians have had in mind the favorable circumstance that chlorpromazine acts as an antagonist on different postsynaptic and presynaptic receptors (dopamine-D1,-D2,-D3,-D4, serotonin-5HT1,-5HT2, histamine H1, α1- and α2-adrenergic, M1- and M2-muscarinic acetylcholine) [10], as used here. Chlorpromazine reduced the anxiety and annulled psychogenic superposition of allergic rhinitis. Here is implemented dual therapy of sneezing.

On the other hand, the prevalence rates of allergic rhinitis are highest in school-age children [2, 3, 6, 7]. Thus, allergic rhinitis and infective rhinitis are logically first suspected in primary health care where, usually, the treatment begins without testing protocols. We were thinking about other types of rhinitis but we did not fit any of the known clinical pictures and received additional diagnostic findings, including sneeze on a full stomach [11]. It is known that the treatment of moderate/severe persistent allergic rhinitis according to the approach titled the ARIA [6] guidelines, and the patient should be checked in 2–4 weeks in primary health care. And, in the end, if there is no improvement, a surgeon should be consulted and specific immunotherapy considered. The treatment of allergic rhinitis according to the ARIA protocol [6] involves stepwise approach [3] but without established order of application of decongestant, oral H1-antihistamine, intranasal H1-antihistamine, intranasal corticosteroids (INS), antileukotriene and cromolyn for any severity of intermittent rhinitis and mild persistent rhinitis lasting up to 4 weeks. If there is no improvement, INS dose should be gradually increased or ipratropium bromide or oral corticosteroids should be introduced [3, 10]. Conditions of controlled persistent allergic rhinitis can be achieved by the treatment of the ARIA approach [6].

However, the ARIA approach [6] implies neither the possibility that allergic rhinitis may be associated with psychogenic or psychological disorders or the attention deficit-hyperactivity disorder (ADHD), nor that the allergic rhinitis is less controlled in the teenagers with tic or ADHD or psychological factors that affect rhinitis. It does not say whether these diseases deteriorate each other, providing no guideline whether the patients should be treated by the ARIA approach [6] or by the DSM-5 protocol [8] first or simultaneously by both approaches.

It is known that low level of vitamin D is associated with the increased frequency of asthma exacerbations, increased allergy markers and asthma severity in children [12], and that vitamin D_3 supplementation may modulate respiratory mucosa. Vitamin D has a role in immunological modulation, aging, gene regulation, brain homeostasis, and neurodevelopment. But, at this moment we do focus on just the condition of tic related conversion associated with allergic rhinitis.

For the doctors in the primary health care, as well as for other doctors, it is important to have the correct initial guidelines for the treatment of a certain disorder in teenagers, which affects the cost of treatment.

Conclusion

This case report emphasizes the necessity of revising the systematization of tic disorders, the DSM-5 protocol and the ARIA approach in terms of their association, emphasizing each other, and clearer diagnostic/treatment/prognostic approaches of these associated disorders with the age of teenagers.

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Hospitals on the territory of Vardar Macedonia as part of Serbia during the Kingdom of Serbs, Croats and Slovenes/Yugoslavia (1918–1941)

Bolnice na teritoriji Vardarske Makedonije u sastavu Srbije za vreme Kraljevine Srba, Hrvata i Slovenaca/Jugoslavije (1918–1941)

Verica Josimovska

"Goce Delcev" University, Štip, Former Yugoslav Republic of Macedonia

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Introduction

The period after the First world war (FWW) is considered as one of the most intensive periods of hospital openings in the Vardar Region of Macedonia as part of Serbia. Vardar Macedonia (today's Republic of Macedonia) was part of the Kingdom of Serbia in the period 1912–1915, the province of Serbia within the Yugoslav Kingdom until 1929, and Vardar Banovina within the Kingdom of Yugoslavia from 1929 to 1941.*

Because the situation was unsatisfactory as a result of the lack of hospitals, minimalistic capacities and the low hospital equipment the hospital's opening started with the new country formation and the foundation of the Ministry of National Health.

If we follow some chronological order we should mention first the hospitals in the cities that existed before the war but because the Vardar Region of Macedonia was practically ruined there was no evidence of them. That is why we use the term new hospitals not the old ones. When we say new hospitals, in the period after the war we do not think of building new hospitals but forming hospitals in places where they did not exist before.

According to the data given in the telegram sent by the Head of the Sanitary to the Management of all hospitals in the Kingdom of Serbs, Croats and Slovenes (SCS), of 14 May 1919, by which it was called on purchasing products and equipment for the hospitals, in the Vardar Region in 1919, four public hospitals only existed in Skopje, Štip, Bitola and Tetovo [1].

According to some other reports from 1920 in the Vardar Region, besides the public hospitals, there were also hospitals of the American mission in Veles, Prilep, Ohrid, Strumica and Gostivar [2].

There were whole areas, as for example the area of Kumanovo, where there were no hospitals at all. Also in Serbia, Montenegro and the Vardar Region of Macedonia at that time, there were no hospitals. In the places where war hospitals did not exist like Štip, Veles, and Ohrid, the injured were treated in the regional hospitals [3].

Hospitals which worked after the war had a status of 'Permanent' or 'Temporary' hospitals. In the Kingdom of SCS the hospitals were public and private depending on the investors.

Public hospitals were divided into state, regional, local and municipal hospitals which later, after regions were formed, became regional hospitals. In the Vardar Region of Macedonia only regional and local hospitals existed without having the status of public hospitals until 1929.

Regional hospitals covered a range of few municipalities and were settled in the centre of the region, while the local hospitals were formed in few villages. That was the situation till 1929 when all hospitals were renamed as regional hospitals.

Regional hospitals were hospitals in Skopje, Bitola, Štip, Tetovo and Ohrid, and the local in Veles, Prilep and Gevgelija.

Besides the regional and local ambulances in the municipalities, there were public ambulances which were free of charge.

There were also hospitals in the Vardar Region that were private and founded by the foreign humanitarian missions.

According to the professional and organizational set up, the hospitals could be common treating all diseases as well as special hospitals where only one disease was treated.

* Vardar Macedonia (today's Republic of Macedonia) was part of the Kingdom of Serbia in the period 1912–1915, the province of Serbia within the Yugoslav Kingdom until 1929, and Vardar Banovina within the Kingdom of Yugoslavia from 1929 to 1941.

Correspondence to: Verica Josimovska, "Goce Delcev" University, Štip, Macedonia. E-mail: vericajo@yahoo.com

The Sanitary Law of the Kingdom of SCS imposed on the state and its bodies to build a hospital at their own expense. When they were not in condition to do that the state must have helped them or finance the building of the hospitals. Thus, to 1930 when the Skopje's hospital became regional, there were no hospitals with public status in the Vardar Region, although, as a result of bad financial conditions, the state fully covered the expenses for the hospitals openings [3].

The hospital staff did not satisfy the hospital needs. There was a lack of doctors and professionals. According to the law, one doctor should cover 25 hospital beds, and in Vardar region he/she covered 32 [3].

Great working difficulty represented the post of a hospital manager and a house keeper which the law did not stipulate. In the regional hospitals, with a few exceptions, the duty manager of the hospitals was led by epidemiologists. The same happened with the local hospitals where the duty of a manager was performed by the local doctor. In places where hospitals were bigger, two doctors were posted as managers. The law had other negative side and that was the administrative work which was manager's duty. That prevented the manager to dedicate himself/herself to the professional and real hospital work. That was the reason for imposing the need for opening the post of a manager with professional qualifications and legal responsibility. The Sanitary Law predicted posts such as a chief of ward, secondary doctor, or honorary doctor. These doctors received free home for living in the hospital surroundings, food, energy and heating materials. Secondary doctors should have spent 3 years in one ward and worked under a supervision of the chief of the ward and after that, they could run the ward by themselves. The greatest difficulty in hospitals was the equipping of helping professional staff, doctor's assistant, and nurses [3]. That was why schools for necessary professional profiles were opened.

According to the Law, for a normal functioning of a hospital, the following was needed: a manager, secondary doctor, honorary doctor, doctor's assistant, nurses, midwives, laboratory assistant, pharmacist, recorder, procurement clerk, technician, electrician, priest and hospital service for hygiene maintenance, preparation of hospital food, bedding and hospital clothes, ironing, and laundering of the clothes [3].

According to the report of the president of the permanent hospital commission and assistant to the minister of national health Dr. Dobrivoje Ger. Popovic, since 1923, all hospital buildings in the Vardar Region of Macedonia, except for the hospital in Veles, did not provide for the hospital needs. For them, the Ministry of National Health (MNH) paid rent because they were placed in private property buildings, except for the hospital building in Skopje which was a state one. Without an exception, for all of them, it was given a recommendation that a new hospital should be built [4].

After 1929, with the division of Kingdom of SCS on 9 regions, so called *banovina*, all state hospitals as well as the public ones were under the supervision of the region where they belonged to. The centres of the regions were appointed as state hospitals. So, the hospital in Skopje became the State hospital. It was actually the unique state hospital in the Vardar Region.

The municipality of the cities managed the municipal hospitals, under which authority were the hospital expenses.

The state was in charge of building new hospitals and their opening for regions and municipalities with over 20,000 citizens. But, at some private hospitals, under special conditions, the minister could give them the right to charge a number of hospital expenses.

Regional hospitals from 1918 to 1929

Regional hospital in Bitola

The regional hospital in Bitola existed from December 1, 1916 and it was placed in an old Turkish building which was a private property the state paid a rent for.

The hospital building was placed in the centre of the town. Besides the main building, the hospital had one smaller building and barracks with 137 beds. The buildings were so destroyed in the last explosion that it was very difficult to readapt them.

Bitola Regional Hospital was reopened in 1919 and it functioned with 3 wards: internal, surgical, and venereal ward. The following posts were fulfilled: a manager, chief – sergeant, 2 secondary doctors, 1 honorary doctor, 1 pharmacist, 1 midwife, 1 procurement clerk, 1 recorder, 11 nurses, other services 6, 1 technician, and 1 priest. According to the stipulations in the law there were no medical assistants, laboratory assistant, supervisor, and electrician.

Professional staff in the regional hospital - Bitola in 1924 was as follows [5]: manager: Dr. Vasilije (Vasa) Petrovic, duty manager -regional physiotherapist; secondary doctors: Dr. Ego Nikolaric, Dr.. PreDr.ag Pop – Simeon, Dr.. Desanka Kirkovic, pharmacist: AnDr.ija Timofejev; procurement clerk: Lazar Premovic.

The hospital, according to the grade given in the Dr. Popovic's report, was not adequate for the hospital needs and it was recommended to build a new one with 125 beds at least, and about 10 million denars should have been provided for it. As cited in the report, it was an old, half ruined building, a property of the ex-Greek hospital called 'Evangelizmos' which should, according to the doctor Popovic's opinion, be rebuilt and transformed into a new hospital.

Those hospital capacities could not satisfy the city needs. In addition to those claims, there was the article in the local newspapers 'Novi Sjaj' [6] in which it was stated: "It can freely be said that Bitola even today does not have its own hospital. The comfort and hospital accommodation should correspond to all hygiene rules. The current hospital capacities should be significantly increased. We had two major hospitals during the war and now we have only one. Because of that, the issue of a hospital in Bitola does not stop to be actual and necessary for the citizenship."

Besides many initiatives, the problem of the hospital remained unsolved. Since 1920, when the regional manager of the hospital was Dr. Vasa Petrovic, an initiative for renovation of "spitaljeto" was raised and as a result the hospital capacities were significantly increased. Unfortunately, this was not realized because '.... one pure philanthropic movement was raised in a political issue [7].

The conditions did not change even though the need for the children ward instead of the internal in the hospital in Bitola, and the gynecology ward instead of the surgical was seen. What was essential in the Bitola hospital, was to open an ophthalmology ward, and to place venereal ward in the barracks as it is today. It was also important to have a tuberculosis ward because it was very difficult to admit people ill with tuberculosis in the hospital. A high percentage of tuberculosis affected in our villages was a result of not having a place in the hospitals where they could have been treated [6].

In the period after 1920, the citizens were convinced that a new hospital in Bitola would be built as a result of the manager's intervention, Dr. Dragoljub G. Popovic. The location was the place 'Devejani' at today's Ohrid Street. It was predicted to have a pavilion system in the hospital and to build one pavilion per year. The idea remained unrealized and the city was forced to be satisfied with the small inadequate hospital.

Because of this, the poor suffered the most, and the rich solved their problems in private hospitals.

In such hospital conditions, the city entered World War II and faced the Bulgarian administration[8–10†].

Regional hospital in Skopje

Right after World War I, the Minister of National Health of the Kingdom of SCS, approved the English Mission 'Lady Paget' to form a hospital in Skopje by a decision number 15517. At that time, Skopje had a population of 144,848 inhabitants. The hospital was opened on September 1st 1919 at the state property buildings in the barracks 'King Petar' at the Skopje fortress. The first venereal ward was formed then and it was led by Dr. Nikola Megeri, a native from Vojvodina, gynecology ward was led by Dr. Ante Anicin, X-ray ward by Dr. Nikola Nastic, and surgical ward was led by Dr. Vladimir Rojic. The manager of the hospital was Dr. Etta Gray, an „American Women's Hospitals" representative [1].

The hospital had 80 beds, without a sanitation system, and rooms did not correspond to the needs of a hospital. In Dr. Popovic's report it was alerted that a new building should be built with a capacity of 300 beds and a sum of 20 million dinars to cover the expenses [8].

The hospital was moved to the barracks of the War hospital in the 2nd army area 'Half-moon' in 1920. It was renamed in Skopje Regional hospital and placed into two new pavilions, seven Decker's barracks, five wooden houses; one part was set up in the army hospital. It had 200 beds, 7 wards: internal ward (56 beds), surgical ward (27 beds), gynecology and lying –in ward (16), venereal ward (33), ophthalmology ward (33), children ward (50 beds) and X-ray ward [1].

The hospital was financed by the English mission and a small amount of the state budget was allocated. Its manager was a regional physicist in Skopje [1].

Staff in Skopje's regional hospital in 1920 were: the manager, Dr. Zivojin Milenkovic, a regional physicist; Venereal ward: the chief Dr. Nikola Megeri, and at the same year doctor Valentin Ivanivic Zarubin was employed, Russian immigrant, temporary honorary doctor [‡]: Surgical ward: Vladimir Rojic was its chief to 1925, when the Minister appointed the surgeon doctor Josif-Jozo Pulizovic, and then Dr. Milisic; Gynecology ward: chief Dr. Kosta Cohadzic. The secondary doctor, doctor Radmila Milicevic-Smiljanic was working with him, and since 1926 the ward was under supervision of Dr. Andre Stojanov from Ohrid; Internal ward: chief was Dr. Ante Anicin, then doctor Sarinka Jovanovic–Najdic, secondary doctor; X-ray ward: chief Dr. Nikola Nastic; Otorhinolaryngology ward: chief Dr.. Petar Zdravkovic, and since 1926 the ward was under supervision of Dr. Bozidar Sekulic; Children ward: Dr. Marija Fjodorovna-Siebold[§], temporary honorary doctor; Pharmacist: Dr. pharmacist Krsta Stavric; Midwife: Katerina Veljkovic; Procurement clerk: Milosav Stojanovic.

Besides doctor staff, 22 nurses, 1 laboratory assistant, 2 recorders, and 7 helping assistants worked in the hospital. There were no doctor assistants [1].

Besides the venereal ward, in the Regional Hospital in 1921, as a result of increased prostitution, a unit for venereal diseases was opened and the sick were treated for free.

In 1923, Skopje Hospital got its own manager, and the regional physicist was released from that obligation. Milan Zankovic was appointed as the manager of the hospital and performed this duty for 9 years.

Initiated by Dr. Etta Gray, who provided the financial part, a chilren pavilion with 80 beds was built the same year.

† Right after setting the Bulgarian management in the Vardar Region of Macedonia the conditions did not change, and it was similar to the previous one. The absurd lies in the fact that there was a promise of building two hospitals in Bitola and one of them should have been workers hospital. The hospital was planned to be built with all the necessary wards in it with 200 beds. (Petar Bojadzievski, Health service in Bitola during the Bulgarian reign, Scientific thought, Bitola, 1981, 577–598.) The municipality provided a place for building the hospital, the allotement of 30 acres in the Bair area. (Newspaper. „Pelistersko eho", number 8, 21 February 1942, Bitola). The other hospital which was promised to the citizens of Bitola by the Mayor of Bitola, Ilija Nencev, the Minister of Health, P. Gabrovski and the head manager of the National Health of Bulgaria, doctor, I. Balkanski, should have been a modern and big state hospital, located in Arnaut place near the war hospital and hygiene laboratory (n. „Pelistersko eho", number. 35, 30 August 1942, Bitola). Unfortunately the hospital was left to be built some other time.

‡ Since receiving Yugoslavian citizenship, all Russian immigrants no matter of the education, after employment received status of temporary honorary doctor.

§ Dr. Marija Fjodorovna-Siebold (1849-1939), German origin, married to Russian, sanitary mayor of the Serbian Army in 1876. She worked as a war doctor during the Balkans wars and the FWW. She received many awards from the Red Cross, Ss Sava, Courage award and White Erl award. Since the FWW she worked in the Skopje's hospital till her death.

Since 1927, the hospital was transferred in Dolno Vodno where it is today, and it was renamed as Regional Skopje Hospital.

Since 1929, when a new reorganization in the Kingdom was done, the hospital was renamed as Regional Hospital.

Skopje Regional Hospital covered a territory where 2 million citizens lived, and that is from Leskovac to Gevgelija, from Albanian to Bulgarian border, the whole Vardar Region and one part of the Zeta Region.

Since 1930 it was renamed as State or Country Hospital. The State Hospital in Skopje had opened new wards since 1930: tuberculosis ward, isolation ward, neurology ward and ophthalmology ward. The staff who worked there were:

Manager, Dr. Radovan Milutinovic; chief of the internal ward, Dr. Andra Stojanovic; chief of the gynecology ward, Dr. Valentin Zarubin; chief of the venereal ward, Dr. Dusan Anastasijevic; assistant of the venereal ward, Dr. Nikola Guknic; chief of the surgical ward, Dr. Dragoljub Katanic; surgical doctor, Dr. Ilija Bojanic; pathologist, Dr. Bozidar Sekulic; chief of the otorhinolaryngology ward, Dr. Klementije Krstic; chief of the children ward, Dr. Zdravko Jeftanovic; chief of the X-ray ward, Dr. Budimka Milojkovic; assistant doctor, pharmacist Dr. Kosta Stavric; Midwife Katerina Veljkovic; manciple Milosav Stojanovic; 18 nurses and 18 servants.

The Great Pavilion for the surgical and gynecological ward started to be built in 1931. Besides the beds for the sick in the building, there was a space for the central laboratory, physical therapy and x-ray diagnosis and a therapy. The schedule of the interior parts, in pavilion, apparatus, and everything else corresponded to the modern technological-medical rules.

Even after having built the new pavilion in 1938 (today's building of the gynecology ward) the space problems were partially solved [11].

All names of the Skopje Hospital from 1919 to 1947 are given below:

1919 – Lady Paget
1920 – 1927 Regional Skopje Hospital
1927 – 1929 Territorial Skopje Hospital
1929 – 1930 Banovina (Regional) Hospital in Skopje
1930 – 1947 State Hospital

Since 1947, with the opening of the Faculty of Medical Sciences in Skopje, until today, the State Hospital has transferred into Clinical Hospital as a scientific base of the Faculty of Medical Sciences.

Managers of the Skopje Hospital from 1919 to 1941
1919–1920 Dr. Etta Gray
1921–1923 Dr. Zivojin Milenkovic
1923–1925 Dr. Milan Zerajic
1926–1932 Dr. Milan Zankovic
1932–1937 Dr. Radovan Milutinovic
1937–1941 Dr. Viktor Sosic

A report dated May 1, 1941 sent by the administrator of the hospital, taken over by the Bulgarian government to the Chief Directorate of National Health in Sofia can serve as a testimony about the Skopje Hospital. In this report, among other things, it is said that the Skopje Hospital was the largest hospital in the "newly liberated country" and that it had 500 beds. It was situated in four hospital buildings:

Administration Building, a two storey massive building built in 1928 with funds from the mission of American women from New York; a three storey massive central building with a basement in the form of a letter "H" built in 1938 with state funds; Infectious Diseases building, a two storey massive building in which the Department for Lung Diseases is situated. It is located 200 meters from the administrative building; pavilion for the mentally ill, one-storey small building.

The Administrator gives a comprehensive description of the apparatus found in the radiology ward. He says that the department is very well established and it has three X-ray apparatuses: one apparatus for diagnosing - a Siemens model "Tuto Heliofos" with four valves and equipped with full automation, an X-ray apparatus for diagnostics, type Galon-Pilon-Pariz, about 16 years old, and a Siemens X-ray apparatus for treatment of the type "Tuto Multi-Volt [12].

Regional hospital in Štip

Štip's Regional Hospital was opened on April 1, 1919 (it was formed in 1914), with 30 beds as one hospital without dividing the wards. A motive for the urgent opening of the hospital was the epidemic of typhus fever in the city. The hospital was in the centre of the city in the so called Ceramidsko neighbourhood, in privately owned buildings, and supplied with water from the city pipe line. The buildings were not appropriate for the hospital needs but they were adapted by the MNH.

According to Dr. Popovic recommendation, it was necessary to build a new hospital** for which 6 million denars were needed.

Staff in the Regional Hospital in 1922 were: manager, Dr. Franjo Navratil; temporary honorary doctor, Dr. Ljudmila Smirnova; procurement clerk, Kosta Ristic; midwife, Paraskeva Jokanovic; 1 recorder, 1 laboratory assistant, 4 nurses and 4 helping staff.

Regional hospital in Tetovo

Tetovo Hospital was opened on July 16, 1919 by the sanitary mission 'Scottish women' located right to the object

** The project for the Stip's Hospital was done in 1931 and the building started in 1936 when a credit from the bank was taken for the costs. In 1941 the hospital was completely equipped, and before it was opened it should have been connected to the sanitary system. But, on April 6, 1941 when Stip was bombardeded all windows of the hospital were destroyed. Right after the bombardement , the city was a victim of robbery and a large number of the equipment was robbed. When the Bulgarians came in Stip, it was started with new reequipping of the hospital and on July 5, 1943 it was prepared to receive the first patients. Hospital capacity was 100 beds and its manager was Dr. Georgi Rahlij (Russian immingrant).

where recently was the geodetic management, in the old private buildings (one of them was the house of Scanderbeg Bey) with 10 rooms, 50 beds which did not correspond to the needs [14]. That was why Dr. Popovic recommended that a new building should be built with 75 beds, which would cost around 5 million dinars [15].

The hospital had one two-storey building, and a smaller building in which was the manager's office, the ambulance and maternity hospital with 10 beds [16].

Its staff were: a manager, Dr. Stojan Stefanivic; regional physicist, Dr. Edit Harley[17, 18††] who was the manager of the hospital pharmacy which was supplied with all the necessary medicaments by the sanitary mission [13]; procurement clerk, Janikije Davinic; midwife, Mara Todorovic; 5 nurses and 4 clerks.

Regional hospital in Ohrid

The Ohrid's Hospital was opened on September 27, 1920 in two privately owned buildings and one building which was the property of the Ministry of the Army. The hospital had 80 beds and 4 hospital wards: surgical, infective, and the ward for internal diseases. The buildings partially corresponded to the hospital needs. As one of the main problems in the hospital was the lack of hospital clothes and bedclothes.

According to Dr. Popovic's report recommendations a new hospital should have been built and for that occasion 4 million dinars was needed. This hospital admitted soldiers because there was no other hospital in the city.

Its staff were: a manager, honorary doctor, 1 doctor assistant, 6 nurses, 1 midwife, procurement clerk, hospital priest and 5 clerks (manager: Dr. Gura Martinovic; regional physicist; temporary honorary doctor, Dr. Sofija Larinova – Leskova; Doctor assistant, Milan Nedeljkovic; Procurement clerk, Aleksandar Siljak).

Territorial hospitals in the period from 1918 to 1929

Territorial hospital in Strumica

In 1919, in the Bey Inn, at the same place where the Bulgarian hospital was from 1912[‡‡] hospital of the American women mission was formed. Professional and assistance staff were all women at the hospital. The head of the hospital was Dr. Etta Gray, a specialist for surgical and ophthalmological diseases, and miss Teppi, a doctor specialist internal, miss Eliot, a general practicioner and two nurses. There were also staff nurses and translators from English into Macedonian: Vasil Panov and Zaev from Strumica. The whole medical help was free and people from distant towns came to be treated here. Koce Pesev from Novo Selo, Stip, had a plastic surgery on his eyebrow.

Unfortunately, the Hospital of the American women did not work long. In 1921, it was burned by a forgery, leaving behind a beautiful memory of one professional team that gained all sympathy of the population in Strumica region [19].

Territorial hospital in Veles

The Veles's Hospital was opened on March 21, 1922, in the buildings of the Children hospital of the American mission which was formed in 1919 and its manager was Dr. Etta Gray.

It was placed in buildings, the property of the Ministry of War and in Dr. Popovic's report, it was evaluated as a hospital appropriate for the hospital needs, but because it did not have its own building it was recommended to build a new hospital for 4 million dinars.

It was placed in a two-storey house on the upland of Veles. The hospital had two helping or in total three buildings: building for patients, building for the staff nurses, washing machine, infective ward, and the other building for the dead.

The hospital had the adult ward, children ward, and the infective ward. Besides the civilians, soldiers were also treated because there was no other hospital in the city.

Of the total amount of 100 beds, 50 were used for adults, 41 for children, and 9 for the zymotic disease. It was connected to the sanitary system. The hospital was financed by MNH except for the children's ward which was financed by the American mission. The whole equipment was supplied by Dr. Etta Gray who was the manager of the hospital until 1922.

Staff that worked in the Territorial Hospital in Veles in 1922 were: a manager, Dr. Antun Saso; honorary doctor, Dr. Stanislava Simonovic – Ilic; procurement clerk, Spasoje Ostojic; 9 nurses and 5 clerks.

†† Edit Harley and her sister Flora, came to Macedonia in 1915, as representatives of the 'Scottish Women's Hospitals Motor Ambulance Column' together with their mother Catherine.

Dr. Catherine Mary Harley, 1853-1917, Scottish origin, was sister of the Field marshal Sir John French, executive commander of the English troupes in France. The mission „the Scottish Women's Hospitals Motor Ambulance Column" known as 'Transport Unit' based in England as special unit with 18 members, 6 Fords, came in Macedonia as chief administrator of the most modern ambulance service with sanitary column. She administrated the Dr.iving department and beside the Dr.ivers the workers were equipped for mechanics of their Dr.iving vehicles. In her unit, beside her two daughters, were many famous women from the English and Scottish circles. She also took care of the wounded, injured, and hungry people after the bombing in Bitola in 1917. She was hit in her head on March 7, 1917 and died immediately while doing that mission. Her tomb is in Serbian cemetery in Zejtinlik (Thessaloniki) as unique woman between all other dead soldiers. Her daughters continued with her work. Right after the Great War Edit Harley worked in the Tetovo Hospital

‡‡ During the presence of the Bulgarian authorities a war hospital was formed where the population received prevention. The hospital was in the Bey Inn and it was equipped with modern medical equipment. Two doctor s worked in it: Dr. Kambacev and Dr. Mircev, and two pharmacists: Ivan Stojanov and Nikola Petrov. Sava Kovaceva worked on the sterilization ward and staff nurses were Zora Trendafilova and Danka Mirceva. This hospital existed to 1918.

The largest number of patients from the hospital in 1922, according to Dr. Popovic's report, were ill with malaria (n = 166), and tuberculosis infected (n = 48). The number of patients who were injured was 49. They completed 21 surgery and 3 autopsies and made 84 ambulance's medical check ups [4].

On August 27, 1922 the American mission left Veles and went to America. Dr. Whiste was the only person that stayed there, one surgeon and a medical nurse.

In the annual report for 1923 the manager Anton Saso gave a description of the hospital: '...the hospital is placed on the hill above the barracks, one kilometre distant, with three buildings, one for the hospital wards, the other for the infective ward, staff nurses and washing machine, and the third building is a chapel. There are three hospital wards with 100 beds, the children ward with 41 bed and infective ward with 9 beds. The heating in the building is with metal stoves on wood, lighting by kerosene, sanitary system. The laundry washing is made manually. The hospital needs hospital equipment (laundry, setting, bed equipment) laboratory and laboratory assistant. There is a pharmacy without a pharmacist, and there is no duty doctor [20]. In this hospital workers who built the railway Veles-Prilep and Veles- Štip were received.

In the annual report to the territorial inspection in Skopje, Dr. Saso reported for the malaria outbreak in 1923. Out of 560 infected people, 46 were infected by malaria quartana, 147 terciana, 201 of tropic malaria and 156 of chronical malaria. Most of the infected were between 20 and 30 years old, and a smaller number was from 1 to 20 years old. In the second half of 1924, the number of the malaria infected increased to 761 [21].

Veles Hospital did not manage to find the appropriate place for its dislocation and the ministry could afford money for a new one. It was at the same place in the Second World War (SWW).

Territorial hospital in Prilep

Territorial hospital in Prilep functioned from 1919, under the management of the American mission 'Hospitals of the American women'. Since January 23, 1922, the management was taken by the Territorial sanitary management. The hospital had only 30 beds and was placed in a private building, located between the house of Pance Magar and the Evangelic church, and water was supplied from the hospital well.

In Dr. Popovic's report, it was noted that the hospital was not the adequate for the needs of a hospital and it was necessary to build another hospital with at least 50 beds and 3 million dinars for building it [4].

Its staff were: a manager, Dr. Cvetko Gorgevic; territorial doctor, temporary honorary doctor, Dr. Lujza Bihari; procurement clerk, Omilj M. Radovanovikc; 4 nurses; 3 clerks and 1 priest.

Territorial hospital in Gevgelija

A Hospital in Gevgelija was opened in 1920 in the old building, which was in a private property of the recent catholic–unite pension). The rent was paid by MNH for a sum of 400 dinars monthly. It had three hospital rooms with 15 beds, an ambulance, office and kitchen. The lighting was with kerosene, heating by wood stoves, and water supply by street taps and wells. There were no enough blankets and sheets, dividers and setting.

The rooms did not correspond to the hospital needs and that is why Dr. Popovic recommended that a new hospital should be built and the amount for it was 3 million dinars.

Its staff consisted of: the manager, territorial doctor, Dr. Milos Jakovlevic; procurement clerk; Rafajlo Petrovic, 3 nurses and 2 servants.

As a conclusion to this report, Dr. Popovic recommended that a new hospital should be built in Kumanovo with a capacity of 100 beds and amount of 10.500 million dinars. Also he recommended that another hospital should be built in Strumica with 30 beds and amount of 3 million dinars [4].

The hospitals in the Vardar Region of Macedonia were financed by the American charity mission which did successful work with its staff. The work of Dr. Etta Gray should be mentioned who had Children Hospital in Veles. The American mission gave 10.000 dollars to support the building of a children's pavilion in Skopje with 80 beds [22].

Besides the fact that hospitals were opened as temporary at first, with recommendation that they were not the adequate for hospital needs, and that new hospitals should be built, many hospitals waited the WW II in this condition.

Hospital in Kumanovo

Kumanovo Hospital was built with resources of Nikola Spasic's fund; he was a respectable merchant and philanthropist from Belgrade. The hospital building was a modern edifice and the amount for its construction was 1,382,022 dinars. Fully equipped with 60 beds, it was opened on July 11, 1935. Today, this building is a part of the hospital in Kumanovo [23].

Conclusion

The opening and construction of hospitals in Vardar Macedonia located within the South Serbia during the Kingdom of SCS/Yugoslavia, in the period between the two world wars were carried out with great intensity because of the urgent need. The fact is that the state budget allocated huge funds to build hospitals that were built by all regulations of the modern architecture of the time. The quality of these buildings was high and many of them are still operational. As an example, I will mention the Hospital in Stip built in 1936, which after 80 years still works in the same premises, all the time as a Regional Hospital and, most recently with the opening of the Faculty of Medicine of the University "Goce Delchev", it has grown into a Clinical Hospital - Štip. As for the provision of experts, particularly from specialist activities, the shortage of these was very obvious because the conditions were incredibly complex and difficult. Mostly, staff was taken on by recruitment and civil mobilization but also by sending doctors on a regular job in order to open new departments and services. The Ministry of Public Health of the Kingdom of SCS / Yugoslavia always took care about the quality of professional staff enabling them to handle difficult and responsible tasks, especially in the areas where infectious diseases, primarily malaria, reigned.

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SCIENTIFIC MEETING REPORT

Third International Congress of Cardionephrology and Hypertension (Pirot, Serbia, May 11–13, 2017)

Treći međunarodni kongres kardionefrologije i hipertenzije (Pirot, Srbija, 11–13. maj, 2017)

Milica Petrović

Military Medical Academy, Clinic of Nephrology, Belgrade, Serbia

The third International Congress of Cardionephrology and Hypertension, "KARNEF" was a gathering of cardiologists and nephrologists of South-Eastern Europe. The Congress was organized by the authorities of the city of Pirot in cooperation with the Serbian Cardionephrology Association and the Serbian Medical Association.

The role of the Serbian Cardionephrology Association in educational and scientific research work in the field of kidney and heart diseases, as well as in the organization of such meetings has been recognized by major world associations. Our organization has been an associate member of the World Association of Nephrologists since March 2016.

Beside nephrologists and cardiologists, the three-day International Congress of Cardionephrology, was also attended by pathologists, clinical biochemists, pharmacologists, endocrinologists, vascular surgeons, cardiac surgeons, and for the first time – pharmacists, chemists and biologists. More than 315 participants attented the Congress; 260 came from the host country and 55 from abroad. There were 33 participants who presented researches from the host country and 16 participants from abroad.

There were 54 lectures in total, out of which 44 were by invitation. The Meeting was organized on the basis of mini symposiums, educational seminars, thematic sessions and plenary lectures.

Thirty five eminent experts in the field of cardiology and nephrology presented their lectures. Apart from the lecturers from Serbia, the experts from the: USA, Canada, Switzerland, Israel, Spain, Italy, Macedonia and Slovenia shared their knowledge and experience, too.

The complex etiopathogenetic mechanisms of formation and development of associated heart and kidney diseases, the multidisciplinary approach to patients suffering from these diseases is of paramount importance for early detection, prevention and treatment.

Abstracts of the presented communications at the Third International Congress of Cardionephrology and Hypertension "Cardionephrology Up To Date" have been published in the book "Cardiology 2017".

The seven chapters present the latest findings, from the etiopathogenetic mechanisms of associated heart and kidney diseases, to diagnostic and therapeutic procedures. The greatest attention was paid to cardiovascular complications in patients with end-stage of renal disease, with an emphasis on combined heart, lung and kidney damage.

Special attention was also paid to studies related to experimental cardiology and medical chemistry.

The book is aimed at educating young doctors, doctors in specialist and subspecialist studies in cardiology and nephrology, as well as students of doctoral studies. The editors of the book are Prof. dr Sonja Radenković and Prof. Andrija Šmelcerović.

There was a ceremony organized at the official opening of the Congress on the occasion of the fifteenth anniversary of the existence and work of the Cardiovascular Association of Serbia, and the awards were given to President of the European Cardionephrology Association, Prof. Mario Timio, Prof. Luca Di Lullo, President of KARNEF Serbia Prof. Sonja Radenković, Mayor of Pirot, Mr. Vladan Vasić, and President of the Serbian Medical Association in Pirot, Dr. Aleksandar Lilić.

The Serbian Cardionephrology Association also presented awards for the three best papers in the field of cardioneprology, published in scientific journals in 2016.

The first prize was awarded to Anderluh Marko and associates, for the paper "Cross-talk between dipeptidyl peptidase-4 and stromal cell derived factor-1 in stem cell homing and myocardial repair: potential impact of dipetidyl peptidase-4 inhibitors" published in the pharmacology and Therapeutics 2016; 167: 100-107 (Impact factor 11.00).

Second prize was awarded to Di Lullo Luca and associates for the paper "Cardiorenal acute kidney injuries:

Correspondence to: Milica Petrović, Military Medical Academy, Clinic of Nephrology, Crnotravska 17, 11 000, Belgrade, Serbia.
E-mail: drmilicapetrovic@gmail.com

Epidemiology, presentation, causes, pathophysiology and treatment" published in the International Journal of Cardiology (doi: 10.1016/j.ijcard. 2016.11.156) (impact factor 4.638).

Third prize was given to Dr. Petrović Milica and associates for the paper "Blood concentrations of B-type natriuretic peptide and N-terminal prohormone B-type natriuretic peptide as markers of left ventricular diastolic function in patients with chronic renal failure" published in the Vojnosanitetski Pregled (DOI: 10.2298 / VSP151019079P) Online April 1st, 2016 (impact factor 0.367).

The participants of the Congress had the opportunity to get acquainted with the natural beauties and rich cultural heritage of the host city, Pirot, which was previously known as „little Jerusalem"

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All measurements should be reported in the metric system of the International System of Units (SI), and the standard internationally accepted terms (except for mmHg and °C).

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

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Časopis „Vojnosanitetski pregled“ izlazi godišnje u 12 brojeva.

Godišnja pretplata za 2016. godinu iznosi: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za strane državljane i ustanove. Pretplate: Žiro račun br. 840-314849-70 MO – Sredstva objedinjene naplate – VMA (za Vojnosanitetski pregled), poziv na broj 12274231295521415. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om). Za zaposlene u MO i Vojsci Srbije moguća je i pretplata u 12 mesečnih rata putem trajnog naloga, tj. „odbijanjem od plate“. Popunjen obrazac poslati na adresu VSP-a.

PRIJAVA ZA PRETPLATU NA ČASOPIS „VOJNOSANITETSKI PREGLED“

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

Pretplata na časopis „Vojnosanitetski pregled“ (zaokružiti):

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

Datum _____	Potpis _____

VOJNOSANITETSKI PREGLED
VOJNOMEDICINSKA AKADEMIJA
Crnotravska 17, 11040 **Beograd**, **Srbija**
Tel/Fax: +381 11 2669689
vsp@vma.mod.gov.rs

Časopis „Vojnosanitetski pregled“ izlazi godišnje u 12 brojeva.

Godišnja pretplata za 2016. godinu iznosi: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za strane državljane i ustanove. Pretplate: žiro račun br. 840-314849-70 MO – Sredstva objedinjene naplate – VMA (za Vojnosanitetski pregled), poziv na broj 12274231295521415. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om). Za zaposlene u MO i Vojsci Srbije moguća je i pretplata u 12 mesečnih rata putem trajnog naloga, tj. „odbijanjem od plate“. Popunjen obrazac poslati na adresu VSP-a.

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