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MARCH 18, 2022
QUALITY SLEEP | SOUND MIND | HAPPY WORLD

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Hosted by World Sleep Society

MARCH 18, 2022

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Since 2008, every third Friday in March, World Sleep Day has been celebrated with the aim of raising awareness of the importance of sleep for human health. This year's World Sleep Day is held on March 18 under the slogan: "Quality sleep, sound mind, happy world!"

Od 2008. godine, svakog trećeg petka u martu obeležava se Svetski dan spavanja sa ciljem podizanja svesti o važnosti sna za ljudsko zdravlje. Ovogodišnji Svetski dan spavanja održava se 18. marta pod sloganom: „Kvalitetan san, zdrav duh, srećan svet!”



Late auditory event-related potential changes after sensorimotor rhythm neurofeedback training

Promene kasnih komponenti auditivnih evociranih potencijala nakon *neurofeedback* treninga senzomotornog ritma

Ivana Stanković*, Tihomir V. Ilić†, Ljiljana Jeličić‡, Miško Subotić‡, Vesna Martić‡§, Mirjana Sovilj*, Nela V. Ilić¶, Miodrag Stokić‡

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Abstract

Background/Aim. Neurofeedback (NFB) is a therapeutic method based on monitoring the electroencephalogram (EEG) and providing feedback on the brain activity of subjects. The aim of the pilot study was to investigate the effect of lower-beta or sensorimotor rhythm (SMR) (12–15 Hz) NFB training on amplitudes and latencies of late auditory event-related potentials (aERP) components N100, N200, P300 in Go-No go task of auditory discrimination. **Methods.** Each of 9 healthy participants aged 25–40 years (4 male) had 20 daily sessions of SMR neurofeedback training. The aERP was recorded 5 times: before NFB, after 5, 10, and 20 sessions, and one month after the last session. **Results.** The results showed a statistically significant decrease in N100, N200, and P300 latencies at Fz, Cz, and Pz regions. No significant effect of NFB training on amplitudes of components N100, N200 and N300 was found. **Conclusion.** The obtained results suggest that NFB training exerts its effect on the processes of auditory cognition.

Key words:

brain; cognition; electroencephalography; event-related potentials, p300; evoked potentials; evoked potentials, auditory; feedback, sensory.

Apstrakt

Uvod/Cilj. *Neurofeedback* (NFB) je terapijski metod zasnovan na praćenju elektroencefalograma (EEG) i omogućavanju povratne sprege moždane aktivnosti osobama. Cilj pilot studije bio je da se ispita efekat NFB treninga senzomotornog ritma (SMR), tj. niskog opsega beta ritma (12–15 Hz) na amplitudu i latencije komponenti kasnih auditivnih evociranih potencijala (AEP) N100, N200 i P300 tokom zadatka auditivne diskriminacije. **Metode.** Svaki od 9 zdravih ispitanika uzrasta od 25 do 40 godina (4 muškog pola) imao je 20 SMR NFB treninga (po jedan svakog dana), a AEP su snimani 5 puta: pre primene NFB treninga, posle 5, 10 i 20 treninga i jedan mesec posle poslednjeg treninga. **Rezultati.** Rezultati su pokazali statistički značajno smanjenje latenci N100, N200 i P300 komponenti u Fz, Cz i Pz regijama. Nije utvrđen statistički značajan efekat NFB treninga na amplitude ovih komponenti. **Zaključak.** Dobijeni podaci ukazuju na potencijalni efekat NFB treninga na poboljšanje kognitivnog procesa auditivne diskriminacije.

Ključne reči:

mozak; saznanje; elektroencefalografija; potencijali povezani sa događajima, p300; evocirani potencijali; evocirani potencijali, auditorni; povratna informacija, senzorna.

Introduction

For the last several decades, research has been dedicated to the detection, quantification, and physiological analysis of discrete electroencephalographic

(EEG) changes associated with a particular event. They provide new opportunities in understanding complex brain functions, normal and pathological, that have been unexplained by classical neurophysiological paradigms. Event-related potentials (ERP) represent changes in the

electrical activity of the central nervous system (CNS) structures that are induced (evoked) by a stimulus (exogenous potentials) or by an event (endogenous potentials).

Since the discovery of the P300 component by Sutton in 1965, much research has focused on the generation of P300 and its association with cognitive functions¹. P300 is an endogenous cognitive neuroelectric phenomenon that occurs under the influence of endogenous stimuli and depends on the state of vigilance, concentration, type of task the subject is required to perform. The ERP components are represented by a series of positive and negative waves (N100, P100, N200, P200, and P300) of different duration and amplitude, the most significant of which is P300. The P300 is considered a manifestation of CNS involvement in processing new information when attention is engaged in memory refresh. The peak amplitude of the response signal and the latency of CNS responses to stimulation are indicators of subjects' cognitive functioning during the specific task.

Attention training is possible through a therapeutic method of learning to control brain activity by EEG recording. This process, known as NFB or EEG biofeedback, captures an aspect of physiological function and provides real-time feedback on these levels to achieve a degree of control or change in the internal state². Neurofeedback (NFB), a form of biofeedback, is a therapeutic method based on monitoring the EEG and providing feedback on the brain activity of subjects, which can be learned to regulate *via* operative conditioning³. The goal of the training is to practice recognizing the extent to which the brain works, how we experience what state of activity, and how to willingly transition into the state we need for a particular activity. NFB protocols are based on amplifying, inhibiting, or harmonizing certain EEG rhythms. Certain rhythms are associated with certain subjective states and behaviors.

Higher-frequency wave training refers to better focus and increased attention and concentration, such as the so-called training of the lower beta activity segment (12–15 Hz), also called sensorimotor rhythm (SMR)⁴. Sensorimotor rhythm or SMR waves (12–15 Hz) are beta waves that occur in the sensorimotor region of the brain regulated by the thalamocortical loop⁵. They reflect a state of alertness and alertness without tension⁶. With the NFB-SMR protocol, the subject trains to gain control in terms of increasing the amplitude of the SMR wave, resulting in increased attention and better focus. Literature data indicate that normal healthy individuals can learn to control and modify the components of their EEG activity and thus contribute to improving attention and cognitive function.

This pilot study aimed to investigate the effect of the lower-beta frequency band (12–15 Hz) called sensorimotor rhythm (SMR) NFB training on amplitudes and latencies of event-related potentials (N100, N200,

P300) in a Go/No go auditory task discrimination in healthy adult participants.

Methods

Participants

The study involved 9 participants, 25 to 40 years old. The participants were recruited from the Institute for Experimental Phonetics and Speech Pathology and the Life Activities Advancement Center in Belgrade, whose Laboratory for Cognitive Research conducted the experiments. Participants were healthy individuals of both sexes (4 male and 5 female), without hearing or speech disorders, with no prior and current neurological or psychiatric illness (based on the participant's verbal report). All participants were right-handed, according to the Edinburg Inventory. Each participant gave his/her written informed consent before the experimental procedure. This study was approved by the local Ethics Committee according to the Declaration of Helsinki (22/19).

Auditory event-related potentials recording

The auditory ERPs (aERP) were recorded using a standard oddball Go/No go paradigm. The EEG data were recorded using a Nihon Kohden Electroencephalograph (model EEG-4314 F) and Neuroscan Acquire 4.0 software.

The participants had a task to react by pressing a control button with the right hand's thumb each time they hear a tone that differs from other tones that are mostly presented. A total of 80% of each presented tone had a frequency of 1,000 Hz, and 20% of tones were the oddballs with the frequency of 2,000 Hz. The tones were randomly presented to the participants. The participants listened to the tones using earphones. Three Ag/Ag-Cl ring electrodes for aERP registration were positioned according to 10–20 International system for electrode placement at the Fz (frontal midline), Cz (central midline), and Pz (parietal midline) regions. The reference electrode was set to the ear lobes and the ground electrode on the forehead. The impedance was kept below 5 k Ω with no more than 1 k Ω difference between electrodes. The software has its own implemented tool for artifact rejection. Each recording section that had more than 20% of rejected trials due to excessive artifacts was discarded and done again. Each participant underwent the experimental procedure in the morning hours (9–11 am). Participants were instructed to have 8 hours of sleep before ERP recording. Additionally, they used no medication or alcohol and no caffeine drinks before recording at least 24 h and 12 h respectively. The participants were nonsmokers. For each participant, averaged amplitude (μ V) and latency (ms) of N100, N200, and P300 waves were obtained for each electrode (Fz, Cz, and Pz).

Neurofeedback SMR protocol training

Statistical analyses

The experimental task for participants was to perform an NFB SMR training – increasing the amplitude of SMR. Each participant participated in 20 sessions of NFB SMR protocol training, three times a week for 33 min: 2 minutes of the resting-state period (watching a blank computer screen) at the beginning, 4 training trials each lasting 6 min, and 2 min resting-state at the end. During the trials, a participant observes physiological responses on a screen in the form of pictures and video games. The information that comes from this process is feedback, which is reflected in changes in the image or sound of the video game used for training. The games are designed to let the participant advance in the game if he or she can bring the physiological function that is being rehearsed to the desired level. After each trial, the participants had a one-min break. The NFB SMR training was performed using BioTrace software for Nexus – 10B2015. The electrode was set to a Cz region (central midline-vertex region).

After 5, 10, and 20 NFB SMR training sessions, as well as one month after the last session, participants were reregistered with aERP using the same procedure as at the beginning.

Due to the small sample size, the comparisons of amplitude and latency differences before and after NFB SMR training were analyzed using nonparametric statistics: the Kruskal-Wallis test for exploring the effect of time point (before NFB, after 5, 10, 20 sessions, and one month after the last training session) and Wilcoxon signed ranks test for *post hoc* multiple comparisons reporting *Z* score and *p*-value. In each comparison, a 95% confidence interval was used.

Results

Figure 1 presents the averaged amplitudes of N100 (left panel), N200 (middle panel), and P300 (right panel) waves for Fz, Cz, and Pz electrodes at different time points. Using the Kruskal-Wallis test for exploring the effect of time point and *post hoc* Wilcoxon signed ranks test for multiple comparisons, no significant differences were found in N100, N200, or P300 amplitude among five-time points for Fz, Cz, and Pz electrodes.

Figure 2 presents the averaged latencies of N100 (left panel), N200 (middle panel), and P300 (right panel) waves for Fz, Cz, and Pz electrodes at different time points. The

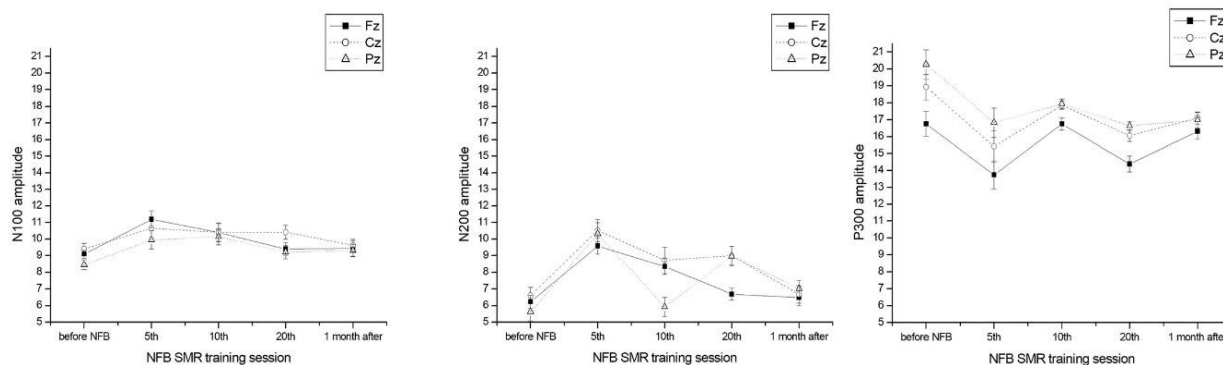


Fig. 1 – Average amplitude value (μV) of N100 (left panel), N200 (middle panel), and P300 (right panel) waves at Fz, Cz, and Pz electrode location at five-time points: before neurofeedback sensorimotor rhythm (NFB SMR) training and after 5, 10, and 20 sessions as well as one month after the last session. No significant differences were found in N100, N200, or P300 amplitude between five-time points for Fz, Cz, and Pz electrodes.

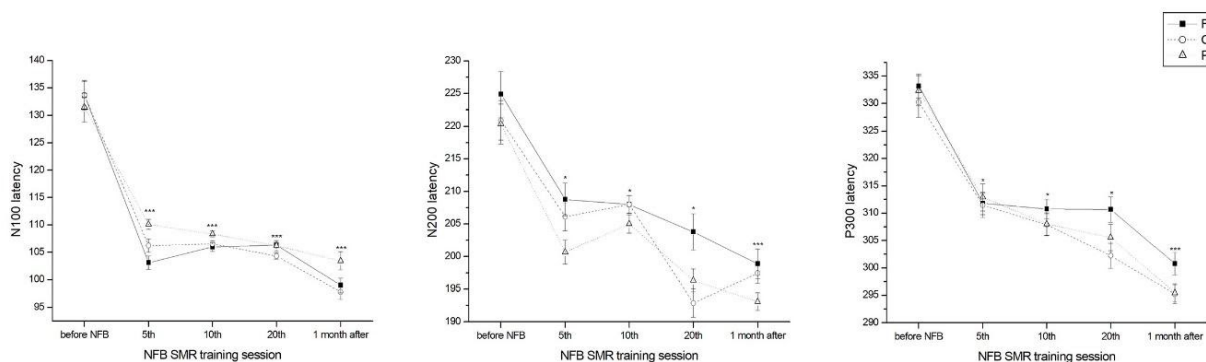


Fig. 2 – Average latency value (ms) of N100 (left panel), N200 (middle panel), and P300 (right panel) waves at Fz, Cz, and Pz electrode location at five-time points: before neurofeedback sensorimotor rhythm (NFB SMR) training and after 5, 10, and 20 sessions as well as one month after the last session. Statistically significant differences are marked with asterisks.

* $p < 0.05$, *** $p < 0.01$.

Kruskal-Wallis test found a statistically significant effect of time point on N100 latency at Fz: $H(40) = 7.695$, $p < 0.01$, Cz: $H(40) = 7.760$, $p < 0.01$, and Pz: $H(40) = 9.418$, $p < 0.01$; N200 latency at Fz: $H(40) = 5.144$, $p = 0.02$, Cz: $H(40) = 8.165$, $p < 0.01$, and Pz: $H(40) = 6.727$, $p = 0.018$; and P300 latency at Fz: $H(40) = 9.118$, $p < 0.01$, Cz: $H(40) = 10.638$, $p < 0.01$, and Pz: $H(40) = 8.119$, $p < 0.01$. The *post hoc* Wilcoxon signed ranks test found that this effect was driven by the significantly shorter latencies after the NFB SMR trainings (after 5, 10, and 20 sessions) and one month after the last session compared to the period before training ($p < 0.05$). No differences in latency of N100, N200, or P300 were found among different sessions (5, 10, and 20 sessions).

Discussion

This study explored the effect of NFB-SMR training on the amplitude and latency changes of aERP recorded at Fz, Cz, and Pz regions during the standard auditory oddball discrimination task. An effect of NFB SMR training was found for aERP latencies. NFB SMR training caused a decrease in latencies of auditory ERP N100, N200, and P300 waves. However, no differences in amplitudes were found. To generate P300 potential, the oddball paradigm was used. It is the acoustic oddball discrimination test, the most commonly used, which involves the use of two types of tone: high-frequency arrhythmic tone and low-frequency rhythmic tone. The difference between the two tones is in frequency and intensity⁷. The respondent is presented with two types of auditory stimuli: the “rare” or “unexpected” arrhythmic tone, which represents the target stimulus and differs in frequency from the “standard” or “expected” tone and occurs in random order. The participant is required to respond to an “unexpected” tone (by counting, pressing a key), and to ignore the “standard” tone, *ie*, to recognize target stimuli in a series of stimuli that differ in a characteristic (volume, duration), and are less likely than standard ones. Oddball experimental paradigm requires the attention and concentration of respondents.

Over the last several decades, a large number of studies in healthy individuals as well as in patients with brain damage have shown that SMR NFB can lead to cognitive improvements, mainly in memory functions and attention⁸⁻¹⁴.

Latency is usually interpreted as the speed of processing sensory stimuli as a consequence of distinction from the other stimuli. Therefore, shorter latencies are considered to reflect more effective mental performance compared to longer latencies. Kober et al.¹⁵ showed in a study in healthy young adults that training with the NFB SMR protocol leads to cognitive improvements associated with changes in the electrophysiological parameters of evoked potentials. The experimental but not the control group showed a linear increase in SMR strength during training, which was associated with improvements in attention, to more pronounced processing of stimuli, as indicated by increased N100 and P300 amplitude after

training compared to the pre-training condition. We found a similar result regarding a sample of healthy individuals. The amplitude of this potential is related to the amount of attention given to a task and the refreshment of working memory content. The P300 latency reflects the speed of stimulus classification, that is, the information evaluation time. ERP waveforms are quantitatively described by amplitude level, latency length, and topographic distribution. The amplitude reflects the magnitude of neural activity and typically ranges from 1 to 30 mV¹⁶. Latency represents the time interval, that is, the period from the moment of stimulation to the appearance of maximum amplitude, *ie*, peak of ERP, and ranges from several hundred milliseconds.

In contrast, a study by Arns et al.¹⁷ showed an increase in N200 and P300 amplitudes in participants who had the SMR protocol. Increasing the amplitude of N200 and P300 indicates the normalization of neural circuits associated with discrimination of stimuli and updating of attention¹⁷. In a study by Reichert et al.¹⁸, an increase in N100 and P300 amplitude was observed in a poststroke patient in an experimental group who had SMR training, whereas the control group showed no difference. This finding is in line with our study that found no differences in N100 and P300 amplitude in healthy participants after SMR NFB training. The earliest wave in the sequence arises about 100 ms from stimulation and is designated as wave N100 due to the negative polarization. The second wave occurs about 150 ms from stimulation, is positive polarity, and is designated P100. Of the early components, the most important is the negative wave, the N200 component, which occurs 200 ms from stimulation and is associated with the process of sensory discrimination. The role of N200 today is mainly focused on “cognitive controls”, a concept that encompasses monitoring and control of motor responses^{19,20}. The longest latency and the highest amplitude registered above the central and parietal regions of the cerebral cortex is the P300 wave. The time span of this P300 component by Coles and Rugg²¹ can range from 250 ms to 900 ms, with an amplitude ranging from at least 5 μ V to the usual limit of 20 μ V for auditory and visually evoked potentials, even though amplitudes up to 40 μ V have also been recorded. The P300 component is thought to be a cognitive neuroelectric indicator of CNS activity that involves processing new information when attention is directed to updating memory performance²². P300 latency can be considered a measure of the relative duration of the stimulus evaluation process, which is an upper bound on the time of stimulus categorization and evaluation²¹, or the time it takes to allocate resources and update memory. It is an endogenous response to a task that is not known, *i.e.*, response to target stimuli²³. Late potentials are used to study multiple modalities of cognition but are most commonly related to memory and attention. Extension of the P300 latency, which reflects the time of assessment and categorization of stimuli, indicates a slowdown in mental functions. The P300 ERP is a determinant of alertness and active attention. The lack of attention causes a decrease in the P300 amplitude or the absence of a P300 wave. Insufficient attention and concentration also make it difficult

to distinguish between standard and target tone, which directly affects P300 latency²⁴.

In a study by Kropotov et al.²⁵ in children with attention-deficit/hyperactivity disorder (ADHD) after beta and SMR training sessions, differences in pretreatment and posttreatment ERP were observed. Successful performers received positive components evoked within 180–420 ms. Differences are distributed in the frontal/central parts and appear to reflect activation of the frontal cortical areas. The use of NFB as an operative conditioning paradigm by the SMR protocol in a study by Kaiser and Othmer²⁶ showed a significant clinical improvement in attention and impulsivity control in 85% of subjects after NFB training²⁶. In the Lubar and Lubar²⁷ study in children after SMR and beta wave training, all children increased SMR or beta values and decreased slow EEG activity, which was also evident in their spectral strengths regarding increased beta and decreased slow activity and improved school success. Normalization of ERP components in participants with ADHD after NFB training is also described by several other authors^{28,29}. These results indicate the possibility of using SMR NFB as a therapeutic method for attention improvement. Several authors have shown that the latency and/or amplitude values of P300 in normal adults are reproducible and stable without statistically significant differences in retesting state at different time intervals^{30–35}. This is in line with our finding that P300 latency is stable even one month after the last training. This might point to a plastic change of the brain's electric activity that can last for a longer period of time. This is important because in generating endogenous potentials, selective attention is paramount. That is, directing alertness and willing activity to complete the information processing.

Selective attention occurs when processing information in situations where it is necessary to select one from several messages to be further processed while the others are ignored. Achieved long-lasting enhancement of selective attention using NFB might be a promising field of research towards its application in neurotherapy in children with developmental disorders as well as adults with neurological cognitive impairments.

Our study has a limitation regarding the small sample size. Hence, the generalization of the results should be done with caution. However, this study showed that participants gained a shorter reaction time – shorter latencies in the auditory discrimination task, after NFB SMR training. This finding implies that NFB might be a useful method of neuromodulatory therapy in improving the auditory processes. Further research should comprise a much bigger sample size in order to explore the potential effect of NFB training on cognitive processes.

Conclusion

The obtained results suggest that NFB training produces its effect on the processes of auditory cognition.

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The use of reperfusion therapy in transition countries without fully applicable pharmacoinvasive strategy

Upotreba reperfuzione terapije u zemljama tranzicije bez potpuno primenljive farmakoinvazivne strategije

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Abstract

Background/Aim. The pharmacoinvasive (PI) therapy is a recommended strategy in patients (pts) with ST elevation myocardial infarction (STEMI) unable to undergo timely primary percutaneous coronary intervention (p-PCI). The aim of the study was to find out the cohorts of pts who are not treated by any reperfusion therapy (RT) as well to determine the outcome of the pts treated with RT in a transition country without fully applicable PI therapy. **Methods.** The study analyzed data from the Hospital National Registry for Acute Coronary Syndrome of Serbia (HORACS). **Results.** The significant predictors of the withdrawing of the application of any RT in the model [c 75.6%, SE 0.004, 95% CI 0.748–0.761] were age (≥ 65 years), heart failure (Killip II-IV), diabetes mellitus, and the time to first medical contact (FMC) (> 360 min). In patients without RT, mortality was 15.7%, in pts treated with fibrinolytic therapy (FT)

was 10.5%, and in pts treated with pPCI, it was 6.2% ($p < 0.000$). Within 3 hours to FMC, higher in-hospital mortality was in FT pts (FT 8.7% *vs* p-PCI 4.3%). FT treated patients were older, had more comorbidities and heart failure (HF). However, after propensity score matching, in order to adjust the differences among the pts, the mortality rate remained higher in FT pts but not statistically significantly higher than in p-PCI pts (FT 8.8% *vs* p-PCI 6.4%). **Conclusion.** The balance of the best cost-benefit strategies for better use of RT is difficult to achieve in transition countries. The possibility for timely p-PCI and PI therapy is especially not applicable in high-risk patients, older pts, pts with HF, and those with diabetes mellitus.

Key words: drug therapy; st elevation myocardial infarction; myocardial reperfusion; risk factors; serbia; treatment outcome.

Apstrakt

Uvod/Cilj. Preporuke za lečenje bolesnika sa akutnim infarktom miokarda sa elevacijom ST segmenta (STEMI), nalažu da se kod bolesnika koji ne mogu blagovremeno da odu na primarnu perkutanu intervenciju (p-PCI) primeni farmakoinvazivna (FI) strategija lečenja. Cilj rada bio je da se utvrde karakteristike bolesnika koji se uopšte ne leče reperfuzionom terapijom (RT), kao i da se analizira ishod lečenja pomoću RT, u zemlji u tranziciji u kojoj mreža za primenu FI terapije nije u potpunosti razvijena. **Metode.** Za istraživanje su korišćeni podaci bolničkog Nacionalnog registra za akutni koronarni sindrom Srbije (HORACS). **Rezultati.** Značajni prediktori za izostanak primene RT su prikazani u modelu (c 75,6%, SE 0,004, 95% CI 0,748–0,761) u koji su uključene godine starosti

(≥ 65), srčana insuficijencija (Killip klasa II-IV), dijabetes melitus, i vreme do prvog medicinskog kontakta (PMK) (> 360 min). Kod bolesnika koji nisu bili lečeni RT, mortalitet je bio 15,7%, kod bolesnika lečenih fibrinolitikom terapijom (FT) iznosio je 10,5%, a kod bolesnika lečenih p-PCI 6,2% ($p < 0,000$). U grupi bolesnika koji su do PMK stizali za 3 sata, mortalitet lečenih pomoću FT bio je veći od mortaliteta bolesnika lečenih p-PCI (FT 8,7% *vs* p-PCI 4,3%). Bolesnici lečeni pomoću FT bili su stariji, sa više komorbiditeta i sa učestalijim znacima srčane insuficijencije. Ipak, posle primenjenog *propensity* skora, sa ciljem da se izbegnu razlike između dve grupe bolesnika, mortalitet u FT grupi ostao je veći, ali bez statistički značajne razlike u odnosu na bolesnike lečene p-PCI (FT 8,8% *vs* p-PCI 6,4%). **Zaključak.** Primena RT, uz postignuti idealan balans potrošnje i koristi, teško je izvodljiva u zemljama u tran-

ziciji. Mogućnosti za blagovremenu primenu p-PCI, kao i FI terapije, posebno su ograničene kod visoko rizičnih, starijih bolesnika, kod bolesnika sa znacima srčane insuficijencije, komorbiditetima i dijabetesom melitusom.

Ključne reči:
lečenje lekovima; infarkt miokarda sa st elevacijom; miokard, reperfuzija; faktori rizika; srbija; lečenje, ishod.

Introduction

The better outcome of patients (pts) with acute myocardial infarction with ST elevation (STEMI) is directly dependent on reperfusion therapy (RT). A timely primary percutaneous coronary intervention (p-PCI) is the preferred therapy for STEMI¹. The prompt coronary reperfusion as early as the symptom onset, p-PCI within two hours, and fibrinolytic therapy (FT) within ten minutes from the first medical contact (FMC) are difficult to achieve, especially in economically undeveloped countries². However, in the European Society of Cardiology (ESC)³ and the American College of Cardiology/American Heart Association (ACC/AHA) STEMI guidelines⁴, the optimal organization of the STEMI systems of care at a community level is needed. Offering p-PCI to the maximum proportion of pts within the recommended time spans provides optimal care in the prehospital setting, including a rapid and accurate diagnosis, the preactivation of the cardiac catheterization laboratory, and the initiation of pharmacological RT by FT if p-PCI cannot be offered in a timely fashion. However, in the last few years, the use of pharmacoinvasive (PI) strategy of the FT and p-PCI, respectively, within 2–24 hours seems to be as good as p-PCI, especially in the areas where p-PCI is not available within the recommended time^{3, 5}. The latest results have shown that the pts treated with the PI strategy of therapy, compared with p-PCI, presented within 3 hours after the symptom onset, but who were unable to undergo p-PCI within 1 hour, had a similar percentage of the composite primary endpoint consisting of death, shock, congestive heart failure, and re-infarction in 30 days⁵. The rates of 1-year overall mortality were similar between the two groups of PI vs p-PCI⁶. However, the conclusion of this study may be controversial since there was a similar risk of the primary end-point in the two study groups and a significantly higher risk of intracranial bleeding with early FT⁶. Thus, p-PCI remains the treatment of choice in such patients who have close access to catheterization laboratory centers.

The strategy of STEMI treatments at the community, regional, and national levels has been supported⁷ and recommended in order to increase the proportion of the pts receiving timely p-PCI by bypassing closer hospitals without interventional facilities⁸.

In Serbia, however, the overall proportion of untimely reperfused eligible STEMI patients remains high⁹. It might be caused by the insufficient PCI network or unused PI therapy^{9–11}. The cardiovascular outcome is different between Eastern and Western European countries^{12–16}, and the performance measures for reperfusion in STEMI have significantly improved with greater use of p-PCI¹⁷. However, it is unclear that PI strategy is as important as p-PCI in develop-

ing and transition countries^{16, 18, 19}. It has not been applied on time in remote regions of developing countries. Moreover, transportation of high-risk patients is particularly difficult. On the other hand, FT is a very expensive therapy. Consequently, the best cost-benefit strategies for the high-risk patients and the patients treated by FT and who need transportation in PCI centers are unclear in these countries.

The primary aim of this study was to find out the cohorts of patients who were not treated by any RT. The second aim was to determine the outcome of the pts treated by RT (p-PCI or FT) in a transition country without a fully applicable PI strategy.

Methods

Data collection and the study population

We used the data of the Hospital National Registry for Acute Coronary Syndrome of Serbia (HORACS)^{9, 11}. The registry was filled in by the attending physicians in the 54 Coronary Care Units (CCU) in Serbia for all the pts with the acute coronary syndrome (ACS). There were 7 primary PCI centers and 2 were open round-the-clock (24 h/7 days). In Serbia, there were 9 University Centers at that time. All pts' data, clinical diagnoses, treatments, and the hospital outcome were collected, and all the definitions were in accordance with guidelines¹. The HORACS registry was designed to reflect an unbiased, representative population of pts with ACS. This observational study included consecutive pts with the diagnosis of a STEMI, according to the European guidelines, hospitalized for 3 years (2007–2009). To further narrow our study population, we excluded the following: pts < 18 years of age; pts who presented the FMC with unknown or invalid date/time of reference for the hospital arrival or the application of RT (p-PCI or FT). Then, only observed the pts were those who arrived within 18 hrs from the symptom onset to the FMC. The patients who arrived within 3 h from the symptom onset to the FMC and who were treated with RT were additionally analyzed.

Statistical analysis

Univariate and multivariate analyses were performed in order to determine the predictors of the patients profiled for receiving the RT. The variables were included and analyzed categorically. A multiple backward regression analysis was performed, with a significance of $p = 0.05$ for the removal of the variables from the model. The Hosmer-Lemeshow statistics for the goodness of fit were calculated. A stepwise logistic regression analysis was performed to assess the significance of the factors generally thought to be related to the clinical decision of RT.

Additionally, because the study was observational and the pts were not assigned randomly to either type of treatment, the events in both treatment groups were matched using the propensity score matching. A propensity score analysis was performed by using a logistic regression model, with the 1 on 1 matching without a replacement for p-PCI vs the FT group in order to adjust the differences among the pts. All statistical analyses were performed by the SPSS statistical package for Windows.

Results

A total of 15,354 consecutive STEMI pts, mean age 63.58 ± 11.97 years [median 64 (55–73) years], from the HORACS registry, were included in our analysis. There were 8,502 (55.4%) pts treated with RT (Table 1). In patients without RT, mortality was 15.7%, in pts treated with FT was 10.5%, and in pts treated with p-PCI was 6.2% ($p < 0.000$). There were 84.3% of pts who arrived within 12 h and 15.7% of those who arrived 12–18 h from the symptom onset to the FMC. The main reasons why the pts did not receive RT are shown in Figure 1. In Figure 2, the model is presented with the prediction value *c* statistic 75.6%, 95% confidence interval (CI) 0.748–0.761, for a decision on whether or not to apply RT (the Hosmer-Lemeshow test, $\chi^2 = 8.899$, $p = 0.351$,

SE = 0.004). The significant predictors for making a decision not to apply RT were age (≥ 65 years), heart failure (Killip II-IV), diabetes mellitus, and the time from the symptom onset (> 360 min.).

A total of 4,986 (58.6%) pts arrived within three hours from the symptom onset to the FMC. Their mean age was 59.6 ± 11.4 yrs, and the median was 59 (IQR 52–69) yrs. More pts received FT [3,277 (65.7%)]. On the other hand, 1,709 (34.3%) patients received p-PCI.

The baseline characteristics, comorbidities, and previous coronary diseases are shown in Table 1. The pts in the FT group were older (60.1 ± 11.3 years vs 58.7 ± 11.5 yrs, $p < 0.000$), with a higher prevalence of diabetes mellitus (20.1%, vs 17.6%, $p = 0.004$), and renal failure (4.3% vs 3.1%, $p = 0.035$). There was a significant difference between the two reperfusion groups regarding the span time from the symptom onset to the FMC ($p < 0.001$).

The significant predictors that determinate the type of RT (p-PCI or FT) were the arrival at the non-PCI capable center, the previous p-PCI, the time from the symptom onset, heart failure ($p < 0.001$), diabetes mellitus ($p = 0.019$), and renal failure ($p = 0.035$), the localization of myocardial infarction ($p = 0.024$), previous angina pectoris ($p = 0.010$). High-risk patients with heart failure, diabetes mellitus, and renal failure were treated more with FT. Significant predic-

Table 1

The baseline characteristics, comorbidities and previous coronary diseases

Baseline characteristics	Without RT n = 6,852	FT (18 h) n = 5,132	p-PCI (18 h) n = 3,370	<i>P</i>	FT (3 h) n = 3,277	p-PCI (3 h) n = 1,709	<i>P</i>
Age (years), mean \pm SD, median (IQR)	67.1 \pm 11.6 69 (59–76)	61.2 \pm 11.3 61 (53–70)	60.0 \pm 11.7 59 (52–69)	< 0.001	60.1 \pm 11.3 59 (52–69)	58.7 \pm 11.5 58 (51–67)	< 0.001
Gender (male/female), %	60.6/39.4	68.0/32.0	70.5/29.5	< 0.000	70.6/29.4	72.1/27.9	0.277
Anterior myocardial infarction, %	44.1	42.4	44.1	0.137	41.1	44.4	0.025
Atypical symptoms, %	12.2	4.5	3.6	< 0.001	4.1	3.1	0.068
Time from symptom onset (min), mean \pm SD, median (IQR)	290 \pm 270 180 (90–420)	160 \pm 152 120 (60–180)	206 \pm 184 150 (90–240)	< 0.001	80.9 \pm 37.4	92.4 \pm 36.4	< 0.001
Transport to hospital, %							
independently	16.1	16.4	18.5	< 0.001	16.0	18.7	< 0.001
emergency	52.8	61.5	60.0		64.0	63.5	
other medical ambulance or hospital	31.1	22.1	21.5		20.0	17.8	
Comorbidities, %							
hypertension	68.7	63.5	65.8	< 0.001	61.3	63.0	< 0.001
hyperlipidemia	41.9	63.5	65.8	< 0.001	43.9	51.4	< 0.001
diabetes	29.9	22.2	20.2	< 0.001	20.1	17.6	0.004
previous stroke	8.2	4.5	5.1	< 0.001	3.9	4.3	0.489
renal failure	6.8	4.3	2.9	< 0.001	4.3	3.1	0.035
anemia	5.9	3.3	2.3	< 0.001	3.0	2.6	0.416
peripheral vascular diseases	8.4	4.8	3.3	< 0.001	4.0	3.2	0.203
Previous coronary diseases or their treatment, %							
angina pectoris	31.6	22.8	20.9	< 0.001	21.8	18.7	0.010
MI	24.8	15.8	14.5	< 0.001	15.9	14.2	0.122
CABG	3.0	2.0	1.8	< 0.001	2.0	1.7	0.442
PCI	5.2	3.1	19.7	< 0.001	3.0	16.0	< 0.001

CABG – coronary artery bypass grafting; MI – myocardial infarction; PCI – percutaneous coronary intervention; p-PCI – primary PCI; FT – fibrinolytic therapy; RF – renal failure; SD – standard deviation; IQR – interquartile range.

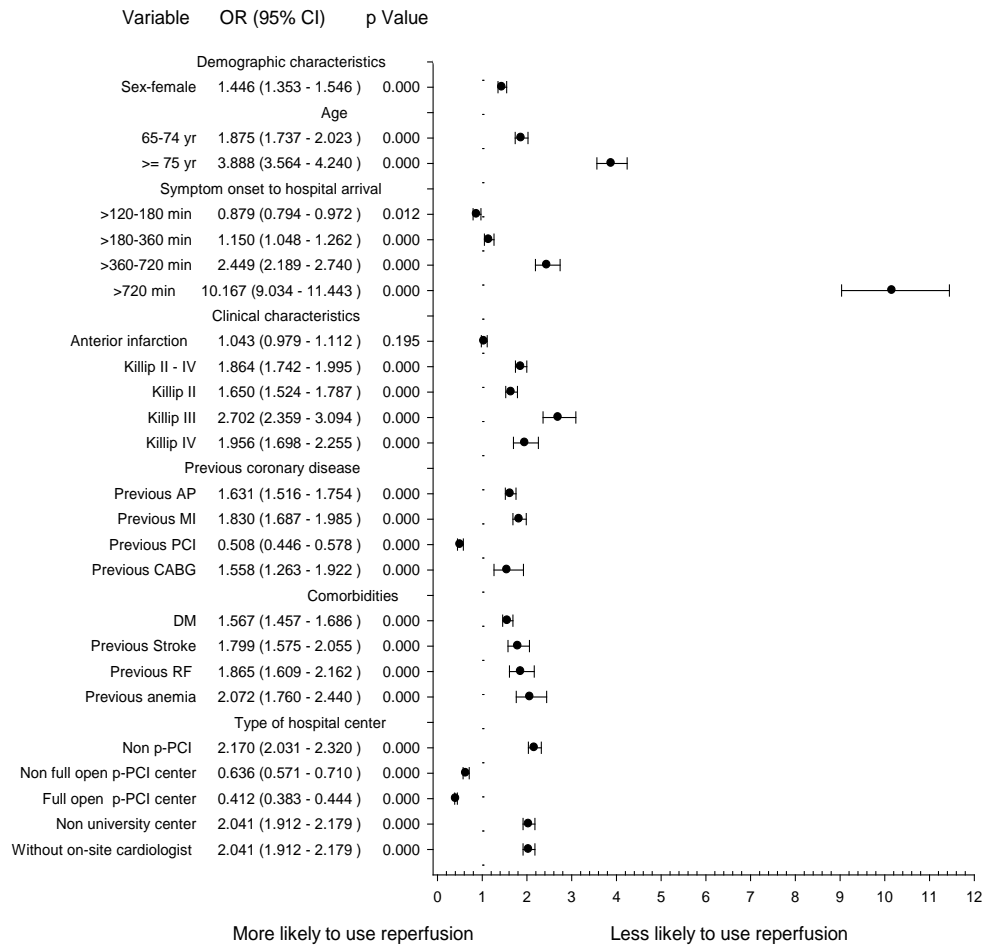


Fig. 1 – The predictors that indicate the absence of the application of reperfusion therapy (univariate analysis). OR – odds ratio; CI – confidence interval; AP – arterial pressure; MI – myocardial infarction; PCI – percutaneous coronary interventions; CABG – coronary artery bypass grafting; DM – diabetes mellitus; RF – reperfusion therapy; p-PCI – primary PCI.

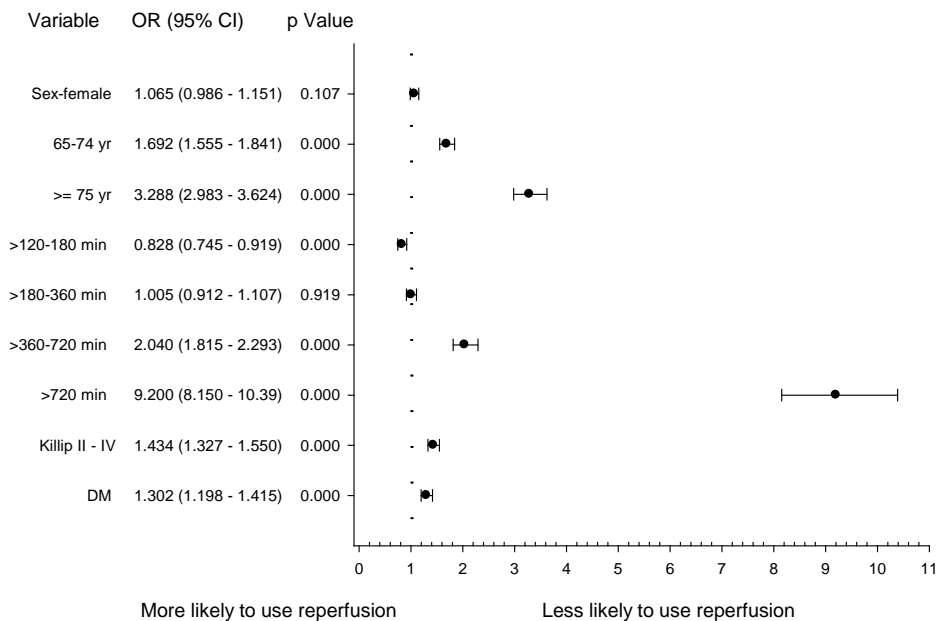


Fig. 2 – The model for predicting the absence of the application of reperfusion therapy. OR – odds ratio; CI – confidence interval; DM – diabetes mellitus.

tors for applying p-PCI were the treatment at the PCI center, the university center, the center with an on-site cardiologist, and the previous PCI in the past medical history (Figure 3).

The mortality rate (8.7% vs 4.3%) and the worse in-hospital outcome: heart failure (27.9% vs 18.5%), the composite of the mortality events and/or re-infarction (11.4% vs 7.2%), cardiac arrest (12.2% vs 7.2%), mechanical postmyo-

cardial complication (4.4% vs 1.7%), postinfarction angina (12.1% vs 5.5%), and arrhythmia (41.6% vs 23.9%) were significantly more often found in the FT group when compared with the p-PCI group, respectively ($p < 0.001$) (Table 2).

After the propensity score, there were 3,256 matched pairs of pts in the two groups treated with RT, who were so

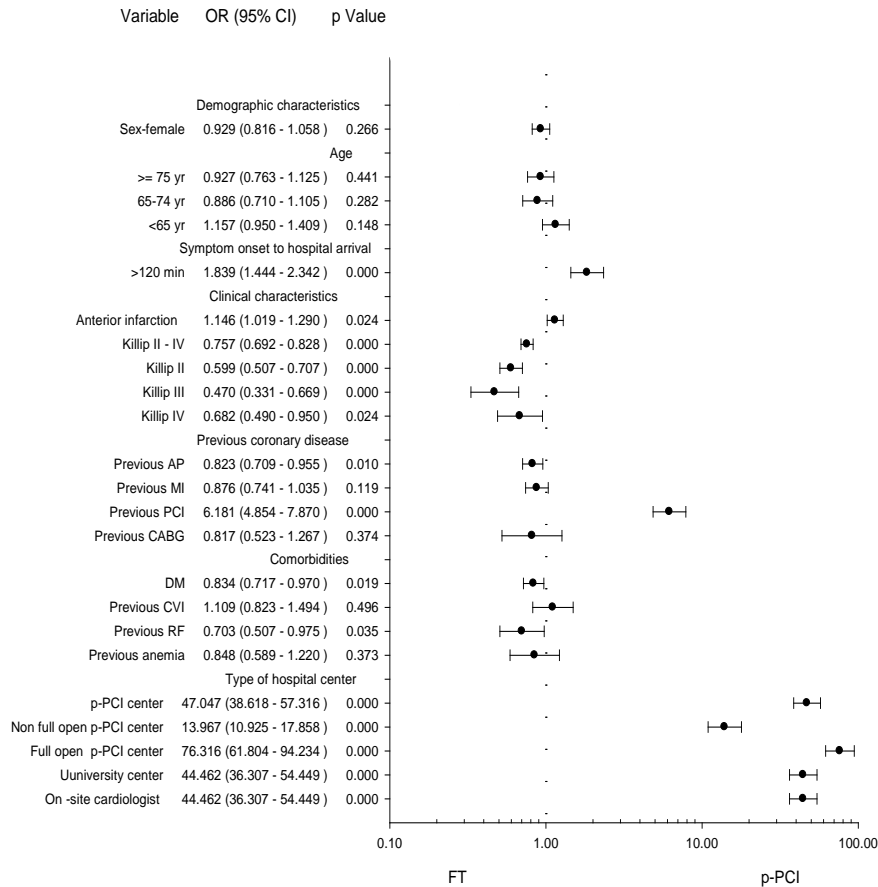


Fig. 3 – The predictors of the doctor’s decision on reperfusion therapy (univariate analysis). OR – odds ratio; CI – confidence interval; AP – arterial pressure; MI – myocardial infarction; PCI – percutaneous coronary interventions; CABG – coronary artery bypass grafting; DM – diabetes mellitus; CVI – cerebrovascular insult; RF – reperfusion therapy; FT – fibrinolytic therapy; p-PCI – primary PCI.

Table 2

The complications and the outcome

Complications	FT (n = 3,277)	p-PCI (n = 1,709)	<i>p</i>
Heart failure (%)	27.9	18.5	< 0.001
Killip II	19.5	13.2	< 0.001
Killip III	4.5	2.4	
Killip IV	3.9	3.0	
Mortality (%)	8.7	4.3	< 0.001
Reinfarction (%)	3.2	3.0	0.795
Composite events (mortality and re-infarction) (%)	11.4	7.2	< 0.001
Cardiac arrest (%)	12.2	7.2	< 0.001
Mechanical complication (%)	4.4	1.7	< 0.001
Postinfarction angina (%)	12.1	5.5	< 0.001
Arrhythmia (%)	41.6	23.9	< 0.001

FT – fibrinolytic therapy; p-PCI – primary percutaneous coronary intervention.

matched according to the categories of age, gender, the time to FMC (≤ 180 min), diabetes mellitus, and heart failure (Killip II-IV) (Table 3). When the two therapy condition groups were compared, the mortality rate was higher in the FT group, but it was not significantly different compared to the p-PCI group: FT 8.8% vs pPCI 6.4% (Figure 4).

benefit strategies for the successfully reperfused patients by FT and the high-risk patients are unclear.

Moreover, according to the studies published in the last ten years, the choice of RT (p-PCI or FT) should not only be based on the time elapsed from symptoms onset to FMC. Reperfusion therapy in Serbia was less applied in high-risk

Table 3

The characteristics of matching 3,256 patients treated with FT and p-PCI

Baseline characteristics	FT n = 1,538	p-PCI n = 1,758
Age (years), mean \pm SD	61.1 \pm 11.0	61.5 \pm 11.6
Gender (male/female), %	69.1/30.9	62.9/37.1
Time from symptom onset (minute), mean \pm SD,	81.54 \pm 32.12	86.4 \pm 40.03
Diabetes, %	28.6	29.6
Heart failure (Killip II-IV), %	29.5	18.7

FT – fibrinolytic therapy; p-PCI – primary percutaneous coronary intervention; SD – standard deviation.

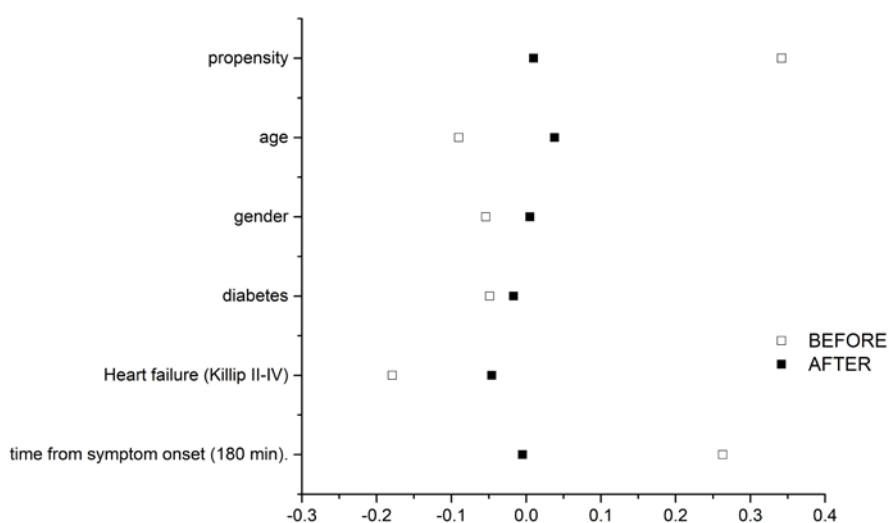


Fig. 4 – Matching 3,256 patients treated with fibrinolytic therapy and primary percutaneous coronary intervention by using the propensity score.

Discussion

The p-PCI remains the treatment of choice in patients who have close access to PCI centers. One-third of STEMI patients have ischemic time from FMC to p-PCI of more than 120 minutes¹⁸. Considering the results of less shock and heart failure in PI-treated pts, it could be a greater clinical benefit in situations where PCI-related delays occur in real-world situations⁶.

The unsolved problem is the strategy of improving RT in remote regions in developing and transition countries particularly. The balance of cost-benefit therapy is difficult to achieve. The advantage of the fibrinolytic agent in a situation where an urgent invasive procedure and transportation of high-risk patients is not possible may be very important. However, it includes the extra cost of the FT. The dilemma is whether the PI strategy is a reasonable and useful option for every patient who cannot undergo timely p-PCI and whether it is available in every region and country. The best cost-

pts. The problems of the treatment of high-risk pts and the gaps seem to persist in a large number of studies and registries in not only low-income countries¹⁹⁻²¹ but in developed countries as well²²⁻²⁶.

In the Strategic Reperfusion Early After Myocardial Infarction (STREAM) study⁵ of 3/5 PI successfully reperfused patients who underwent scheduled angiography approximately 18 hours after FT, excellent angiographic, 12-lead electrocardiography (ECG) metrics, and clinical outcomes were achieved. On the other hand, the pts requiring rescue PCI after contemporary FT, aspirin, clopidogrel, and enoxaparin in combination, with completed PCI within 140 minutes after FT, had high 30-day composite event rates of death, shock, chronic heart failure, and reinfarction (18.7%)²⁷. These data support findings that although PI-treated, pts requiring rescue angiography had greater baseline risk with more comorbidities and worse 30-day outcomes compared with pts successfully treated by FT. The patients requiring rescue PCI should be immediately transported to a

center capable of completing rescue intervention after the administration of FT ²⁷.

In Serbia, there were 37.3% STEMI pts presenting within 12 hours from the symptom onset to the FMC and ECG who did not receive any type of RT. The other 62.7% were treated with RT, of which 24.4% were treated with p-PCI, and 38.3% were treated with FT. In other countries' registries, the results were similar ^{16, 28, 29}. In Serbia, pts without RT were older (≥ 65 yrs), came later after the symptom onset (> 360 min), had heart failure (Killip II-IV), comorbidities such as diabetes mellitus, previous stroke, renal failure, anaemia, previous coronary diseases, except previous PCI and the arrival at a non-PCI capable and non-University center without a cardiologist on site. The situation was similar in other registries: in the SNAPSHOT ACS registry ²⁸, patients without RT were older, there were more those of the female gender, with comorbidities, and atrial fibrillation. In the Global Registry of Acute Coronary Events (GRACE) Registry ¹⁰, they were older and of the female gender; a history of heart failure, prior myocardial infarction, or diabetes mellitus were found to be independently associated with a lack of RT ¹⁰. In the CRUSADE ³⁰ and TETAMI registries ³¹, the results were similar.

In our study, the average time span from the symptom onset to the FMC was significantly shorter in the FT group than in the p-PCI group (80.9 ± 37.4 min vs 92.4 ± 36.4 min, respectively). If pts arrived within 2 hours, FT was applied in 45.3% and p-PCI in 22.8% of the pts. However, if they arrived later, more than 2 hrs, the percentage of the applied RT became similar: FT in 23.7% and p-PCI in 21.3%.

The important predictors that influenced the strategy of treatment with RT were the time span from the symptom onset, the anterior localization of myocardial infarction, the previous PCI, the arrival at the PCI center, the arrival at the university center, where cardiologists were on site. If pts had heart failure, previous coronary diseases, diabetes mellitus, and renal failure, pts were treated more by FT. Further, if pts arrived 2 h after the symptom onset, with the anterior localization of myocardial infarction, and the previous PCI, the doctors decided to a greater extent to apply p-PCI.

Throughout the history of applying RT from prehospital fibrinolysis to PI therapy, it seems that FT has a very important position in STEMI pts, especially in the regions where PCI centers are farther ³²⁻³⁶. From 1995 to 2015, there was a decrease in applying FT (from 40% to 6%) and an increase in p-PCI (from 12% to 77%) in France ³⁵, and also a decrease in FT (from 66% to 7%) and an increase in PCI (from 12% to 61%) in SWEDHEART/RISK-HIA registry ³⁷. In Serbia, it seems that pts with a higher risk, who needed a more efficient therapy sooner, were not treated well enough,

and they received FT rather than the undeveloped PI strategy. Finances may be the reason why the network of PI therapy has not been fully applied yet.

After analyzing patients treated with FT in the STREAM study, it was determined that PI-treated pts with greater baseline risk and with more comorbidities required rescue angiography. These pts had worse 30-day outcomes compared with successful FT pts and scheduled PCI ²⁷.

In Serbia, the mortality of the pts who arrived within 3 hours was different between the groups of the pts treated with FT (8.7%) and the pts treated with pPCI (4.3%). However, after using the propensity score in the two similar groups of patients, FT vs p-PCI, concerning age ≥ 65 (35.1% vs 37.1%), gender (male 30.1% vs female 30.6%), the time from the symptom onset < 180 min (47.6% vs 47.5%), diabetes mellitus (20.6% vs 19.9%) and Killip-class heart failure > 1 (25.9% vs 21.8%), the mortality rate was higher, but not significantly in the FT group compared to the p-PCI group (FT 8.8% vs p-PCI 6.4%).

In the last years, the situation in Serbia has been better after a full opening of 7 p-PCI 24/7 day-centers. However, the percentage of pts who were not treated with RT is the same, the percentage of pts treated by FT is lower, and the percentage of pts who go to p-PCI is higher. In the last years, mortality of reperfused and non-reperfused pts has not been significantly improved ²⁷.

Limitation of the study

Not a fully applicable network of PI therapy in Serbia is a possible limitation of the present study. Furthermore, the differences between the two reperfusion groups were observed. There were more high-risk patients in the FT group, which was solved by using the propensity score.

Conclusion

The possibility for timely p-PCI and PI therapy is especially not applicable in high-risk patients, older patients, those with heart failure, and with diabetes. The unsolved problems were the reperfused pts by FT who should go to the PCI center after the therapy and especially high-risk patients. The strategy of STEMI pts must be modified and defined in the developing and transition countries without the possibility for timely p-PCI and without a network of fully applicable PI therapy. The improvement of treating these pts has two pathways: opening more p-PCI centers and, as important, using more PI therapies. Achieving the balance of the best cost-benefit strategies is difficult in remote regions and developing countries.

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Children and youth in the institutional setting – mental health characteristics of children from the Center for Protection of Infants, Children, and Youth in Belgrade

Deca i mladi u institucionalnom okruženju – karakteristike mentalnog zdravlja dece u Centru za zaštitu odojčadi, dece i omladine u Beogradu

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Abstract

Background/Aim. Unfavorable life experiences of children and youth, unmet emotional needs at an early age may lead to difficulties in emotional life and mental health or externalizing problems. The aim of the study was to determine the number of children in the Center for Protection of Infants, Children, and Youth (hereinafter Center) in Belgrade with mental health issues, their life circumstances, and the way their issues are manifesting. **Methods.** The study included all children in residence ($n = 486$) at the Center during 2015. The study was conducted in the period from February to April 2016. For the needs of the research, a questionnaire examining the children's mental health issues was developed. Both health workers and social workers participated in the research. The children's health status questionnaire was filled in from their health records, while social anamnestic data were taken from the social worker's records. The research included four group homes for children without parental care, ages between 7 and 18 years, 3 children's shelters for children from birth to 18 years, and the organizational unit infirmary (children with developmental difficulties, aged between 4 and 18 years). **Results.** Out of the total number of children placed in institutions during 2015 ($n = 486$), 96 (19.8%) children were diagnosed with mental health issues. The most frequent diagnoses were F90-F93 (behavioral and emotional disorders usually occurring in childhood and adolescence); F70-F72 (mental retardation); F30-F32.3 (mood disorder –

affective disorder). The percentage of children with mental health issues diagnosed prior to their admission was 41.7% with one diagnosis, while 21.8% had two, three, or more diagnoses. After being received to the institution, 36.5% of children and youth got their diagnosis. Prior to their arrival at the Center, 74% of children had more than one life change. The average age of children was 11 years, although there were deviations in two group homes (15 years). After the paramedic emergency intervention, 21% of children were hospitalized. Inadequate parental care was the most prominent reason for institutionalization into the organizational units of children's homes, as well as the inability of parents to exercise their parental rights (76%). Inadequate parental care was neglected in 2/3 of children, while in 1/3 of children, it was abuse. **Conclusion.** Living in an institutional setting can lead to a number of problems in child development. The results of the research unequivocally show that children and adolescents placed in the social protection system are a vulnerable population and at a significantly higher risk of developing mental disorders, considering the negative influences and the absence of positive emotional stimuli from the earliest childhood. The type of consequence due to the stay at the institution depends on the age of the child, the length of the stay, their previous family experience, and the life changes that the child had.

Key words: adolescent; child; mental disorders; mental health; psychology, social; risk assessment.

Apstrakt

Uvod/Cilj. Nepovoljna životna iskustva dece i mladih, nezadovoljene emocionalne potrebe u ranom uzrastu mogu dovesti do teškoća u emotivnom životu i mentalnom zdravlju ili do eksternalizacije problema. Cilj rada bio je da se utvrdi broj dece u Centru za zaštitu odojčadi, dece i omladine (u daljem tekstu Centar) u Beogradu sa

problemom mentalnog zdravlja, njihove životne okolnosti, kao i način na koji se problemi manifestuju. **Metode.** Ispitivanjem su obuhvaćena sva deca na smeštaju u Centru ($n = 486$), u toku 2015. godine. Istraživanje je sprovedeno u periodu od februara do aprila 2016. godine. Za potrebe istraživanja sačinjen je upitnik za ispitivanje problema mentalnog zdravlja dece. U istraživanju su učestvovali zdravstveni i socijalni radnici. Upitnik o zdravstvenom

statusu dece popunjava se podacima iz zdravstvene dokumentacije, a socioanamnestički podaci su preuzeti iz dokumentacije socijalnog radnika. Istraživanjem su obuhvaćena četiri doma za decu bez roditeljskog staranja uzrasta od 7 do 18 godina, 3 prihvatilišta za decu uzrasta od rođenja do 18 godina i Radna jedinica (RJ) Stacionar (deca sa teškoćama u razvoju), uzrasta od 4 do 18 godina.

Rezultati. Od ukupnog broja dece na smeštaju u toku 2015. godine ($n = 486$), 96 (19,8%) dece je imalo dijagnostikovane probleme mentalnog zdravlja. Najčešće dijagnoze bile su F90-F93 (poremećaji ponašanja i emocija koji se obično ispoljavaju u detinjstvu i adolescenciji); F70-F72 (mentalna retardacija) i F30-F32.3 (poremećaj raspoloženja – afektivni poremećaj). Sa već dijagnostikovanim problemima mentalnog zdravlja u ustanovu je primljeno 41,7% dece sa jednom dijagnozom, dok je 21,8% dece imalo dve, tri ili više dijagnoza; 36,5% dece i mladih dobilo je dijagnoze nakon smeštaja u ustanovu; 74% dece imalo je više od jedne životne promene do dolaska u Centar. Prosečno životno doba dece na prijemu bilo je 11 godina, iako je bilo odstupanja u dva doma za decu (15 godina). Ukupno 21%

dece bilo je hospitalizovano posle intervencije Hitne medicinske službe. Neadekvatno roditeljsko staranje bilo je najčešći razlog za smeštaj dece u RJ domske zaštite, kao i sprečenost roditelja da vrše roditeljsko pravo (76%). Neadekvatno roditeljsko staranje se kod 2/3 dece ispoljavalo kao zanemarivanje, a kod 1/3 dece kao zlostavljanje. **Zaključak.** Život u instituciji može dovesti do mnogobrojnih problema u dečjem razvoju. Rezultati istraživanja nedvosmisleno pokazuju da su deca i adolescenti smešteni u sistem socijalne zaštite vulnerabilna populacija i pod značajno većim rizikom od razvoja mentalnog poremećaja, s obzirom na činjenicu da od najranijeg detinjstva trpe negativne uticaje i nedostatak pozitivnih emotivnih stimulusa. Kako će se boravak u sistemu socijalne zaštite odraziti na dete zavisi od uzrasta deteta, dužine boravka u instituciji, prethodnog porodičnog iskustva deteta i životnih promena koje je imalo.

Ključne reči:

adolescenti; deca; psihički poremećaji; mentalno zdravlje; psihologija, socijalna; rizik, procena.

Introduction

A pediatrician Henry Kempe was the first to describe and use the term “The Battered Child Syndrome” in 1962 and provided the first epidemiologic data for this occurrence, introducing it into the clinical practice¹. Noting the increasing number of abused and neglected children and the consequences it exerts on children's health, the World Health Organization (WHO) adopted the General Definition of Violence at the 1999 Consultation on Child Abuse Prevention in Geneva². An estimated 40 million children worldwide suffer abuse and/or neglect, requiring removal from family and health care and treatment³.

Childhood abuse affects five important interrelated areas: neurological and intellectual development, school success and life expectations, socio-emotional development, social relationships and behavior, and mental health in general⁴. What is more, trauma can affect all aspects of life that are also closely linked to psychological functioning, behavioral problems, depression, and social competence.

The consequences of abuse and neglect in early childhood can occur soon after the act, can develop during the development of the child, and have long-lasting effects^{5,6}. The consequences to mental health include the onset of mental issues in childhood, adolescence, and later in adulthood. Typical symptoms of traumatism are reflected in the occurrence of somatization, anxiety, depression, hostility, aggression, posttraumatic symptoms, dissociative disorders, but also severe mental health issues, such as paranoid ideation, psychosis, self-harm, suicide, personality disorders, interpersonal dysfunction, abuse and addiction to psychoactive substances (PAS)⁷⁻¹⁰.

Abuse and neglect are the most common reasons for separating children from the family^{11, 12}. Children whose right to live in a family is most often affected by parental violence in situations where the parent faces multiple

challenges, such as poverty, social exclusion, poor living conditions, disability, physical or mental health problems, or substance abuse¹³. These families are long-term stressed, exhausted, marginalized, and socially excluded, without a supportive social network. They are characterized by a lack of impossibility to apply parental knowledge and skills in the conditions in which they live or the knowledge and skills are inadequate concerning the child's developmental needs. The parents of these children are often unable to recognize their developmental needs, do not respond to them, or do not respond in a way that is stimulating for the children¹⁴. A child expects protection and love from the adults, trusts them, and approaches their world with confidence which means that inadequate actions will affect his/her mental health¹⁵.

Research in developmental psychology shows that the absence of parental care, especially in childhood, puts children in a state of high risk on the existential, psychophysical, social, and educational level¹⁶⁻¹⁸.

Mary Ainsworth examined the way in which patterns of emotional attachment manifest. She examined the connection between the individual's style of affective attachment and the mother's parenting style in an experimental situation¹⁸. Based on the obtained results, she singled out three types of bonding patterns in children: type A – insecure-avoidant bonding, type B – secure bonding, type C – uncertain ambivalent bonding. Conditions that contribute to inadequate models of emotional attachment were examined¹⁶. Type IV, the so-called disorganized/disoriented bonding, mostly found in children who have been abused and experienced severe traumatic experiences¹⁹, was discovered later. Mary Ainsworth's research enabled other researchers to monitor the effects of adverse developmental conditions (trauma, abuse, separation/loss, relocation, and lack of stability in life) on bonding styles. According to the theory of affective bonding, a human infant is born as a social being whose

primary need is the need for an emotional connection with an adult¹⁶. Many researchers cite a reduced ability to bond and fulfill true, deeper, and lasting relationships with others as a common feature of children growing up under (unfavorable) conditions in the institution, which may result in unsuccessful socialization of the child or disorders in social behavior and taking on social roles¹⁶.

There is some evidence to suggest that institutional care, especially in the early stages of life, is detrimental to all areas of a child's development and that it predetermines intellectual²⁰, behavioral²¹, and socioemotional problems later in life²². Evidence suggests that institutionalization in children up to three years of age is very likely to affect the development of brain functions, leaving long-term consequences on a child's social and emotional behavior.

Radojevic¹⁸ states: "Some research shows that even after the first year children can form affective attachments to a certain degree". However, from the perspective of research in the field of social protection, children who are adopted or placed in long-term foster care after spending the first year of life in institutions are at increased risk of poor outcomes in the psychological sense.

The results of numerous research unequivocally show that children and adolescents placed in the social protection system are a vulnerable population and at significantly higher risk for the development of mental disorders, considering that they suffer from negative influences and the absence of positive emotional stimuli from the earliest childhood. Moreover, numerous studies indicate an increased prevalence of mental disorders in children and adolescents placed in social care institutions¹⁶.

Research shows that children who are placed in institutions have slower motor development, exhibit different cognitive deficits and unusual behaviors (repetitive and stereotypical or self-stimulating) more often²³.

Adolescents in institutions often experienced loss, neglect, or violence in their relationship with caregivers, and quite certainly separation as well, which are all situations that signal the activation of the attachment system, whereas the security base is inaccessible or represents a threat itself²⁴. The conclusions of research about institutionalized children stated that "despite the good physical care that institutionalized children receive, the lack of continuous and close emotional contact with the mother produces retardation in emotional and cognitive development, adoption of speech and habits, and that these children are more insecure"²⁵. Bowlby²⁶ was particularly interested in children undergoing long-term treatment in healthcare institutions and how separation from parents (mother) or loss of parents affect children. Working with children who have been deprived of parental care, Bowlby has theorized that young children establish a strong emotional connection and bond with the main caregiver; in case of that bond breaking, it causes great distress and suffering to the child.

Two forms of attachment disorder have also been identified, characteristic primarily of institutionalized children or children who lived without parents for a long period of time in their earliest childhood. The first disorder

describes inhibition; the second disinhibition of attachment and the accompanying nondiscriminatory sociability²³. Both occur significantly more often when it comes to very early and long separations from the attachment figure, with the former involving withdrawal, depressive appearance, rejection, and inability to establish attachment relationships, while the latter includes manifestation of excessive physical contact, verbal and social crossing of borders, unusual spontaneity and dealing with strangers as if they were figures of attachment²⁴. Why does a percentage of children who have experienced early separation develop one or the other or none of these disorders has yet to be explained, and it is suggested that there may be some biological or epigenetic causes in the background. From the perspective of psychoanalytic theory, perhaps we can think of the underdevelopment of the "capacity to be alone"²⁷, as a consequence of the experience of hurts in early childhood and the loss or failure of a mother to meet the child's needs.

At the beginning of the 21st century, there had been increasing discussions in Serbia about the consequences on the development and health of children and youth who were separated from their families. It was the reform of the social protection system that began in the first years of the last decade that had a goal to reduce the number of children in residential accommodation, *ie*, deinstitutionalization. The Social Protection Development Strategy was adopted, which represented the policy framework for all future initiatives, including activities on the reform of the childcare system. Over the last ten years, the Republic of Serbia has made great efforts to move from a model that has relied heavily on residential institutions to a model of community services for children and families. The basic principle of the reform of the social protection system is to respect the rights of children – the right to live in a family. However, during this period, much emphasis has been placed on strengthening foster families and much less attention has been paid to supporting the natural family. Only recently, the state has begun to realize that the rights of the child are best being realized through the empowerment of families and children.

Today, the protection of children in residential care faces numerous challenges. As mentioned, the transformation of residential institutions was undertaken, the number of children in residential care was reduced, but the user structure was also changed. Faced with serious issues of children and youth, Center employees collaborate with mental health professionals. Understanding the current level and prospects of further development of child protection and the challenges that lie along the way is not possible without knowing and understanding the current situation in the Center. Adverse life experiences of children and youth and unmet emotional needs at an early age can lead to difficulties in emotional life or mental health or externalization of problems. Children and young people with behavioral problems are those who are trying to overcome psychological suffering and tension through violent, destructive, and self-destructive behavior.

Methods

The research was conducted at the Center for the Protection of Infants, Children, and Youth in Belgrade, Serbia. The goal was to determine the number of children with mental health problems, the way the problems manifest themselves, as well as their life circumstances. To this end, the children's records and documentation in the institution were analyzed.

The research was conducted between February and April 2016; the sample included all documentation of children in residential care during 2015 (486 children). The study was conducted in four homes for children aged 7 to 18, these being: Residential Home for Children and Youth "Jovan Jovanović Zmaj" (54 children); Home "Drinka Pavlović" (50 children); Home "Moša Pijade" (54 children); Home for High School and University Youth (32 children). At the Shelter for Urgent Protection of Abused Children, aged 7 to 18 years, 53 children were included in the research, while within the Children's Home "Dragutin Filipović Jusa" containing the Small Home Community for children with disabilities and a receiving station for children aged 4 to 7, records of 58 children were included. Within the organizational infirmary unit, the children are in the care of the Intensive support department, 127 children from birth to 18 years old, including the receiving station for children from birth to 4 years, a total of 74 children (Table 1).

For the needs of the research, a questionnaire was developed examining the mental health issues of children. The research involved health professionals and social workers. The questionnaire on the health status of children was filled in from the health records by health workers; socio-anamnestic data were entered by the social worker/workers from the social worker records. Following the procedure, the permission to conduct a research at the Center was obtained by the Director of the Institution who was contacted by the researcher himself¹.

The International Classification of Diseases (ICD) of the WHO, 10th revision (ICD-10) was used in this study²⁸.

Results

The survey showed that out of the total number of respondents in 2015 (n = 486), 96 (19.8%) children were diagnosed with mental health issues. Table 1 shows the distribution of children within the Center by organizational units and incidence. In some organizational units, 56.3% and 39.5% of children, respectively, were diagnosed with mental health issues, while 8.6% of children aged 4 to 7 years were diagnosed with such problems.

Out of the 96 children, 57% were male and 43% were female. On admission to the Center, 63.5% of children had previously been diagnosed with mental health problems.

Most commonly diagnosed were the issues from the F92 group – a mixed disorder of behavior and emotions (21), while in 17 children and youth, the diagnoses from F90 – hyperkinetic disorder to F93 group – anxiety disorder in childhood were registered. The following most commonly reported disorders were from the F70–F79 group – mental retardation, with the most frequent incidence of F72 – severe mental retardation (8), F70 – mild mental retardation (4), F71 – moderate mental retardation (3), and F70.1 – mild mental retardation—a significant behavioral disorder that requires attention and treatment (2). F30–F39 mood disorders were registered from bipolar disorder to severe depression, as follows: F31 – affective bipolar mental illness (4), F32 – mild depressive episode (3), F30 – manic episode (2), F31.7 – affective bipolar mental illness in remission (1), F32.1 – moderate depressive episode (1), F32.3 – a severe depressive episode with symptoms of psychosis (1). Between two and six diagnoses were present in 21.8% of children, while 41.7% of children had one diagnosis.

At admission to the institution, 36.5% had not been previously diagnosed with mental health issues, but during their stay in the institution, these children and youth were

Table 1
Children with mental health difficulties (MHD) at resident care at the Center during 2015

Organizational unit (OU)	Total number of children in OU	Number of children with MHD		Total number (%) of children with MHD
		male	female	
Shelter for urgent protection of abused children	53	3	6	9 (17)
"Drinka Pavlović"	50	12	5	17 (34)
Home for High School and University students	32	10	8	
Infirmary				18 (56.3)
intensive support	127	13	9	22 (17.3)
infirmary shelter	74	2	1	3 (4.1)
"Moša Pijade"	54	3	4	7 (13)
"Jovan Jovanović Zmaj"	38	8	7	15 (39.5)
"Dragutin Filipović Jusa"	58	4	1	5 (8.6)
Total	486	55	41	96 (19.8)

¹In mid-2016, at the proposal of the Expert Council of the Center (the author of the paper is a member of the Expert Council), a Commission for giving opinions on professional training and conducting research at the Center was formed. This Commission was formed and modeled like other ethics committees in the health system.

diagnosed with disorders in the F10–F19 group (mental disorder and behavioral disorder caused by the use of PAS), which was not the case upon admission to the Center (this diagnosis had almost been missed). F30–F39 disorders were diagnosed in ten children and young adults; disorders F40–

F48 (neurotic, stressful, and body manifest disorders), F80–F89 (mental development disorder), F50–F59 (behavioral disorder syndromes associated with physiological disorders and physical factors), and F60–F69 groups (personality disorder and behavioral disorder of adults) were recorded as well. During their stay in the institution, 23.96% of children received more than one diagnosis.

During the placement in the institution, 55% of the children had psychopharmacotherapy introduced by the prescribing specialist. During 2015 (the survey took the whole year, from January to the end of December), 82 (85.4%) children used psychopharmacotherapy (Table 2). The survey showed that 65.5% of children had between two and six administered medicines, 19.79% had one psychopharmacotherapy, while 14.58% had no therapy regardless of the confirmed diagnosis. In 68% of cases, administered psychopharmacotherapy was not reduced; in 17% of cases it was adjusted (reduced number of medications); in only 10% of cases, it was discontinued. The age of children and young people who used psychopharmaceutics in 2015 ranged from 10 to 18 years in 77.9% of cases. Looking at organizational units, 41.5% were aged 10–15 and 36% were aged 15–18. This age prevailed in all organizational units. More than seven percentage (7.5%) of children who used psychopharmaceutics were aged 7–10, most of them were in Intensive Care and Infirmary, while 14.6% of young adults (who were still in school) were mostly in the work unit of Center for high school and student youth (Table 3).

Table 2
Number of children using psychopharmacotherapy in 2015 (by number of psychopharmacotherapy)

Number of medications	Number of children		Total
	male	female	
1	10	9	19
2	15	8	23
3	9	11	20
4	11	5	16
5	1	1	2
6	1	1	2
no medication	8	6	14
Total	55	41	96

Table 3
Number of children using psychopharmacotherapy in 2015 (by age and gender)

Age (years)	Number of children		Total children (%)
	male	female	
7–10	2	4	7.5
10–15	22	12	41.4
15–18	17	13	36.5
18+	6	6	14.6
Total children (%)	57.3	42.7	100

Out of the total number of children hospitalized due to mental health issues in 2015, the largest percentage had been hospitalized only once, while one child had as many as 13 hospitalizations. During 2015, 36% of children with mental health issues were hospitalized during the year

(Table 4). Emergency medical interventions addressed 34% of children and youth in the Center placement, and 21% of children remained hospitalized after emergency medical intervention. Aggressive behavior towards property, other children, and adults was exhibited in 22.9% of children and young people.

Table 4
Number of hospitalizations of children as a consequence of mental problems during 2015

Number of hospitalizations	Children (%)
1	22
2	8
3	2
4	2
5	1

Systemic protection of children, upon separation from the inadequate environment in which the children were residing, showed that 73% of children had more than one major life change before being placed in the Center (Table 5), while a history of abuse and neglect was present in 69.7% of children and youth.

Table 5
Number of children/youth experienced changes prior to admission to the Center

Number of changes	Children (%)
1	22
2	28
3	29
4	11
5	5

Problems of children and youth in the placement in their everyday functioning, related to running away from the institution, were recorded in 23% of the examined; use of physical force in solving issues with other children was also present in 23% of cases; use of PAS in 9%.

The average length of stay for these children was 4 years, while 28.12% of children were staying in an institution between 6 and 13 years. 61% of children had contact with family and other significant persons, whether regular or occasional, while 33% of children maintained no contact with family and other significant persons. Children and young people diagnosed with F72 in 62.5% of cases did not have contact while others had regular (12.5%) or occasional (12.5%) contact with other significant persons (parents, relatives, foster parents, etc.) after being placed in the institution.

Discussion

Institutions were once considered the best solution for taking care of vulnerable children, children from risk groups, and children with disabilities. However, it has been shown that the care services provided by the institutions constantly give worse results, especially if the children are placed at an early age. The institutional environment can leave numerous consequences on children's development in

the sphere of slow physical, cognitive development, speech development, intellectual development and motivation to achieve school success, depression, anxiety, behavioral disorders. Psychosomatic difficulties can be manifested to a significant extent. On the emotional level, there is a habit disorder (most often enuresis), aggression, and hyperactivity^{16, 29, 30}.

The institutional environment itself is such that it creates additional inconveniences that can accompany a person staying in an institution for the rest of his/her life. Lack of personal life, autonomy, and disrespect for a person's personal integrity can jeopardize their emotional and social development. Expressions such as "social deprivation" and "learned helplessness" were coined to describe the psychological effects of being in an institution²⁹.

The results of the research show that children in residential care have been diagnosed with mental health problems in certain Center organizational units between 39.4% and 56.2%. These units are Homes for Children Without Parental Care, ages 7 to 18, where the F70 group diagnoses were very rare. Most children with F70 diagnosis were placed in the organizational unit of the Infirmary – Intensive Support. Researches show that children in institutions for children in Serbia have a reported confirmation of some psychiatric disorder at the beginning of placement in 7.3% of cases, and 9.7% of children have a diagnosis in the last report. Among the problems reported, the hyperactive disorder (1.4%) is most commonly reported at the beginning of treatment, which is almost equally present in the last report (1.2%), while attention deficit, behavior, mood, and habit disorders, as well as autism were reported for 0.6% of children each. According to the latest report, the number of children diagnosed with a behavioral disorder has increased, and the proportion of children with a mood disorder has increased slightly¹¹.

The obtained results are in line with numerous research on the negative impact of child development institutions. Numerous studies indicate that children and adolescents placed in social care institutions are significantly more reminiscent of the clinical population with mental health problems than of the general population of the same age¹⁶. The results showed that, already during the placement in the institution, 21.8% of children had from two to six years diagnoses relating to mental health problems. Many studies show that the prevalence of psychopathology is higher in children and adolescents placed in homes for children without parental care, in contrast to children placed in foster care. The prevalence of psychopathology in foster children and adolescents ranges between 44% and 96%, while the prevalence of foster children and adolescents in foster care varies between 30% and 63%¹⁶. In several studies, it has been observed that older children and adolescents are more likely to have mental health problems, while there are no clear differences according to gender. In addition to older age, the main predictors of mental health problems are the age at which one enters the social protection system, previous mental health problems, physical health, developmental

characteristics, living conditions in institutions, access to health care, frequent changes in social protection (frequent change of guardians or caregivers)¹⁶.

The results obtained in Damjanović's¹⁶ doctoral dissertation show that "Anxiety and depressive problems were present in a higher percentage in children and adolescents in foster care – 41.4% of children and adolescents from the home showed anxiety problems, and 29.5% of children and adolescents from foster care. Nearly 60% of children and adolescents from home and 33% of children and adolescents from foster care had depressive symptoms. Concerning gender and age, female subjects aged 13–18 years had more pronounced anxiety and depressive problems. Outsourcing problems were present in a higher percentage of respondents of both sexes and ages, in dormitory accommodation; 12.6–29% of children and adolescents from the home have externalizing mental problems, and in foster care, about 3–4%; 67% of children and adolescents in foster care had at least one mental disorder, while that percentage of foster children is about 44%".

Numerous studies prove that children who grow up in a foster family achieve better results than their peers who grow up in institutions, not only in terms of physical and cognitive development but also when it comes to success in education and integration into the community as adults²⁹.

That is the reason why Serbia started the process of deinstitutionalization. Since 2001, the number of children in residential care without parental care has decreased, but the user structure has changed, and the number of children in foster families has increased (a survey for the period between 2006 and 2011 shows that 47% of children were placed in foster families), while kinship foster care was poorly developed¹¹. However, changes in the placement of children with disabilities and children in conflict with the law are slow. Specialized foster care is making progress but not to a satisfactory extent.

It may be concluded that a large number of children and youth, at the moment of admission, have a diagnosed emotional disorder originating in childhood. Externalizing the problem is a basic feature of children and youth in placement, but a group of children and young people who have been diagnosed with the bipolar affective disorder is also evident. These disorders are characterized by impulsiveness, poor wish control, violent behavior, autoaggressive behavior, self-harm, and mood swings with depressive episodes. Most of these disorders occur early in life and are related to poor school performance and results, aggressive behavior, and joining the high-risk groups.

Moreover, the disorders in group F10–F19, which were not registered at the admission of children, are recorded during their stay at the institution. One of the contraindications for admission of children is the diagnosis from the F10–F19 spectrum. These children are not admitted to the institution until they are first treated, but there is a possibility that the admission documentation does not contain their health records, which occurs in reality. After entering the institution, problems become registered and a treatment procedure is initiated.

The data show that a total of 85.4% of children have prescribed therapy in the form of two, three, and sometimes four medicines, while only 14.5% of children and adolescents do not have psychopharmacotherapy introduced. Once the therapy is introduced, it is rarely changed or reduced. In the majority of children and young people (68%), it remains the same, and only 17% of children received reductions in their prescribed therapy. This is significant information given that 85.4% of children are administered multiple-drug pharmacotherapy. In only 10% of cases, the therapy was discontinued. These data coincide with worldwide data that children in care homes are 16 times more likely to receive medication than children living in their families³¹, and that is why caution should be taken when prescribing drugs in psychiatry³². The report of the Initiative for the Mental Disability Initiative of Serbia (MDRI) Rights states: "The worrying fact is that even when it comes to children, the use of psychopharmaceuticals is very common". The excuse of this practice is very debatable if the diagnoses of children are taken into account – severe developmental disabilities, autism, behavioral disorders. In Zvečanska (Center), 20–30% of children received neuroleptics. According to the employees, "children are overwhelmed with diagnoses, controls, and medication." It is positive that the employees of this institution carefully monitor the impact of drugs on children and point out possible problems³³. However, the introduction of drugs into therapy, in addition to the targeted antipsychotic effect, reduces aggression, anger attacks, stereotypes, and hyperactivity³⁴. When it comes to the abuse of psychopharmaceuticals, there is evidence that some institutions (in the world) for children and adults with disabilities arbitrarily use psychiatric drugs to control behavior, when a psychiatric diagnosis does not exist, without regular drug review²⁹.

Emergency medical intervention is associated with urgent hospitalization of children. It can be assumed that high anxiety, with acting out behavior is the reason for hospitalization but also self-harm or suicide threats. These are mostly situations where professional practitioners contact the emergency medical service.

The fact that the children had undergone several changes before coming to the Center shows that the children are being "dragged" through the system. A large number of children have experienced changing multiple families (foster care families, relatives) until placement in a social care facility. Often, children were exposed to traumas of early separation, frequent changes and relocations, unreliable and unpredictable upbringing and raising, hospitalizations, life in institutions. These circumstances are among the risk factors that increase the possibility of developing mental health disorders³⁴.

Once children enter residential care, they become "forgotten" by the system, as evidenced by the fact that some children stay in institutions between 6 and 13 years. Most often, these children are diagnosed with F70–F72, which is confirmed by the fact that in 62.5% of cases, children with the F72 diagnosis do not have any contact with other significant persons (parents, relatives, foster parents, etc.)

after being placed in an institution. The placement of these children practically from the maternity ward, that is, the abandonment of these children by their parents, indicates the need for the development of preventive services as well as synchronized functioning of health and social institutions in the community, in order to provide timely assistance to pregnant women at risk that may result in abandonment of a child, as well as additional psychosocial and material support after childbirth or the first months of a child's life.

All organizational units included in the research within the Center provide accommodation services. The difference is whether the accommodation is short-term within the shelter (up to 6 months) or is a home accommodation that can sometimes last for years. Children in accommodation are very different in relation to age, disability, length of stay in the institution, and different family experiences, which can be a limiting factor of this research. However, it is characteristic of all children to experience abandonment, abuse, and neglect. All children have parents or relatives, and experiencing separation from family is always a traumatic experience.

Conclusion

The research shows that institutional care for children can have a number of consequences for children's development. Young children and children who have spent many years in the institution are, particularly, at risk. Children in such accommodations represent a vulnerable group, and the data from the conducted research show that 19.8% children were diagnosed with mental health problems. In 63.5% of children, these problems were diagnosed at the time of admission to the institution, and in 36.5%, during their stay in the institution. A history of abuse and neglect was present in 69.7% of children and young people, while 45% had multiple life changes before being placed in the Center (three, four, five), and 28% had two life changes.

Given the growing body of evidence, modern researchers think that institutional care means poorer outcomes for children of all ages, as well as a lower quality of life.

In order to adequately respond to the needs of children and young people in accommodation, it is necessary to reduce the number of life changes, reduce accommodation capacities, improve the quality of care, enable individualized approach as much as possible, shorten children's stay in institutions, develop short-term treatment programs together with mental health professionals and professional assistance who will help with the implementation of psychosocial support programs. Furthermore, it is necessary to strengthen the biological family in order to create conditions for the faster return of children to the family. Developing family support programs based on their strengths and needed community support is crucial.

Investing in services such as early response, family support, reintegration, and high-quality alternative care can help prevent poor outcomes, such as early school leaving, unemployment, homelessness, addiction, antisocial behavior, and crime.

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The influence of diabetes mellitus type 2 on the central corneal thickness

Uticaj dijabetesa melitusa tip 2 na centralnu debljinu rožnjače

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Abstract

Background/Aim. Complications of diabetes mellitus (DM) in the eye are the leading cause of blindness in the world. Although research on eye complications of DM is mainly focused on retinal damage, changes in the cornea are also associated with DM. Central corneal thickness (CCT) reflects the metabolic status of the cornea and is also affected by DM. Knowledge of CCT changes that occur within DM is important for accurate IOP measurement, diagnosis, and monitoring of patients with glaucoma. The aim of the study was to examine the effect of DM type 2 on the central corneal thickness. **Methods.** The study was designed as a clinical, cross-sectional, observational study. It consists of 96 patients, divided into two groups. The first group consisted of 49 patients diagnosed with DM type 2. The second group was the control group and consisted of 47 healthy subjects. The DM group was divided into subgroups depending on the status of diabetic retinopathy, the length of DM treatment, and the levels of glycosylated hemoglobin (HbA_{1C}). **Results.** A statistically significant dif-

ference in CCT was observed among DM patients and the control group. Analyzing only DM patients, the highest CCT values were observed in patients who had HbA_{1C} > 7.0%, as well as those who have treated DM for more than 15 years, with a statistically significant difference in relation to the corresponding patient subgroups ($p = 0.002$ and $p = 0.037$, respectively). No statistically significant difference was observed depending on the status of retinopathy. Intraocular pressure (IOP) was statistically significantly higher in patients with DM compared to the control group. **Conclusion.** Our research demonstrated that the status of retinopathy had no statistically significant influence on CCT. Knowing that the increase in CCT also affects the measured IOP values, this research will be useful in better understanding and control of the patients who have glaucoma in addition to DM type 2.

Key words:

cornea; corneal pachymetry; diabetes mellitus, type 2; diabetic retinopathy; intraocular pressure; risk assessment.

Apstrakt

Uvod/Cilj. Komplikacije dijabetesa melitusa (DM) u oku su vodeći uzrok slepila u svetu. Iako su istraživanja o očnim komplikacijama DM uglavnom usmerena na oštećenje mrežnjače, promene na rožnjači su takođe povezane sa DM. Centralna debljina rožnjače (*central carneal thickness* – CCT) odražava njen metabolički status, a na njega utiče i DM. Poznavanje promena CCT koje se dešavaju u sklopu DM važno je za tačno merenje intraokularnog pritiska (IOP), dijagnozu i praćenje bolesnika sa glaukomom. Cilj studije bio je ispitivanje uticaja DM tipa 2 na CCT. **Metode.** Studija je dizajnirana kao klinička, opservaciona studija preseka, a obuhvatila je 96 ispitanika podeljenih u dve grupe. Prvu grupu činilo je 49 bolesnika sa dijagnozom DM tipa 2. Dru-

ga grupa bila je kontrolna grupa i sastojala se od 47 zdravih ispitanika. Grupa obolelih od DM bila je podeljena u podgrupe u zavisnosti od statusa dijabetesne retinopatije, dužine lečenja DM i nivoa glikoziliranog hemoglobina (HbA_{1C}). **Rezultati.** Statistički značajna razlika u CCT utvrđena je između bolesnika sa DM i kontrolne grupe. Analizirajući samo bolesnike sa DM, najveće vrednosti CCT uočene su kod bolesnika sa HbA_{1C} > 7,0%, kao i kod onih koji su lečili DM duže od 15 godina, sa statistički značajnom razlikom u odnosu na odgovarajuće podgrupe bolesnika ($p = 0,002$ i $p = 0,037$, redom). Nije nađena statistički značajna razlika u zavisnosti od statusa retinopatije. Takođe, IOP je bio statistički značajno viši kod bolesnika sa DM u poređenju sa kontrolnom grupom. **Zaključak.** Naše istraživanje je pokazalo da status retinopatije nije imao statistički značajan uticaj

na CCT. Znajući da povećanje CCT utiče i na izmerene vrednosti IOP, ovo istraživanje će biti od koristi boljem shvatanju i kontroli bolesnika koji, pored DM tipa 2, imaju i glaukom.

Ključne reči:
rožnjača; rožnjača, pahimetrija; dijabetes melitus, tip 2; dijabetična retinopatija; intraokularni pritisak; rizik, procena.

Introduction

Diabetes mellitus (DM) represents a systemic disorder that affects many different tissues and organs¹. Ocular complications of DM are the leading cause of blindness worldwide. The most common complications are diabetic retinopathy, neovascular glaucoma, and diabetic cataract¹. Morphological and functional changes in corneal tissue can also occur in DM patients². These patients are at a higher risk of developing recurrent corneal erosions, neurotrophic corneal ulceration, stromal edema after intraocular surgical procedures, dry eye, slowed wound healing, and decreased corneal sensitivity²⁻⁴.

Hyperglycaemia causes chronic metabolic stress which seems to be the reason for the disturbance of corneal endothelial cells. These cells are presented as a monolayer of hexagonal cells, and they do not possess the ability of regeneration⁵. They are responsible for maintaining the dryness of the cornea by pumping the ions and water into the anterior chamber. DM decreases the activity of Na⁺-K⁺ATPase pump in corneal endothelium². By decreasing the number of corneal endothelial cells and interrupting their function, DM causes corneal edema followed by increased central corneal thickness (CCT) and eventually decreased visual acuity⁶.

Changes in CCT can cause difficulties in the measurement of the intraocular pressure (IOP). It represents the most important and treatable risk factor for glaucoma. During the Goldmann applanation tonometry, due to stronger impedance, thicker cornea expresses falsely increased IOP values⁷. Some studies revealed that the raise of 25 µm of CCT increases the IOP value for 1 mmHg⁸. IOP, alongside visual field testing, gonioscopy, and fundus examination, is the most important criterion in diagnosing and monitoring the progression of glaucoma. That fact ensures the importance of precise measurement of IOP⁹. Some investigators claimed that the duration of DM and the level of glycated hemoglobin A_{1C} (HbA_{1C}), as well as the status of diabetic retinopathy, have a huge influence on the CCT in DM patients^{3,10,11}.

The aim of this study was to investigate the influence of DM type 2 on CCT.

Methods

The study was designed as a cross-sectional, observational study. It was approved by the local Ethics Committee and carried out at the Clinic for Ophthalmology, Clinical Center of Kragujevac, Serbia. The research was performed in February 2020. It included patients of both sexes with the confirmed diagnosis of DM type 2, as well as the health participants recruited in the control group.

The inclusion criterion was confirmed DM type 2 before the research. The study exclusion criteria were: the presence of other DM types, incomplete information of the DM status, corneal pathology (edema, degeneration, dystrophy), patients with previous intraocular surgeries or trauma, cataract, dry eye, pterygium, glaucoma, myopia, uveitis. Patients on long-term anti-inflammatory therapy, those with a history of photocoagulation in the last three months, contact lens wearers, and pregnant women were also excluded from the study. According to the tenets of the Declaration of Helsinki, all patients gave their written consent at the beginning of the study. Once the consent was obtained, demographic and clinical data were collected: gender, age, duration of DM, antidiabetic therapy, associated diseases, and HbA_{1C}.

A detailed ophthalmological examination was performed in every patient: the best-corrected visual acuity (BVCA), slit-lamp biomicroscopy, IOP measurement using Goldman applanation tonometer, detailed fundus examination in maximal mydriasis, ultrasound pachymetry (Palm-Scan P2000 FastPach, Micro Medical Devices, Inc., Calabasas, CA, 91302 USA), and in indicated cases fluorescein angiography and ocular ultrasound.

The investigation included 96 participants divided into two groups. The first group involved 49 patients with diagnosed DM type 2. The second group was the control group and consisted of 47 healthy participants. According to the clinical characteristics, based on the results from the Early Treatment Diabetic Retinopathy Study (ETDRS)¹¹, patients from the DM group were further divided into three groups: group without diabetic retinopathy (DR, n = 23 patients), group with nonproliferative DR (NPDR, n = 15 patients), and group with proliferative DR (PDR, n = 11 patients).

The DM group was also divided according to the duration of DM in the subgroup of patients who have treated DM for more than 15 years (19 patients) and the subgroup of patients who have treated DM less than 15 years (30 patients).

Finally, depending on the level of HbA_{1C} diabetic patients were divided into the subgroup of patients with HbA_{1C} < 7.0% (25 patients) and the subgroup of patients with HbA_{1C} with > 7.0% (24 patients).

A complete ophthalmological examination was conducted. We calculated CCT as the mean value of the three consecutive measurements.

IBM SPSS version 22.0 was used for the statistical analysis. According to the normality of distribution, the paired *t*-test, χ^2 , Person's test, Mann-Whitney test were performed in analyzing the association between the values of continuous variables (CCT, patients' demographic characteristics). In analyzing statistical differences of CCT between the more than two subgroups, we used ANOVA. The Pear-

son's correlation test was used to calculate the relationship between continuous variables. The results were shown as mean \pm standard deviation (SD). Values $p < 0.05$ and $p < 0.001$ were considered statistically significant.

Results

The DM group consisted of 25 female and 24 male patients. The control group included 47 healthy participants, 26 females and 21 males. No statistically significant difference was recorded between groups in sex distribution ($p = 0.668$). The mean age in the control group was 58.5 ± 11.6 years (range 47–70 years), while it was 57.7 ± 11.6 years (range 38–72 years) in the DM group. No statistically significant difference was noticed between these two main groups ($p = 0.701$).

However, a quite different age distribution was presented between DM subgroups (Table 1). According to the status of DM, a statistically significant difference in age distribution was recorded between patients without DR compared to those in the NPDR subgroup ($p = 0.041$) and to patients who had PDR ($p = 0.038$). No statistical significance was seen between those with NPDR and PDR ($p = 0.067$). High statistical significance was noticed among participants who were treating DM for more than 15 years, compared to those who treated it for less than 15 years ($p = 0.027$). Depending on the level of HbA_{1c} statistical significance was not found in patients' age ($p = 0.058$).

Table 1

Age distribution in diabetic subgroups	
Patients	Mean age \pm SD (years)
Without DR	46.9 ± 3.1
NPDR	54.4 ± 6.4
PDR	56.5 ± 4.2
DM > 15 years	56.4 ± 9.1
DM < 15 years	48.1 ± 4.6
HbA _{1c} < 7.0%	47.8 ± 4.4
HbA _{1c} > 7.0%	50.2 ± 5.1

SD – standard deviation; DR – diabetic retinopathy; DM – diabetes mellitus; HbA_{1c} – glycated hemoglobin A_{1c}; DR – diabetic retinopathy; NPDR – non-proliferative DR; PDR – proliferative DR.

CCT was carefully measured in every participant. The DM group had CCT of $558 \pm 27 \mu\text{m}$, while it was $523 \pm 14 \mu\text{m}$ in the control group. High statistically significant difference was noticed between groups ($p = 0.013$). Examining CCT in DM patients and comparing them to each other, the differences between the DM subgroups were revealed. Depending on the status of DR, the highest CCT value was measured in patients with PDR ($553 \pm 13 \mu\text{m}$). Patients with NPDR had CCT $550 \pm 12 \mu\text{m}$, while those without DR had CCT $544 \pm 12 \mu\text{m}$. However, the difference among these subgroups was insignificant. Patients treating DM for more than 15 years had obviously thicker cornea ($568 \pm 18 \mu\text{m}$) compared to those who have treated DM less than 15 years ($551 \pm 12 \mu\text{m}$). This difference was statistically significant ($p = 0.037$). In patients with HbA_{1c} > 7.0%, the highest CCT values was found ($574 \pm 15 \mu\text{m}$), while the patients with HbA_{1c} < 7.0% had CCT $548 \pm 13 \mu\text{m}$. The difference between these subgroups was highly statistically significant ($p = 0.002$).

Analyzing all participants, the highest CCT value was measured in 5 patients who had HbA_{1c} > 7.0% and had treated DM for more the 15 years. Their mean CTT was $578 \pm 17 \mu\text{m}$.

IOP was significantly higher in the DM group ($16.2 \pm 2.1 \text{ mmHg}$) in comparison with the control group ($12.4 \pm 2.3 \text{ mmHg}$) ($p = 0.034$). Analyzing only DM patients, subgroups with DM duration > 15 years and HbA_{1c} > 7.0% had the highest IOP values (Figure 1). Results in these patients were significantly higher compared to the adequate subgroups ($p < 0.001$). We calculated a moderately positive correlation between IOP and CCT values in patients treating DM for more than 15 years ($r = 0.34$), and strongly positive correlation in patients with HbA_{1c} > 7.0% ($r = 0.57$).

Discussion

The average CCT in the healthy population is about $540 \pm 30 \mu\text{m}$ ⁸. CCT represents the metabolic status of the cornea as well. Due to that fact, CCT is prone to change in various metabolic disorders, such as DM. Many previous studies indicated that CCT is higher in patients with DM^{12, 13}. These results are in a correlation with the results collected from our

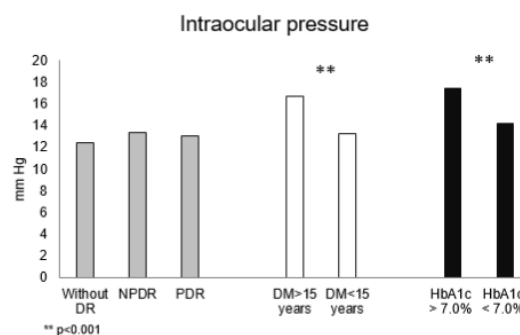


Fig. 1 – Mean intraocular pressure (IOP) in diabetes mellitus (DM) subgroups – group without diabetic retinopathy (DR), group with non-proliferative DR (NPDR), and group with proliferative DR (PDR). HbA_{1c} – glycated hemoglobin A_{1c}.

study. We noticed a statistically significant difference in CCT between the control and the DM group. It could be concluded that the changes in corneal tissue were directly associated with DM.

Some authors believe that increased values of CCT are among the first changes that occur in people with DM^{5,6}. A possible pathophysiological mechanism is that hyperglycemia leads to endothelial pump dysfunction, which leads to stromal hydration and increased CCT values¹⁴. Experimental studies have shown that DM reduces Na⁺-K⁺ATPase activity in the corneal endothelium. One of the proofs of interrupted endothelial pump function is the decreased ATP production¹⁵. That is caused due to the slowed Krebs cycle, which is present in diabetic cornea¹¹. Another postulated mechanism is that hyperglycemia increases the level of aldose reductase, an enzyme involved in sorbitol synthesis. That activates the sorbitol pathway and increases its level in the cornea. Sorbitol acts as an osmotic agent and causes stromal hydration¹⁶. By increasing the corneal permeability, these mechanisms lead to morphological changes, such as decreased endothelial cell density, decreased percentage of the hexagonal cell below 50%, and increased coefficient of variation of cell area^{17,18}. That is subsequently followed by increased CCT.

Earlier studies showed quite different CCT values among DM patients depending on glycemic control, presence of retinopathy, or duration of disease^{3,10}. We found no statistically significant difference among patients with PDR, NPDR, and patients without DR. That finding was in accordance with the results of Canan et al.¹⁶. However, we examined statistically significantly thicker cornea in patients treating DM for more than 15 years. It could be concluded that mechanisms mentioned to be responsible for swelling the cornea in DM patients had an accumulative effect during the years. Regarding the HbA_{1C} level, our research found a correlation with elevated CCT. These results were quite similar to the investigation of Yazgan et al.¹⁹. Opposite of these findings, Scheler et al.²⁰ have not reported a significant dif-

ference comparing the healthy population and DM patients with good metabolic control, with DM patients who had HbA_{1C} > 7%.

IOP stands as the most important risk factor in glaucoma development. Unlike age, gender, race, refractive error, IOP is a risk factor that can be corrected. Prescribing adequate antiglaucoma therapy, the progression of glaucoma can be stopped or at least delayed⁹. Therefore, accurate IOP measurement is extremely important in diagnosing and treating glaucoma. Changes in CCT in DM patients can affect IOP values. Many earlier studies advocated that IOP values were higher in DM patients compared to the healthy population^{3,7,14}. Our results were similar to these findings. Highly statistically significant difference between the healthy participants and the DM patients was noticed. The highest IOP values we recorded were in patients with PDR and DM > 15 years and patients who had HbA_{1C} > 7%.

Conclusion

Previous research mostly investigated the influence of DM on the posterior eye segment. Our research throws light on the complication that DM can produce in the cornea. Regarding that the increase of CCT also affects the measured values of IOP, this study could be useful in better understanding and better control of patients with DM type 2 who also have glaucoma. Our results indicated that the strongest effect on CCT and subsequently IOP had patients with poor glycemic control, as well as those who have treated DM for more than 15 years. We can assume that the presence of a positive correlation between IOP and CCT values in these two DM subgroups points out that some other, still unknown mechanisms, might have an influence on IOP and CCT in these patients. That hypothesis will be better understood when some future investigations are done. In addition, our research demonstrated that the status of retinopathy had no statistically significant influence on the central corneal thickness.

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Graft and patient survival after renal transplantation in the period from 1996 to 2017 at the Military Medical Academy, Belgrade, Serbia

Preživljavanje graftova i bolesnika sa transplantiranim bubregom u periodu od 1996. do 2017. godine na Vojnomedicinskoj akademiji, Beograd, Srbija

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Abstract

Background/Aim. Renal transplantation is the best and preferred way of treating patients with end-stage renal disease, as it offers improved survival and better quality of life compared to dialysis. The aim of this study was to present single-center (Military Medical Academy in Belgrade, Serbia) results of the kidney allograft and patient survival from 1996 to 2017. **Methods.** A retrospective 22-year cohort study was conducted. Variables of interest were graft and patient survival in kidney transplanted patients. Age, gender, serum creatinine levels, and induction therapy after transplantation were analyzed in this group of patients as well. **Results.** Among 386 transplanted patients, 316 had a living donor and 70 patients had a deceased donor. Pre-emptive renal transplantation was done in 29 (7.5%) patients and ABO-incompatible kidney transplantation in 21 (5.4%) patients. One-year, 5-year, 10-year, and 20-year overall patient survival after kidney transplantation in the observed group was 97.7%, 95.3%, 93.8%, and 91.7%, respectively. One-year, 5-year, 10-year, and 20-year graft survival in our patients were 93.8%, 85.5%, 78.5%, and 73.3%, respectively. **Conclusion.** The outcome of graft and patient survival in the Belgrade Military Medical Academy kidney transplantation program is good and in line with the leading world medical centers.

Key words:

age factors; creatinine; allografts; graft, survival; kidney transplantation; sex factor.

Apstrakt

Uvod/Cilj. Transplantacija bubrega je najbolji i poželjan način lečenja bolesnika sa završnim stadijumom bubrežne bolesti, s obzirom na to da omogućava bolje preživljavanje i kvalitet života u poređenju sa dijalizom. Cilj rada bio je da se prikažu rezultati jednog centra (Vojnomedicinska akademija u Beogradu, Srbija) u preživljavanju bolesnika i alografta posle transplantacije bubrega tokom perioda od 1996. do 2017. godine. **Metode.** Sprovedena je retrospektivna kohortna studija. Parametri od interesa bili su preživljavanje alografta i bolesnika sa transplantiranim bubregom, a analizirani su i starost, pol, nivoi kreatinina u serumu i indukciona terapija posle transplantacije. **Rezultati.** Od ukupno 386 bolesnika sa transplantiranim bubregom, kod 316 bolesnika je urađena transplantacija od živog donora, dok je kod 70 bolesnika urađena transplantacija bubrega od kadaveričnog donora. Predijalizna transplantacija bubrega je urađena kod 29 (7,5%) bolesnika, dok je transplantacija bubrega od donora sa nepodudarnom krvnom grupom urađena kod 21 (5,4%) bolesnika. Jednogodišnje, petogodišnje, desetogodišnje i dvadesetogodišnje preživljavanje bolesnika posle transplantacije bubrega u ispitivanoj grupi bilo je 97,7%, 95,3%, 93,8% i 91,7%, redom, dok je preživljavanje grafta u istim periodima iznosilo 93,8%, 85,5%, 78,5% i 73,3%, redom. **Zaključak.** Rezultati preživljavanja grafta i bolesnika u programu transplantacije bubrega na Vojnomedicinskoj akademiji su dobri i u skladu su sa onima u vodećim svetskim medicinskim centrima.

Ključne reči:

životno doba, faktor; kreatinin; alograft; graft, preživljavanje; transplantacija bubrega; pol, faktor.

Introduction

Chronic kidney disease is an important health problem worldwide since it is associated with an increased risk of morbidity and mortality in this large population group¹. Renal transplantation is the best and preferred way of treating patients with end-stage renal disease, as it offers improved survival and better quality of life in comparison to dialysis^{2,3}.

In most transplantation centers, one-year kidney graft survival in transplant patients with living-donor and deceased-donor is between 90%–98%⁴. However, despite such good short-term results, the results of long-term graft survival are still unsatisfactory and have not been improved sufficiently over the last 20 years⁵. Data shows that hazard rates of graft failure at 10 years after transplantation is 64%, and terminal graft dysfunction is, by frequency, one of the 5 most common reasons for starting a chronic dialysis program in countries in which a large number of kidney transplants have been performed in the past period^{6,7}. This fact represents a major health, social and economic problem. Factors that affect graft survival are numerous and can be divided into immunological and non-immunological ones⁸. Furthermore, results of graft and patient survival can also vary among individual regions due to the difference in certain patients and health care system characteristics, which may be important for the outcome of the transplantation.

The aim of this study was to show single-center results of the kidney allograft and patient survival during the period from 1996 to 2017.

Methods

This retrospective, 22-year cohort study was conducted from 1996 (when first kidney transplantation was performed at the Military Medical Academy) till 2017. The study was performed at the Clinic for Nephrology and the Center for Solid Organ Transplantation at the Military Medical Academy, Belgrade, Serbia. All transplant patients who were regularly controlled in our Clinic were included in this study.

Variables of interest were graft and patient survival in kidney transplanted patients. Age, gender, serum creatinine levels, and induction therapy after transplantation were recorded in this group of patients as well.

Although it changed over time, standard immunosuppressive protocol after kidney transplantation in our hospital included steroids (according to hospital practice), azathioprine until 1998, later replaced with mycophenolate (mofetil and myfortic acid), and cyclosporine or tacrolimus (with C0 and C2 therapeutic monitoring for cyclosporine and C0 monitoring for tacrolimus). The mTOR inhibitors were administered sporadically, initially as a replacement for calcineurin inhibitors (this practice was later stopped), in the cases of tumor formation after transplantation and, in recent years, in reduced doses with low doses of tacrolimus in some patients. For removal of ABO isoagglutinins from the blood in ABO-incompatible kidney allograft recipients, an original method

was performed^{9–11}. During the last 10 years, patients were usually discarded from the hospital after kidney transplantation with steroids, mycophenolate, and tacrolimus. In patients who are considered to have a higher immunological risk, after cadaveric transplantation and in the cases of delayed graft function, induction therapy was applied in the form of anti-tymocyte globulin or interleukin (IL)-2 antagonist.

Complete statistical analysis was done with the statistical software package PASW Statistics 18. Attribute variables were presented as the frequency of certain categories, while the statistical significance of differences was tested with the χ^2 test. Numerical variables were presented as mean with standard deviation, while the statistical significance of differences was tested with the Mann-Whitney test or Independent samples *t*-test (normal or not normal distribution). All the analyses were estimated at a $p < 0.05$ level of statistical significance. Unadjusted graft and patient survivals were calculated using Kaplan-Meier plots and *p*-values derived from the univariate Log-rank test.

Principles of the International Conference of Harmonization (ICH) Good Clinical Practice were strictly followed, and Ethical approval No. 01/31-01-13 from the Ethics Committee of the Military Medical Academy in Belgrade was obtained for the study protocol No. 910-1.

Results

During the observed period, a total of 445 kidney transplantations were done at the Military Medical Academy in Belgrade. However, analysis in this study included 386 kidney transplant patients since patients who were transplanted but not regularly controlled in our hospital were not analyzed [59 (13.7%) patients]. Among 386 transplanted patients, 316 (81.9%) patients had a living donor and 70 (18.1%) patients had a deceased donor. Preemptive renal transplantation was done in 29 (7.5%) patients and ABO-incompatible kidney transplantation in 21 (5.4%) patients.

Out of all transplanted patients, 32.6% were females and 67.4% were males. The average age in all patients was 44.65 ± 10.46 . The average age in male patients was 43.96 ± 10.12 and in female patients 45.35 ± 10.80 (Mann-Whitney test; $p = 0.604$).

Induction therapy was performed in 98.5% transplantations in deceased-donor group and in 41.1% in living-donor group (χ^2 test; $p < 0.001$).

The 1-year, 5-year, 10-year, and 20-year overall patient survival after kidney transplantation in the observed group were 97.7%, 95.3%, 93.8%, and 91.7%, respectively (Figure 1). Survival was better in the living-donor in comparison to the deceased-donor transplant recipients: 98.7%, 96.2%, and 94.3% in the living-donor group vs 92.9%, 91.4%, and 91.4% in the deceased-donor group, respectively (Figure 2). However, 20-year overall patient survival in the living-donor group was better for only 3.8% compared to the deceased-donor group (living-donor transplant recipients 92.4% vs deceased-donor transplant recipients 88.6%; Log Rank test, $p = 0.090$).

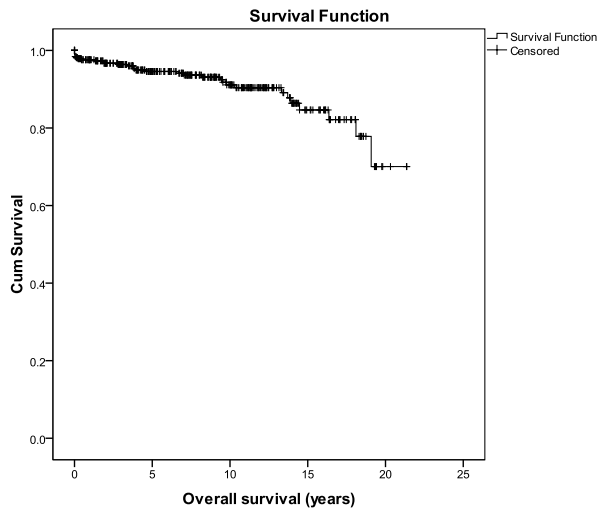


Fig. 1 – Overall survival of the patients with renal transplantation.

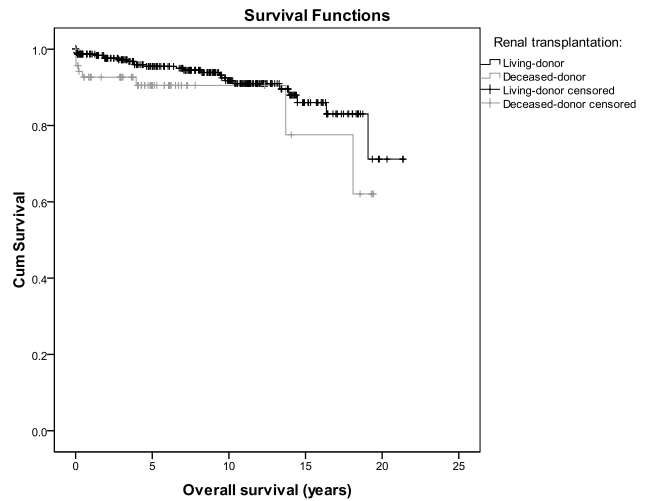


Fig. 2 – Overall survival of the patients with renal transplantation according to living-donor or deceased-donor graft.

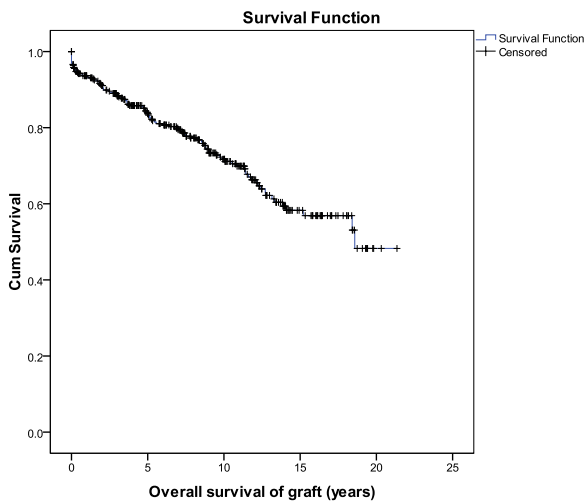


Fig. 3 – Overall survival of graft in the patients with renal transplantation.

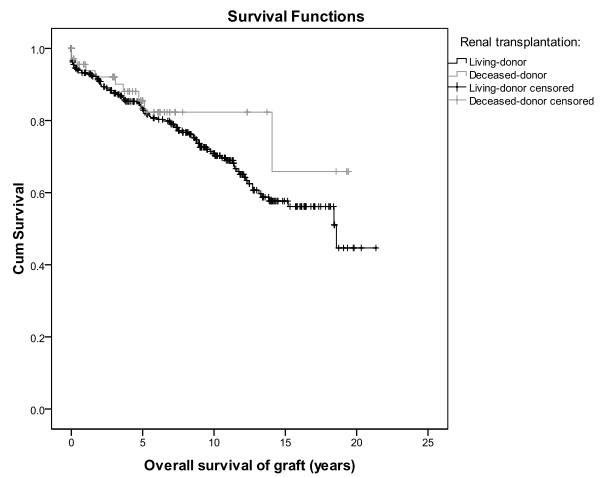


Fig. 4 – Overall survival of graft in the patients with renal transplantation according to living-donor or deceased-donor graft.

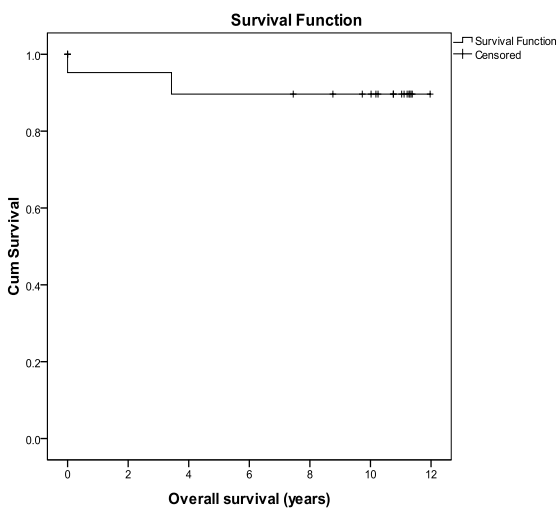


Fig. 5 – Overall survival of the patients with renal transplantation who did not an AB0-compatible donor.

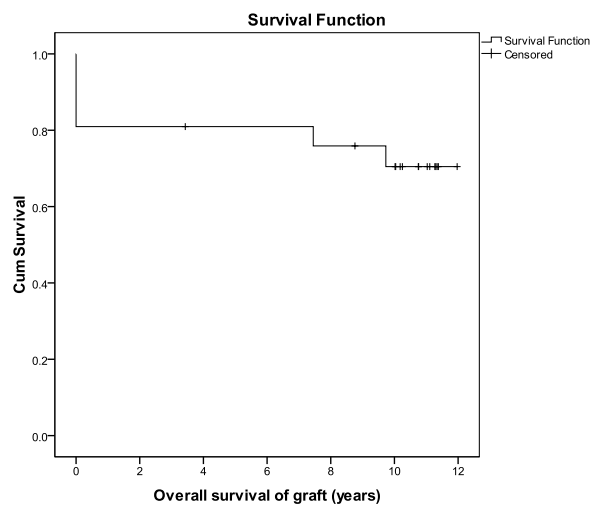


Fig. 6 – Overall survival of graft in the patients with renal transplantation who did not an AB0-compatible donor.

The 1-year, 5-year, 10-year, and 20-year graft survival in our patients were 93.8%, 85.5%, 78.5%, and 73.3%, respectively (Figure 3). The 1-year, 5-year, 10-year, and 20-year graft survival in the patients with living-donor renal transplantation were 93.3%, 84.8%, 76.6%, and 70.6%, respectively, and 94.3%, 88.6%, 87.1%, and 85.7% in the group of deceased-donor renal transplantation, respectively (Log Rank test, $p = 0.295$) (Figure 4).

The 1-year, 5-year, and 10-year patient survival in ABO-incompatible kidney transplant recipients were 100.0%, 90.5%, and 90.5%, respectively (Figure 5). The 1-year, 5-year, and 10-year graft survival in these patients were 80.9%, 76.2%, and 71.4%, respectively (Figure 6).

In the group of preemptive kidney transplant recipients, 1-year, 5-year, and 10-year patient survivals were 100.0%, 100.0%, and 96.5%, respectively (Figure 7), and the 1-year, 5-year, and 10-year graft survival in these patients were 100.0%, 86.2%, and 75.9%, respectively (Figure 8).

Average serum creatinine level during the last follow-up examination in the living-donor group was $130.63 \pm 63.73 \mu\text{mol/L}$, while in the deceased-donor group was 140.08 ± 60.22 (Independent samples t -test; $p = 0.333$).

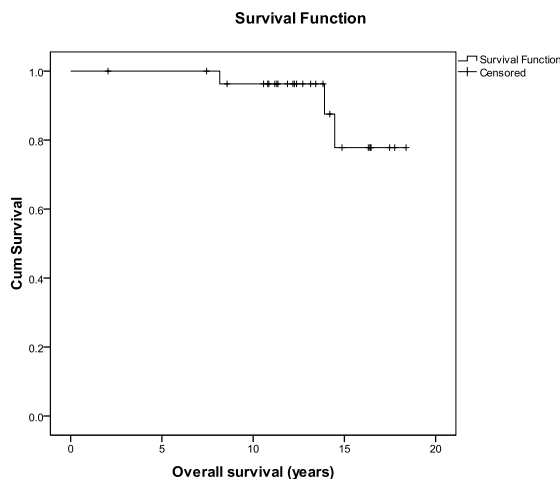


Fig. 7 – Overall survival of the patients with pre-emptive renal transplantation.

Discussion

In our study, graft loss among living-donor and deceased-donor groups showed no significant difference. In our study, the 1-year, 5-year, 10-year, and 20-year overall patient survival after kidney transplantation in the observed group were 97.7%, 95.3%, 93.8%, and 91.7%, respectively. On the other hand, the 1-year, 5-year, 10-year, and 20-year graft survival in our patients were 93.8%, 85.5%, 78.5%, and 73.3%, respectively. In Europe, for deceased-donor renal transplant, overall 1-year graft survival was 90.6%, compared with three American populations: 91.5% for Hispanic Americans, 91.1% for white Americans, and 88.7% for African Americans¹². The 5-year graft survival was 77.0%, 72.9%, 71.3%, and 62.5%, respectively, and the 10-year graft survival was 56.5%, 48.2%, 45.7%, and 33.7%, respec-

tively¹². For example, in a Columbian study, in 164 patients with renal transplantation, patient survival at 5 years was 92.1%, but graft survival at 5 years was 88.4%¹. The 20-year patient survival rate was 37%, but graft survival rate was 13%¹³. Wang et al.⁴ showed that 5-year patient survivals with deceased-donor in the US, Australia and New Zealand, and Europe was 86.1%, 90%, and 87.1%, respectively⁴. Five-year allograft survival with deceased-donor in the US, Australia and New Zealand, and Europe was 72.4%, 81%, and 77.8%, respectively⁴. On the other hand, 5-year patient survival with living-donor in the US, Australia and New Zealand, and Europe was 93.1%, 95%, and 94.3%, respectively, while five-year allograft survival with living-donor in the US, Australia and New Zealand, and Europe was 84.6%, 90%, and 86.9%, respectively⁴.

Results of our study showed that the short and long-term grafts and patient survivals in our patients were comparable or even better in regard to the results in reputable centers worldwide. These results are particularly interesting because they include the beginnings of the kidney transplant program in our hospital¹⁴. Important reasons for this are doubtless, skilled surgical techniques, reliable tissue typing,

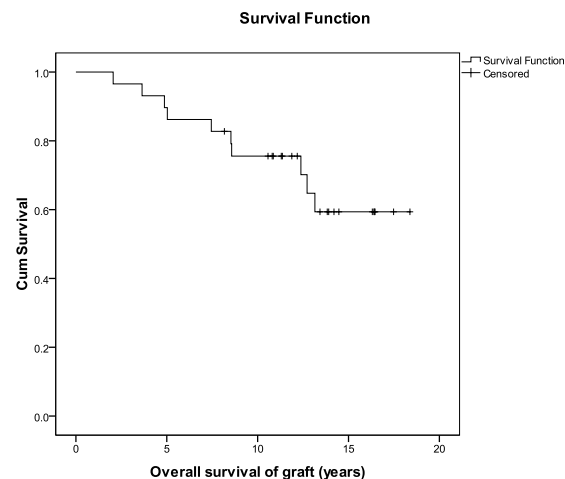


Fig. 8 – Overall survival of graft in the patients with pre-emptive renal transplantation.

careful patient care, frequent controls, and contemporary immunosuppressive therapy^{15, 16}. However, certain demographic characteristics that may be somewhat specific and which may affect the results of transplantation should certainly be mentioned as well. First of all, it can be concluded that patients in this study represent a rather typical sample of transplanted patients in our region: they are Caucasians (in our center, there were no African-American patients) and are mostly of younger-age (patients at the time of transplantation were 44.5 years in average). Our patients were younger compared to patients in some other areas¹⁷ and the average age of transplanted patients corresponded to those patients in other centers in Serbia¹⁸, as well as patients in our earlier study¹⁹. There were more men in our group, which is also in line with our previous research¹⁹ but also with the experiences of other authors^{4-6, 17, 18}. Epidemiological data shows

that chronic renal failure and uremia occur more frequently in males^{8, 20}. No statistically significant difference concerning age was found in comparing men and women ($p = 0.604$).

When analyzing our patients from the immune aspect, it can be concluded that their immunological risks were not high: the majority of transplants were made from live donors. Kidney transplantation from living donors certainly has its advantages – one of the most important is significantly shorter cold ischemia time and, consequently, lower incidence of delayed graft function and acute rejection, which can result in better long-term graft survival²¹. In some patients, kidney transplantations were performed preemptively, which can be associated with better graft survival^{21–23}. However, in our patients there was nevertheless a certain immunological risk: they were relatively young, and it is well-known that these patients react immunologically stronger to the transplanted organ²⁴. Furthermore, in the majority of patients in Serbia, the cause of terminal renal insufficiency was immunological (chronic glomerulonephritis)²⁵. Moreover, it should be noted that the shortage of organs and a higher number of transplantations from living donors results in the acceptance of the so-called “border” living donors, *ie*, older donors with a greater number of comorbid conditions²⁶. However, in relation to kidney transplanted patients in some other regions where African-American ethnicity patients are predominant and where a significant number of patients are retransplanted and/or sensitized²⁷, we can conclude that in our patients, immunological risk was still moderate or lower. Less frequent application of induction therapy in living kidney transplantation programs in our patients indirectly confirms this.

Globally, more than 30% of patients awaiting renal transplant do not have an ABO-compatible donor in the family and, in circumstances when there is not enough kidney transplantation from a cadaveric donor, realistic options for such patients are paired donor exchange and ABO-incompatible (ABOi) kidney transplantation²⁸. In the data from 2001 to 2010 in the ABOi kidney transplantation group in Japan, patient and graft survival rates for the 1,427 analyzed patients were an excellent 98% and 96%, respectively, for the first year, and 91% and 83% after 9 years, respectively²⁹. In our study, the 1-year and 10-year survival of patients with ABOi kidney transplant and allograft survival were 100.0% and 80.9%, and 90.5% and 71.4%, respectively. According to the conclusions from several transplant centers, this therapeutic option is acceptable for treating patients with

end-stage renal disease³⁰, although it has been shown that these patients receive higher doses of immunosuppressive therapy, which puts them at increased risk not only of early but also of delayed complications²⁹.

Preemptive kidney transplantation is considered the best available form of renal replacement therapy³¹. This option is associated with improved patient and graft survival, a better quality of life, and lower long-term medical costs compared with transplantation after dialysis initiation. In a systematic literature review, it was shown that patient survival, graft survival, and acute rejection rate were better in preemptive versus transplantation after the start of dialysis³¹. In an Australian study³², the 5-year survival in the preemptive kidney transplantation group was 97% and 10-year survival was 93%, similar to our data. Therefore, preemptive transplantation should be the preferred modality of renal replacement therapy in patients who have a living donor³².

Conclusion

The outcomes of graft and patient survival in the Military Medical Academy kidney transplantation program are good and in line with the most eminent world centers. Further studies are needed in order to clarify in more detail the influence of different factors on graft and patient survival in our patients.

Acknowledgment

We owe special gratitude to our teachers, former heads of clinics (for nephrology, vascular surgery, urology, neurology, anesthesiology, infectious diseases, pulmonology, endocrinology), centres (for clinical pharmacology), institutes (of radiology, medical research, microbiology), Pharmacy Department as well as other branches and services in the Military Medical Academy in Belgrade without whose active participation and help such complex and multidisciplinary field as kidney transplantation would not be possible. In addition, we feel the greatest gratitude to a great number of our colleagues, medical technicians, and other medical staff who participated in the transplant program with great desire and dedication from the very beginning to the present. All of them had a pioneering vision of the importance of kidney transplantation and have enabled these results with their commitment, faith, knowledge, and care for each patient. The possibility of safe and routine performance of the kidney transplantations in our hospital presently stands on their shoulders.

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Is there a difference in the phthalate exposure between adults with metabolic disorders and healthy ones?

Da li postoji razlika u izloženosti ftalatima između odraslih osoba sa metaboličkim poremećajima i zdravih osoba?

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Abstract

Background/Aim. Phthalates are recognized as endocrine-disrupting compounds and are extensively present in a variety of everyday products. Chronic exposure to phthalates is suspected to be associated with a range of health disorders. The aim of the study was to examine the abundance of phthalate metabolites in the urine samples among adults in the Autonomous Province of Vojvodina, Serbia, and to determine the prevalence of phthalate metabolites in healthy individuals and those with metabolic disorders such as obesity and newly diagnosed type 2 diabetes mellitus (T2DM). **Methods.** For the study purpose, the first morning urine sample of 308 participants was screened for the presence of 10 phthalate metabolites: mono-ethyl phthalate (MEP), mono-(2-ethylhexyl) phthalate (MEHP), mono-n-butyl phthalate (MBP), mono-iso-allyl phthalate (MiAP), mono-n-allyl phthalate (MnAP), mono-cyclohexyl phthalate (MCHP), mono-benzyl phthalate (MBzP), mono-n-octyl phthalate (MOP), mono-n-propyl phthalate (MPP) and mono-methyl phthalate (MMP). **Results.** At least one

phthalate metabolite was detected in the first morning urine sample in 50.32% of the examined population. The most frequently detected phthalate metabolites were MEP and MEHP. Out of all phthalate-positive participants, 38.3% of them had one, 10.7% had two, while 1.3% of participants had three phthalate metabolites in the first morning urine sample. A significant difference ($p < 0.05$) between groups was observed on MEP and MMP frequency, while borderline significant difference ($p < 0.1$) between groups was observed on MEHP and MCHP frequency. **Conclusion.** In the Vojvodina region, both healthy adults and those with metabolic disorders such as obesity and newly diagnosed T2DM are predominantly exposed to di-ethyl phthalate and di-(2-ethylhexyl)phthalate since MEP and MEHP were the most frequently detected phthalate metabolites. Further research is required in order to provide more details of the phthalates influence on the adverse health effects.

Key words: endocrine system diseases; phthalic acid; monoethyl phthalate; risk assessment; serbia.

Apstrakt

Uvod/Cilj. Ftalati predstavljaju grupu jedinjenja za koje je poznato da imaju štetan uticaj na endokrini sistem, a nalaze se u različitim potrošačkim proizvodima. Hronična ekspozicija ftalatima dovodi se u vezu sa nastankom mnogobrojnih oboljenja. Cilj rada bio je da se utvrdi zastupljenost ftalatnih metabolita u uzorcima urina odraslih osoba u Vojvodini, Srbija, kao i da se utvrdi prevalenca ftalatnih metabolita kod zdravih ispitanika i onih sa metaboličkim poremećajima kao što su gojaznost i novootkriveni dijabetes melitus tip 2 (T2DM). **Metode.** Za

ispitivanje je korišćen prvi jutarnji uzorak urina 308 ispitanika koji je analiziran na prisustvo 10 ftalatnih metabolita: mono-etil ftalata (MEP), mono-2-etilheksil ftalata (MEHP), mono-n-butil ftalata (MBP), mono-izo-amil-ftalata (MiAP), mono-n-amil ftalata (MnAP), mono-cikloheksil ftalata (MCHP), mono-benzil ftalata (MBzP), mono-n-oktil ftalata (MOP), mono-n-propil ftalata (MPP) i mono-metil ftalata (MMP). **Rezultati.** Kod 50,32% ispitivane populacije u uzorku urina detektovan je najmanje jedan ftalatni metabolit. Najzastupljeniji ftalatni metaboliti bili su MEP i MEHP. Među ispitanicima pozitivnim na prisustvo ftalatnih metabolita, 38,3% ispitanika imalo je detektovan jedan,

10,7% imalo je dva, a 1,3% ispitanika imalo je prisutna 3 ftalatna metabolita u uzorku jutarnjeg urina. Utvrđeno je postojanje značajne razlike ($p < 0,05$) između grupa u prisustvu MEP i MMP ftalatnih metabolita, kao i granične značajnosti ($p < 0,1$) između grupa u prisustvu MEHP i MHCP ftalatnih metabolita. **Zaključak.** U populaciji Vojvodine, i zdrave osobe i one sa metaboličkim poremećajima, kao što su gojaznost i novootkriveni T2DM, domi-

natno su izložene di-etil ftalatu i di-2-etilheksil ftalatu, s obzirom na to da su najzastupljeniji ftalatni metaboliti bili MEP i MEHP. Neophodna su dalja istraživanja koja će omogućiti bolji uvid u štetan uticaj ftalata na zdravlje.

Ključne reči:
endokrini sistem, bolesti; ftalna kiselina; monoetil ftalat; rizik, procena; srbija.

Introduction

Phthalates represent a large group of omnipresent industrial chemicals, ordinarily used as plasticizers, and can make up to 40%–50% of the polyvinyl chloride plastic product's weight. They are known to act as endocrine-disrupting chemicals (EDCs) ¹. Phthalates can be found in food packaging, furniture, toys, and many other household products, but also in medical devices, such as tubing and intravenous bags. Phthalates are also popular in the cosmetic industry. Since they are not covalently bound to the plastic, phthalates can leach and transfer to the air, food, and water, and thus become inhaled, ingested, or absorbed through the skin ². After being absorbed in the circulation, phthalates are metabolized in two phases: hydrolysis (monoester phthalates are produced) and conjugation. Phthalates are mainly excreted through urine, but they can also be detected in various fluids like blood (serum and plasma), breast milk, saliva, feces, etc. ³.

Monoester phthalates have a fairly short half-life in humans. Despite this fact, numerous scientific evidence implies that phthalate diesters and monoesters can lead to health disorders such as mental retardation, body composition problems, as well as endocrine, pulmonary, and cardiovascular diseases ⁴.

Phthalates can activate different pathways, but nuclear receptors (NR) are recognized as a primary target ⁵. Acting as a partial or complete agonist or antagonist, phthalates can alter NR signaling involved in the regulation of the metabolism and energy homeostasis. The main NRs targeted by phthalates are peroxisome proliferator-activated receptors (PPAR α , γ), androgen receptors, thyroid hormone receptors (TR α , β), pregnane X receptor (PXR), estrogen receptors (ER α , β), and estrogen-related receptors ^{6,7}.

By binding to the different components of the PPARs involved in the regulation of adipose tissue and lipid homeostasis, phthalates affect the fat distribution and alter the lipid status ^{8,9}. Moreover, through the PPAR- γ receptor component, phthalates could induce insulin resistance and impair glucose homeostasis. Besides genetic inheritance and lifestyle, chronic exposure to environmental pollutants, including chronic phthalate exposure, may attribute to the global epidemics of obesity and type 2 diabetes mellitus (T2DM) ¹⁰.

The aim of the study was to examine the presence of phthalate metabolites among adults in the Autonomous Province (AP) of Vojvodina (both healthy ones and those with metabolic disorders) in order to find the most abundant

metabolites. An additional aim was to determine the prevalence of phthalate metabolites in the control group, obese and a group of participants with newly diagnosed T2DM.

Methods

A total of 308 participants aged 18–50 years from the Vojvodina region, Serbia, were enrolled in a cross-sectional study. The participants were divided into 3 groups: 103 in the control [healthy persons with normal body mass index (BMI)], 104 in the obese (BMI > 30 kg/m²), and 101 in the group of patients with newly diagnosed T2DM (fasting plasma glucose value > 7.0 mmol/L), without medical treatment.

Participants with a history of chronic diseases such as dyslipidaemia, autoimmune disease, chronic infections, malignant disease, or those with possible or proven pregnancy or lactation were not involved in the study. Participants treated with any kind of medication that could affect the lipid status or the body composition (such as hypolipidemics, glucocorticoids, oral contraceptives, or immunosuppressive drugs) were not included in the study.

The study participants provided written informed consent, and the study protocol was approved by the Ethics Board of the Faculty of Medicine, University of Novi Sad, Serbia. All subjects who decided to withdraw their informed consent were excluded from the study.

Firstly, all participants were surveyed and asked specific questions about their medical and personal history. Afterward, anthropometric values such as weight, height, waist circumference were taken, and BMI was calculated using the following formula – weight/height² (kg/m²). Waist circumference was measured in the middle of the line joining the anterior superior iliac spine and rib arc.

The first morning urine sample of the volunteers who participated in this study was screened for the presence of 10 phthalate metabolites: mono-ethyl phthalate (MEP), mono-(2-ethylhexyl) phthalate (MEHP), mono-n-butyl phthalate (MBP), mono-iso-allyl phthalate (MiAP), mono-n-allyl phthalate (MnAP), mono-cyclohexyl phthalate (MCHP), mono-benzyl phthalate (MBzP), mono-n-octyl phthalate (MOP), mono-n-propyl phthalate (MPP), and mono-methyl phthalate (MMP).

After enzymatical treatment of collected urine samples, methyl-tert-butyl-ether was used as a solvent for the extraction of phthalate metabolites. The samples were prepared and analyzed by the previously developed method

accurately described by Milošević et al.¹¹. Gas chromatography coupled to mass spectrometric detection (Agilent GC 7890A, 5975C VLMSD) equipped with a fused silica capillary column (30 m, 0.25 mm id. and 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA) was used for the determination of phthalates residues in urine. The limit of detection (LOD) for 10 phthalate metabolites was 0.25 ng/mL.

Separate groups were designed for each phthalate metabolite dividing them between phthalate-free and phthalate positive samples (binary distribution), as the span of positive values was too wide so that standard deviations would exclude valuable patients.

Statistical analysis

The data were analyzed using the Pearson's χ^2 test (in cases of a low number of positive phthalate values coefficient of contingency was used) with the significant results being recorded at $p < 0.05$ and $p < 0.1$. The statistical analyses and graphical representation were done using SPSS 23.0 (SPSS Inc., Chicago, Illinois, USA) and MS Excel Package.

Results

General characteristics of the analyzed population such as male to female ratio, gender, height, age, body weight, waist circumference, and BMI values are shown in Table 1. Statistically significant differences in age, body weight, waist circumference, and BMI were

observed among the studied groups.

Phthalate metabolite abundance is shown in Table 2. The most frequently detected phthalate metabolites were MEP and MEHP, while MnAP was the least represented.

Out of 308 participants, 155 (50.32%) had at least one phthalate metabolite detected in the first morning urine sample.

The abundance of phthalate metabolites in each group is shown in Figure 1.

In the control group, the most abundant phthalate metabolites were MEP and MEHP, while MCHP and MMP were not detected. Analyzing the frequency of all metabolites separately, 26 (25.3%) out of 103 participants in the control group had MEP in their urine, 17 (16.5%) had MEHP, and 4 (3.9%) participants were MBP or MOP exposed. Additionally, MiAP and MPP were detected in two samples in the control group (1.7%), while MnAP and MBzP were above the limit of detection in only one sample (0.9%).

In the group of obese participants, MEP and MEHP were also the most abundant phthalate metabolites, while the presence of MCHP again was not determined. In terms of frequency of detection, MEP was determined in the urine of 30 (28.8%) participants, MEHP was found in 27 (25.9%) obese participants, while 4 (3.9%) of them had MBP in their urine sample. Moreover, only one participant was MiAP or MnAP exposed. Three (2.9%) participants were positive with MBzP presence, and 2 (1.9%) of them had MPP in their urine sample. Only 1 (0.9%) participant was positive with MOP presence, while 6 (5.7%) participants had MMP in the urine sample.

Table 1

General characteristics of the entire cluster

Participants	n	Gender (n)		Age (years)	Height (cm)	Weight (kg)	Waist circumference (cm)	BMI (kg/m ²)
		male	female					
Control	103	51	52	35.91 ± 8.00 ^b	173.37 ± 8.44	69 ± 10.71 ^{a, b}	78.38 ± 7.99 ^{a, b}	22.60 ± 2.07 ^{a, b}
Obese	104	51	53	38.61 ± 8.69 ^c	174.10 ± 9.94	106.36 ± 20.37 ^{a, c}	110.29 ± 14.79 ^{a, c}	35.23 ± 6.74 ^{a, c}
T2DM	101	57	44	44.94 ± 7.38 ^{b, c}	171.56 ± 10.83	91.79 ± 23.21 ^{b, c}	103.01 ± 16.81 ^{b, c}	31.18 ± 7.25 ^{b, c}
Total/ Average	308	108	96	39.75 ± 8.87	173.03 ± 9.82	89.08 ± 24.33	97.21 ± 19.36	29.91 ± 7.88

n – number of volunteers; **T2DM** – type 2 diabetes mellitus; **BMI** – body mass index; **Statistically significant difference ($p < 0.01$): a – between control and obese, b – between control and T2DM; c – between obese and T2DM. All values are expressed as mean ± standard deviation or number (n).**

Table 2

Phthalate metabolites abundance in the examined population

Participants	MEP	MEHP	MBP	MiAP	MnAP	MCHP	MBzP	MOP	MPP	MMP
Control (n = 103)	26 (25.3)	17 (16.5)	4 (3.9)	2 (1.9)	1 (1)	0 (0)	1 (1)	4 (3.9)	2 (1.9)	0 (0)
Obese (n = 104)	30 (28.8)	27 (26)	4 (3.8)	1 (1)	1 (1)	0 (0)	3 (2.9)	1 (1)	2 (1.9)	6 (5.7)
T2DM (n = 101)	13 (12.8)	29 (28.7)	5 (4.9)	1 (1)	0 (0)	3 (2.9)	3 (2.9)	1 (1)	0 (0)	9 (8.9)
Total (n = 308)	69 (22.4)	73 (23.7)	13 (4.2)	4 (1.2)	2 (0.6)	3 (0.97)	7 (2.2)	6 (1.9)	4 (1.2)	15 (4.8)

T2DM – type 2 diabetes mellitus; **MEP** – mono-ethyl phthalate; **MEHP** – mono-(2-ethylhexyl) phthalate; **MBP** – mono-n-butyl phthalate; **MiAP** – mono-iso-allyl phthalate; **MnAP** – mono-n-allyl phthalate; **MCHP** – mono-cyclohexyl phthalate; **MBzP** – mono-benzyl phthalate; **MOP** – mono-n-octyl phthalate; **MPP** – mono-n-propyl phthalate; **MMP** – mono-methyl phthalate. All results are presented as number (percentage).

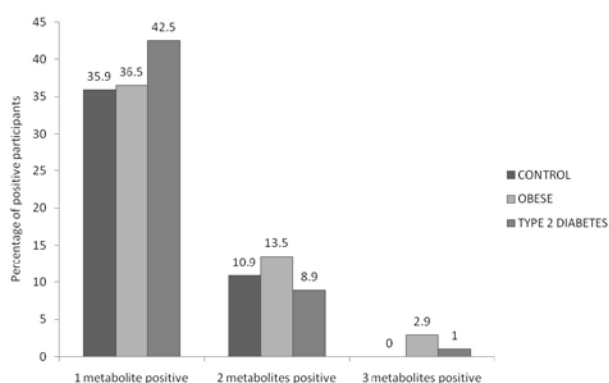


Fig. 1 – Abundance of phthalate metabolites in each individual group.

In the group of participants with T2DM, MEP and MEHP were again the most frequently detected metabolites in the urine, while the presence of MPP and MnAP was not determined. Out of 101 participants with T2DM, 13 (12.8%) had MEP, while 29 (28.7%) of them had MEHP in their urine sample. MBP was detected in the urine of 5 (4.9%) participants, while only one participant was positive with MiAP or MOP presence. Both MCHP and MBzP were detected in the urine of 3 participants, and 9 participants had MMP phthalate metabolite in their urine sample.

The distribution of analyzed metabolites in the phthalate-positive participants is presented in Figure 2. Out of 308 participants, 118 (38.3%) had one phthalate metabolite, 33 (10.7%) of them had two, while 4 (1.3%) participants had three phthalate metabolites in their urine sample. There were no participants with four or more phthalate metabolites detected in their urine samples.

All phthalates were tested for significant differences between the control and obese/T2DM groups of participants. MEP and MMP had p values less than 0.05, while MEHP and MCHP had p values less than 0.1 and, therefore, were considered significant and will be further discussed.

In the examined population of 308 participants, a significant difference was seen in MEP frequency between the control group and T2DM group ($\chi^2 = 5.058$, $df = 1$, $p = 0.025$). Precisely, 26 (25.2%) out of 103 participants in

the control group were MEP positive, exactly double in comparison with the number of exposed T2DM patients (13 out of 101; 12.9%).

Considering the MMP occurrence in urine samples, a significant difference in frequencies was observed between the control group and T2DM group ($\chi^2 = 8.491$, $df = 1$, $p = 0.004$), and the obese group and control group ($\chi^2 = 6.120$, $df = 1$, $p = 0.013$), but one must stress the relative significance of this finding as T2DM group had 9 positive values (9/101, 8.9%), the obese group had 6 (6/104, 5.8%), and the control group had none (0/103, 0%).

MEHP was present in 29 (28.9%) of 101 T2DM participants, in 27 (26%) of 104 in the obese group, and in 17 (16.5%) of 103 in the control group, where a borderline significant difference was observed between the control and T2DM group ($\chi^2 = 3.435$, $df = 1$, $p = 0.064$), and between the obese group and control group ($\chi^2 = 2.765$, $df = 1$, $p = 0.096$).

This metabolite was detected in 3 (3%) of 101 T2DM patients and in none of 103 (0%) participants in the control group with moderate significant difference observed ($\chi^2 = 3.105$, $df = 1$, $p = 0.078$).

Again, among the groups, no statistically significant differences were observed between the characteristics of the MBP, MiAP, MnAP, MBzP, MOP, MPP subgroups.

Discussion

Many chemicals whose presence in nature has been increased after the industrial revolution can act as endocrine disruptors by interfering with endogenous hormonal pathways. Epidemiological studies^{12–14} have shown the link between exposure to these chemicals and the development of common disorders and diseases such as obesity and T2DM. Taking into consideration that the pathogenesis of these disorders depends on the combination of lifestyle habits and genetics, it is lately hypothesized that exposure to endocrine disruptors during or after pregnancy can play a significant role in the onset of some diseases¹⁵. Phthalates are usually found in large quantities in daily products. The number of publications that investigate the positive linkage between

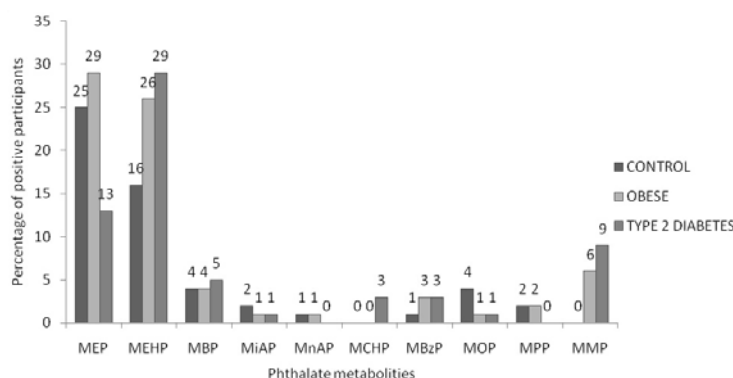


Fig. 2 – Number of phthalate metabolites detected in the urine samples.

MEP – mono-ethyl phthalate; MEHP – mono-(2-ethylhexyl) phthalate; MBP – mono-n-butyl phthalate; MiAP – mono-iso-allyl phthalate; MnAP – mono-n-allyl phthalate; MCHP – mono-cyclohexyl phthalate; MBzP – mono-benzyl phthalate; MOP – mono-n-octyl phthalate; MPP – mono-n-propyl phthalate; MMP – mono-methyl phthalate.

phthalates exposure and adipogenesis and T2DM, some of the largest epidemics of the modern world, increases continuously. Although chemical industry representatives assert that levels of phthalates found in the human body are well below the “safe” concentrations by some regulatory agencies, endocrinologists consider that phthalates exposure, even at low doses, during vulnerable periods, can lead to adverse health effects¹⁶. Although it is estimated that the average level of human exposure to DEHP is around 0.0024 mg/kg/day, much below the current DEHP “No Observed Adverse Effect Level” (NOAEL) by the European Food Safety Authority, the chronic exposure even at low doses could be more harmful than single acute exposure to high dose¹⁷. Natural hormones are active at the pico- to nanomolar range. Hence, phthalates as EDCs might ameliorate hormone homeostasis and cause biological impact at low doses¹⁸. According to literature data^{12, 19}, fetuses, newborns, and adolescents are vulnerable groups and particularly susceptible to phthalate exposure, which is explained by the high levels of cell activity in those age groups.

The obtained results showed that the urine sample of 50.32% of participants was positive for the presence of at least one phthalate metabolite.

The ubiquitous presence of phthalates in human urine samples is documented in the study published by Zota et al.²⁰. Eleven phthalate metabolites were analyzed in the urine sample of more than 11,000 adults and children, and data from five cycles of NHANES (National Health and Nutrition Examination Survey) study from 2001–2010 were used. MEP, MBzP, and MnBP were detected in 98% of participants, while MiBP was detected in 72% of participants in the period 2001–2002 and in 96% participants in the period 2009–2010.

Earlier, Stahlhut et al.²¹ concluded that the urine of more than 95% of participants was positive with the following phthalate metabolites: MEP, MBP, and MBzP, while 80% of participants had MEHP in urine. In the same study, MEP metabolite had noticeably the highest level, followed by MBP and MBzP, while MEHP had the lowest level. In this research, MEHP (detected in 23.7% of cases) and MEP (in 22.4%) were the most represented in the urine sample. The high frequency of the detection of MEHP could be due to the wide use of products that contain di-(2-ethylhexyl)phthalate (DEHP), such as plastic food packaging, toys, and many other household products, while MEP presence is probably the consequence of the increased use of different cosmetic and beauty products, as well as medications, containing diethyl phthalate (DEP)²².

Similar to the findings of previous studies, Hoppin et al.²³ found the highest levels of MEP, MBzP, MBP, and MEHP in the two consecutive morning urine samples of 46 Afro-American women. There was no significant difference in the level of phthalate metabolites between the two urine samples, which indicates that urine is a suitable medium for the measurement of phthalates with a short half-life. Additionally, high urinary levels of MEP, MnBP, and MBzP were found in a study conducted on 289 adult persons by

Blount et al.²⁴, while MEHP was measured in much lower concentrations.

Similar to this research, detectable levels of MEP, MBP, MBzP, MEHP, MiNP, MOP, and MCHP were found in the urine sample of 2,540 volunteers, but with a higher frequency of detection (75%)²⁵. A possible reason for the difference in the distribution is the much smaller sample size in our research (308 vs 2,540 volunteers).

When exposure to six urinary phthalate metabolites was examined in 370 healthy Czech preschool and school children, MEHP, mono (2-ethyl-5-hydroxyhexyl) phthalate (5OH-MEHP), mono (2-ethyl-5-oxohexyl) phthalate (5oxo-MEHP), MBzP, MiBP, and mono-n-butyl phthalate (MnBP) were analyzed. Among all samples, the two latter mono-butyl phthalate isoforms dominated²⁶.

Comparable to similar studies were the results obtained by Frederiksen et al.²⁷, who conducted research on 60 young men to examine the correlation between 13 phthalates metabolites levels in different mediums, such as urine, semen, and serum. DEHP phthalate metabolites, accompanied by MEP, MiBP, MBzP, and MnBP, were detected in the urine samples in the highest amount.

In this research, MEP and MEHP were the most frequently detected metabolites in the control, obese, and T2DM groups. Significant differences were observed for MEP frequency between healthy and T2DM participants ($p = 0.025$). Regarding MEHP, a borderline significant difference was observed between the control and T2DM group ($p = 0.064$), and between the obese and control group ($p = 0.096$).

It is known that activation of PPAR receptors plays an important role in different steps of glucose homeostasis, including insulin secretion and insulin resistance. It can also affect circulating levels of lipids thereby modulating the quantity of subcutaneous and visceral fat. Phthalate metabolites are well-known ligands to PPAR receptors and, therefore, could influence both homeostases of glucose and lipid metabolism. Through the PPAR-signaling pathways deterioration, phthalate metabolites could contribute to the development of obesity and diabetes²⁸. The precise mechanism by which phthalates influence these PPAR-mediated actions is expected to be explained with further experiments.

Limitations and advantages of the study

This study focused only on a middle-aged cluster of white (Caucasian) persons, hence, the results can not be extrapolated to other ethnic and other age groups. Being conducted as a cross-sectional study, this research has a risk of selection bias. Thereby, further studies are needed to confirm the present data. Further studies are also needed for clustering the geographical, age, ethnic, sexual, and other characteristics.

In the current study, urine was used as a matrix for measurements of phthalate metabolites. The advantage of urinary measurements is that apart from low cost and noninvasive methods of obtaining samples, usually higher

levels are found compared with serum and, thereby, more phthalate metabolites could be measured above the lower detection limit.

Conclusion

Approximately half of the examined participants (50.32%) had at least one phthalate metabolite in their urine sample. Our study showed that the most abundant phthalate metabolite present in the group of obese participants was MEP, while MEHP was the most common phthalate metabolite in the T2DM group. A group of healthy individuals had the highest percentage of presence of MEP amongst examined phthalate metabolites. The obtained

results indicate that in the Vojvodina region, both healthy adults and those with metabolic disorders such as obesity and newly diagnosed T2DM are predominantly exposed to widespread DEHP and DEP phthalates.

Further research that will provide more detailed insight into phthalate interference with glucose and lipid metabolism and their influence on the endocrinological balance is needed.

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Relationship among the quality of cognitive abilities, depression symptoms, and various aspects of handgrip strength in the elderly

Odnos između kvaliteta kognitivnih sposobnosti, simptoma depresije i različitih aspekata snage stiska šake kod starijih osoba

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Abstract

Background/Aim. Both the cognitive and physical functioning changes occur within the normal aging, suggesting possible common biological processes. The aging process is often characterized by a reduction of adaptive responses, an increasing vulnerability and functional limitations. The aim of this study was to determine if there were correlations between particular cognitive abilities (verbal ability, spatial ability, processing speed, memory, verbal fluency, divergent thinking, memory, attention, executive functions, conceptualization, orientation, computation), depression symptoms and different dynamometric parameters of muscle contraction, during handgrip (HG) of both hands, in the elderly population. **Methods.** The sample consisted of 98 participants, 16 males and 82 females, aged from 65 to 85. Neuropsychological assessment included Montreal Cognitive Assessment (MoCA), Frontal Function Test (Go/No-Go), Categorical and Phonemic fluency tests and Geriatric Depression Scale Short Form (GDS-SF). Physical measures were assessed by Handgrip Dynamometry Tests (HG), and included: the maximum force

(F_{max}), maximal rate of force development (RFD_{max}), static endurance HG time realized at 50% of maximal HG force ($tF_{max50\%}$) of dominant (Do) and non-dominant (NDo) hand. **Results.** Higher MoCA score was followed by higher values of muscle endurance of dominant hands. Higher values of F_{max} of dominant hand were associated with higher values of Alternating Trail Making that is by visuoconstructive abilities (MoCA). The variable Categorical fluency was in a small, positive correlation with F_{max} . No correlation of depressive symptoms with HG parameters was found except in the subgroup of female subjects. **Conclusion.** Better cognitive performance was associated with better HG muscle strength. Therefore, HG strength can be a useful tool in geriatric practice in monitoring not only physical, but also cognitive function status and decline. The link between lower cognitive functioning and lower values of HG variables, emphasize the need for increased awareness about it in clinical practice.

Key words:

aged; aging; cognition; depression; hand strength; risk assessment.

Apstrakt

Uvod/Cilj. Tokom procesa normalnog starenja javljaju se promene u kognitivnom i fizičkom funkcionisanju koje ukazuju na moguće zajedničke biološke osnove. Proces starenja često karakterišu smanjenje adaptivnih odgovora, sve veća ranjivost i funkcionalna ograničenja. Cilj rada bio je da se utvrdi povezanost određenih kognitivnih sposobnosti (verbalna sposobnost, prostorna sposobnost, brzina obrade, memorija, verbalna tačnost, divergentno razmišljanje, pamćenje, pažnja, izvršne funkcije, konceptualizacija, ori-

jentacija, računanje), simptoma depresije i različitih dinamometrijskih parametara kontrakcije mišića tokom stiska obe šake – *handgrip* (HG) u starijoj populaciji. **Metode.** Ispitivanjem je obuhvaćeno 98 ispitanika, 16 muškog i 82 ženskog pola, starosti od 65 do 85 godina. Neuropsihološka procena uključila je Montrealsku skalu kognicije (MoCA), test frontalnih funkcija (Go/No-Go), testove kategorijalne i fonemske fluentnosti i kratku formu Gerijatrijske skale depresije (GDS-SF). Fizičke mere su procenjene dinamo metrijskim testovima HG i uključivale su: maksimalnu silu (F_{max}), maksimalnu brzinu razvijanja sile (RFD_{max}), vreme

statičke izdržljivosti HG ostvareno na 50% maksimalne HG sile ($tF_{\max}50\%$) dominantne (Do) i nedominantne (NDo) ruke. **Rezultati.** Bolji rezultat na MoCA skali bio je praćen višim vrednostima mišićne izdržljivosti dominantne ruke. Veće vrednosti F_{\max} -a dominantne ruke bile su povezane sa višim vrednostima alterniranog povezivanja brojeva i slova odnosno vizuelnokonstrukcionim sposobnostima (MoCA). Nije nađena korelacija simptoma depresije sa HG parametrima, osim u podgrupi ispitanica. **Zaključak.** Bolje kognitivne performanse bile su povezane sa većim stepenom

mišićne snage HG. Stoga, snaga HG može biti korisno sredstvo u gerijatrijskoj praksi za praćenje stanja ne samo fizičke već i kognitivne funkcije i njihovog opadanja. Veza između nižeg kognitivnog funkcionisanja i nižih vrednosti HG varijabli, naglašava potrebu za povećanom svešću o tome u kliničkoj praksi.

Ključne reči:
stare osobe; starenje; saznanje; depresija; ruka, snaga; rizik, procena.

Introduction

Aging is a process of gradual deterioration of the physiological functioning that a human experiences over time. It is often characterized by a reduction of adaptive responses, increasing vulnerability, and functional limitations. Both the cognitive and physical functioning changes that occur within the normal aging process are often mentioned in the scientific literature. Some cognitive abilities, such as vocabulary, are resistant to brain aging and might even improve with age. Other capabilities, such as conceptual monitoring, memory, and processing speed, decline over time. A noticeable effect of the aging process is observed in the more complex tasks of attention, short-term working memory, and visual constructive skills, as well. Research has shown that concept formation, abstraction, and mental flexibility decrease, especially after the age of 70. Aging is also coupled with a deficit of inhibitory processes in cognitive functioning¹ and accompanied by a decline in physical functions. Changes occur both in muscle mass² and the functionality of the muscle and thus decrease explosive strength, muscular endurance, fine motor control, force steadiness, and the capacity for rapid muscle force production (RFD)³. Thus, age brings changes in the neuromuscular system that result in decreased muscle strength, balance, and proprioception, causing a slower motor response rate⁴. Among the elderly, cognitive impairment is known to occur frequently along with a decline in muscle strength, indicating a close relationship between these two phenomena^{5,6}. All this suggests that both cognitive and physical functioning are vitally important for successful functioning in old age and independent living. Therefore, the need to monitor, test, and correlate them is fully justified.

Numerous tests and batteries allow us to assess and monitor the cognitive and physical decline in the elderly. A wide range of tools has been developed to examine cognitive abilities in the elderly. These tools range from short screening tools to comprehensive neurophysiological assessments. On the other hand, physical functioning in the elderly population is usually examined through a muscle strength test, especially the muscular strength of the upper extremities (handgrip)⁷. Handgrip (HG) strength is a non-invasive measure widely used in research and clinical settings. Easily applicable, it is an indicator of muscle function, nutritional status, and physical health. HG is a reliable overall indicator of health in population-based studies⁸. Since it is also sensitive to changes related to aging processes, it is often used not only as a marker for muscle

strength but also for biological vitality⁹. It is important to note that some researchers have found that the HG test is also associated with individual differences in cognitive functioning^{5,6,10}. Thus, Buchman et al.¹¹ noticed that a decline in HG was associated with an increase in risk for Alzheimer's disease. Taekema et al.¹² concluded that weak HG predicted a decline in cognitive ability in the elderly, while Sternäng et al.¹³ detected a longitudinal association between HG and cognition.

In previous studies, different cognitive abilities, such as fluid cognition, measures of crystallized cognition, and mental state examinations, were generally related to only one parameter of HG strength (HGS), eg, the maximum force F_{\max} ¹⁴. To our knowledge, few studies have considered other aspects of muscular contraction (rate of force development, duration of contraction, endurance, and the like). Therefore, our study aimed to determine if there were correlations between achievement in particular cognitive abilities (verbal ability, spatial ability, processing speed, and memory, verbal fluency, divergent thinking, memory, attention, executive functions, conceptualization, orientation, computation) and different parameters of muscle contraction concerning both hands in the elderly population.

Methods

The study was conducted as a prospective nonrandom clinical study of the cross-sectional study type. The sample consisted of 98 participants aged 65 to 85, 16 males aged [mean \pm standard deviation (SD): 68.63 \pm 5.15 years] [minimum (Min) – maximum (Max): 65.0–81.0 years], and 82 females (mean \pm SD: 68.60 \pm 4.35 years; Min–Max: 65.0–85 years). More detailed characteristics of the sample are shown in Table 1. Before taking part in the research, participants were introduced to the nature of the research and provided their informed consent.

Following inclusion criteria were used: absence of previous diseases and central nervous system (CNS) impairment including cerebrovascular injury (stroke), traumatic brain and spinal cord injury, trauma to the peripheral nerves; participant's ability to cooperate during examination and testing; the normal range of blood pressure, with or without therapy (controlled hypertension); the absence of severe diseases of the heart, lung, liver, kidney, and other organs; the absence of/or well-controlled diabetes mellitus. Exclusion criteria were: previous stroke or other severe neurological brain diseases; dementia (the presence of damage to at least two cognitive areas accompanied with

everyday functional impairments); decompensated cardiomyopathy, unregulated arterial hypertension, presence of malignant disease, hepatic, or renal, or pulmonary insufficiency; uncontrolled diabetes mellitus with hypo- and hyperglycaemia, and quarterly glycosylated haemoglobin (Hb A1c) above 7%.

Procedures

The research was conducted in the Gerontology Centre of Belgrade in Daily centres and clubs located on the territory of Belgrade, as well as in the Department of Geriatrics of the Clinic for Internal Medicine of the Zvezdara University Clinical Centre, Belgrade. Testing procedures were implemented so that a respondent was tested and procedures completed in one day. The testing schedule included taking anamnestic data, followed by the cognitive and mental assessment. After completion of cognitive testing, physical testing was completed. The study was conducted in line with the Declaration of Helsinki and was approved by the Ethics Committee of the Zvezdara University Clinical Centre, Belgrade, Serbia.

Instruments and measures

Montreal Cognitive Assessment (MoCA)¹⁵ is designed as a screening instrument for detecting mild cognitive dysfunction¹⁶. MoCA consists of a variety of tasks measuring different cognitive domains: executive functions, attention and concentration, memory, language, visuoconstructional skills, conceptual thinking, calculation, and orientation. The instrument contains following item clusters measuring aforementioned functions: Alternating Trail Making (ATM), Visuoconstructional Skills [(Cube), VCS_cube)], Visuoconstructional Skills [(Clock) VCS_clock)], Naming (M_N), Memory (M_M), Attention: Forward Digit Span (A_FDS), Attention: Backward Digit Span (A_BDS), Attention: Vigilance (A_V); Attention: Serial 7 subtraction (A_S7), Sentence repetition (SR), Verbal fluency (VF), Abstraction (M_A), Delayed recall (DR), Orientation (M_O).

Administration time is approximately 10 min. The total score is obtained by summing scores for individual items and by adding one point for the individuals who have 12 years of formal education or less, for a possible maximum of 30 points. A final total score of 26 and above is considered normal. The battery demonstrates good psychometric properties and a six-factor structure¹⁷. For the purposes of this research, we used the Serbian version of MoCA¹⁸.

The Go/No-Go (GNG) test requires a subject to emit a simple motor response to one cue while inhibiting the response in the presence of another cue. The test was performed as a contrast program by asking the patient to hold one hand upright, and when the examiner raises one finger, the examinee should respond by raising two fingers. When the examiner raises two fingers, the examinee should not react at all. The answer should be as fast as possible, and always before the next time, the respondent should put his fingers down. Ten samples are given according to a pseudorandom schedule 1,1,1,2,1,2,2,1,1,2. The number of correct reactions from ten attempts is scored.

Testing Go/No-Go (GNG) is often used as a component of a behavioral neurological examination to assess inhibitory

control. Performance in this test is associated with the preservation of the ventrolateral prefrontal cortex (lower frontal gyrus, fronto-insular region) and pre-supplementary motor area that is part of the response inhibition system¹⁹.

It is one of the tests of verbal fluency and includes a listing of words that belong to a specific category (animals). Respondents are asked to name (enumerate) as many animals as possible for a limited time. The task is interrupted after 1.5 min (90 s). The respondent must state as many words in this category (the names of animals within the same species cannot be taken into account, *eg* chicken, rooster, hen, etc.). The number of correct, nonrepeated responses constituted the raw score. The limit value is 18 animal names¹⁶.

This is a test of divergent thinking because there is more than one correct answer. Respondents need to formulate a specific word recall strategy. Categorical fluency reflects retrorolandic functions. Therefore, categorical fluency will be impaired in retrorolandic disorders, such as in the early phase of Alzheimer's disease¹⁶.

The short form is the 15-item form. The score ranges from 0 to 15 and if it is six or more, then indicates signs of depression¹⁶. In a validation study comparing the long and short forms of this self-report scale for depression symptoms, it was found that both were successful in distinguishing depressed vs. non-depressed adults with a high degree of correlation, $r = 0.84$, $p < 0.001$ ²⁰.

Phonemic fluency tests require the respondent to indicate as many words as possible, beginning with a specific letter, for a specified time. The given initial letters of the words required by the respondents are selected according to the frequency in a particular language. In the Serbian version of the test that we applied, the letters (phonemes) s, k, l, are used because they are the most frequent in the Serbian language¹⁶. The authors state the limit values of 7 words for less educated and 9 words for educated respondents¹⁶. Respondents are asked to list as many nouns that begin with a certain letter in one minute. Personal names, geographical terms, and numbers are excluded.

Phonemic fluency predominantly reflects prerolandic functions. Impaired phonemic fluency indicates the onset of vascular dementia¹⁶.

The evaluation of the contractile characteristics²¹ of the hand muscles and finger flexors was conducted using a piece of standardized equipment – dynamometer [*ie*, a sliding device that measures isometric hand and finger flexor force with a tensiometric probe fixed inside the device (All4gym d.o.o., Serbia)]. During the test, subjects were sitting on a chair (without leaning their backs) while holding the device in the tested hand. Testing was performed first on one hand and then on the other one. The arm of the tested hand was stretched out alongside the body, without leaning on the trunk and a chair. The other hand was either leaning against the body or resting on the thigh. During the testing procedure, subjects were not allowed to move. Before the testing, the procedure was explained to the subjects, and they had the opportunity to try it several times. With verbal encouragement, subjects were asked to make the strongest and fastest pressure on the lever (probe). The test was conducted with a dominant and non-dominant hand twice

with a break of one minute between attempts. The maximal force (F_{max} , expressed in N), maximal rate of force development (RFD_{max} , expressed in N/s), and static endurance HG time realized at 50% of maximal HG force ($tF_{max50\%}$, expressed in s) were obtained from this test for both hands – dominant (Do) and non-dominant (NDo). After a ten minutes rest period, the endurance test was performed, too. The test was operated by setting a value on the level of 50% of the maximal force. Subjects were asked to maintain a defined level of force (50%) with visual feedback on the computer for as long as possible (time was measured in s). Due to possible fatigue, only one attempt was carried out. The achieved force impulse ($I_{mpF50\%} = F_{max50\%} \cdot tF_{max50\%}$, expressed in Ns) was calculated as a measure of HG strength endurance.

Statistical analyses

Descriptive statistics were used to characterize the sample and the outcomes. In order to test the relationships between the selected variables, the Spearman's rank correlation was used (two-tailed). Coefficients (ρ) were interpreted as small (0.10–0.29), moderate (0.30–0.49), or strong (0.50–1.00) correlation. All analyses were performed using the SPSS for Windows software program, version 23, using a 0.05 level of significance.

Results

Sociodemographic characteristics of all participants are displayed in Tables 1, 2, and 3 showing motor and cognitive abilities of subjects. Tables 4 and 5 show the relationship

Table 1

Basic characteristics of subjects

Characteristics	Mean	SD	Median	Minimum	Maximum
Age (years)	68.60	4.46	66.50	65.00	85.00
BMI (kg/m ²)	27.14	4.06	26.55	17.93	38.58
G score	1.52	2.26	1.00	0.00	10.00

BMI – body mass index; G – Geriatric Depression Scale; SD – standard deviation.

Table 2

Motor characteristics of subjects

Characteristics	Mean	SD	Median	Minimum	Maximum
F_{max_NDo} (N)	247.69	77.51	241.50	90.00	635.00
RFD_{max_NDo} (N/s)	1,129.50	491.89	1,078.00	349.00	3,601.00
$tF_{max50\%_NDo}$ (s)	61.36	36.73	60.29	4.53	173.48
$I_{mpF50\%_NDo}$ (Ns)	7,914.55	5,459.49	6,908.72	425.63	24,538.31
F_{max_Do} (N)	272.18	79.12	259.50	88.00	651.00
RFD_{max_Do} (N/s)	1,262.35	483.75	1,232.50	446.00	3,569.00
$tF_{max50\%_Do}$ (s)	66.62	37.18	64.00	8.91	248.10
$I_{mpF50\%_Do}$ (Ns)	8,920.45	5,037.93	8,456.49	1,147.99	25,912.08

F_{maxNDo} – maximum force of non-dominant hand; RFD_{max_NDo} – maximal rate of force development of non-dominant hand; $tF_{max50\%_NDo}$ – static endurance handgrip (HG) time realized at 50% of maximal HG force of non-dominant hand; $I_{mpF50\%_NDo}$ – achieved force impulse of non-dominant hand; F_{maxDo} – maximum force of dominant hand; RFD_{max_Do} – maximal rate of force development of dominant hand; $tF_{max50\%_Do}$ – static endurance handgrip time realized at 50% of maximal HG force of dominant hand; $I_{mpF50\%_Do}$ – achieved force impulse of dominant hand; SD – standard deviation.

Table 3

Cognitive abilities of subjects

Characteristics	Mean	SD	Median	Minimum	Maximum
MoCA score	23.96	3.20	24.00	15.00	31.00
Alternating Trail Making	0.51	0.50	1.00	0.00	1.00
Visuoconstructional Skills (Cube)	0.91	0.29	1.00	0.00	1.00
Visuoconstructional Skills (Clock, Contour)	0.98	0.14	1.00	0.00	1.00
Visuoconstructional Skills (Clock, Numbers)	0.74	0.44	1.00	0.00	1.00
Visuoconstructional Skills (Clock, Hand)	0.54	0.50	1.00	0.00	1.00
Naming	2.91	0.29	3.00	2.00	3.00
Attention: Forward Digit	0.92	0.28	1.00	0.00	1.00
Attention: Backward Digit Span	0.77	0.45	1.00	0.00	2.00
Attention: Vigilance	0.97	0.17	1.00	0.00	1.00
Attention: Serial 7 subtraction	2.69	0.58	3.00	1.00	3.00
Verbal fluency	0.70	0.46	1.00	0.00	1.00
Abstraction	1.33	0.73	1.00	0.00	2.00
Orientation	5.82	0.54	6.00	2.00	6.00
Go/No-Go	84.08	14.70	90.00	0.00	100.00
Categorical fluency	23.49	7.95	22.00	7.00	59.00
Phonemic fluency	25.60	8.80	24.50	2.00	55.00

MoCA – Montreal Cognitive Assessment; SD – standard deviation.

Table 4

Relationship between depression, cognitive ability, and various aspects of handgrip strength

Variable	G score	MoCA score	Go/noGo	Categorical fluency	Phonemic fluency
F _{max} _NDo, ρ/p	-0.041 / 0.689	0.051 / 0.615	-0.099/0.333	0.158/0.120	-0.095/0.350
RFD _{max} _NDo, ρ/p	-0.030 / 0.770	0.108 / 0.289	-0.060/0.558	0.111/0.277	-0.141/0.167
tF _{max} 50%_NDo, ρ/p	-0.146 / 0.155	0.040 / 0.694	0.024/0.813	-0.121/0.239	-0.045/0.664
I _{mp} F50%_NDo, ρ/p	-0.092 / 0.369	0.088/0.391	-0.003/0.979	-0.025/0.810	-0.083/0.417
F _{max} _Do, ρ/p	0.064 / 0.533	0.045/0.658	0.089/0.384	0.290/0.004	-0.097/0.342
RFD _{max} _Do, ρ/p	-0.054/ 0.598	0.035/0.735	0.070/0.492	0.171/0.092	-0.154/0.130
tF _{max} 50%_Do, ρ/p	-0.196 / 0.054	0.202/0.046	0.010/0.922	-0.157/0.123	0.033/0.745
I _{mp} F50%_Do, ρ/p	-0.170 / 0.094	0.247/0.014	0.020/0.849	-0.012/0.910	-0.057/0.574

F_{max}_NDo – maximum force of non-dominant hand; RFD_{max}_NDo – maximal rate of force development of non-dominant hand; tF_{max}50%_NDo – static endurance handgrip (HG) time realized at 50% of maximal HG force of non-dominant hand; I_{mp}F50%_NDo – achieved force impulse of non-dominant hand; F_{max}Do – maximum force of dominant hand; RFD_{max}_Do – maximal rate of force development of dominant hand; tF_{max}50%_Do – static endurance HG time realized at 50% of maximal HG force of dominant hand; I_{mp}F50%_Do – achieved force impulse of dominant hand; G – Geriatric Depression Scale Short Form; MoCA – Montreal Cognitive Assessment.

Bolded values are statistically significant.

Table 5

Relationship between MoCa and various aspects of handgrip strength

Variable	F _{max} _NDo (N)	RFD _{max} _NDo (N/s)	tF _{max} 50%_NDo (s)	I _{mp} F50%_NDo (Ns)	F _{max} _Do (N)	RFD _{max} _Do (N/s)	tF _{max} 50%_Do (s)	I _{mp} F50%_Do (Ns)
ATM								
ρ	0.244	0.327	0.138	0.226	0.282	0.272	0.078	0.276
p	0.016	0.001	0.178	0.026	0.005	0.007	0.443	0.006
VCS_cube								
ρ	-0.182	-0.158	-0.106	-0.121	-0.014	-0.073	0.038	0.040
p	0.072	0.120	0.302	0.237	0.891	0.473	0.714	0.693
VCS_clock C								
ρ	0.050	0.158	0.033	0.033	0.091	0.145	0.090	0.122
p	0.625	0.120	0.745	0.749	0.372	0.156	0.378	0.230
VCS_clockN								
ρ	-0.014	-0.043	0.088	0.063	-0.089	-0.085	-0.025	-0.040
p	0.894	0.677	0.389	0.539	0.382	0.408	0.810	0.696
VCS_clockH								
r	0.148	0.099	0.080	0.130	0.169	0.102	0.182	0.251
p	0.146	0.332	0.438	0.201	0.096	0.319	0.073	0.013
M_N								
ρ	-0.022	0.022	-0.094	-0.071	0.015	0.051	0.180	0.163
p	0.827	0.831	0.359	0.484	0.883	0.617	0.076	0.109
A_FDS								
ρ	-0.080	0.007	-0.129	-0.148	0.010	-0.032	0.090	0.067
p	0.434	0.944	0.208	0.146	0.925	0.753	0.379	0.513
A_BDS								
ρ	-0.082	0.113	-0.074	-0.075	-0.033	0.082	0.264	0.216
p	0.421	0.269	0.473	0.460	0.749	0.419	0.009	0.032
A_V								
ρ	0.043	0.017	-0.078	-0.031	0.135	0.047	-0.061	0.017
p	0.674	0.866	0.449	0.760	0.185	0.645	0.548	0.872
A_S7								
ρ	0.070	0.064	0.082	0.097	0.076	0.023	0.092	0.130
p	0.494	0.529	0.425	0.344	0.458	0.819	0.365	0.202
VF								
ρ	-0.038	-0.031	-0.170	-0.148	-0.092	-0.043	0.059	-0.007
p	0.708	0.763	0.096	0.147	0.367	0.678	0.562	0.944
M_A								
ρ	0.025	-0.018	0.136	0.114	0.017	-0.046	0.047	0.069
p	0.805	0.858	0.183	0.263	0.869	0.653	0.647	0.501
M_O								
ρ	0.067	0.064	-0.006	0.013	-0.019	-0.056	0.187	0.160
p	0.511	0.531	0.951	0.896	0.854	0.581	0.065	0.116

ATM – Alternating Trail Making; VCS_cube – Visuoconstructional Skills Cube; VCS_clockC – Visuoconstructional Skills Clock Contour; VCS_clockH – Visuoconstructional Skills, Clock Hands; VCS_clockN – Visuoconstructional Skills Clock Number; M_N – Naming; A_FDS – Attention: Forward Digit Span; A_BDS – Attention: Backward Digit Span; A_V – Attention: Vigilance; A_S7 – Attention: Serial 7 subtraction; VF – Verbal fluency; M_A – Abstraction; M_O – Orientation; F_{max}NDo – maximum force of non-dominant hand; RFD_{max}_NDo – maximal rate of force development of non-dominant hand; tF_{max}50%_NDo – static endurance handgrip time realized at 50% of maximal HG force of non-dominant hand; I_{mp}F50%_NDo – achieved force impulse of non-dominant hand; F_{max}Do – maximum force of dominant hand; RFD_{max}_Do – maximal rate of force development of dominant hand; tF_{max}50%_Do – static endurance handgrip time realized at 50% of maximal HG force of dominant hand; I_{mp}F50%_Do – achieved force impulse of dominant hand.

Bolded values are statistically significant.

between depression, cognitive ability, and various aspects of HG strength.

The variable Categorical fluency was in a small, positive correlation with F_{\max_Do} ($\rho = 0.290, p < 0.01$), indicating that the higher values of the this variable are associated with higher values of the F_{\max_Do} (Table 4). Similarly, the variable MoCA score was in small, positive correlations with both $tF_{\max 50\%_Do}$ ($\rho = 0.202, p < 0.05$) and $I_{\text{imp}F50\%_Do}$ ($\rho = 0.247, p < 0.05$), with higher MoCA score associated with higher values of $tF_{\max 50\%_Do}$ and $I_{\text{imp}F50\%_Do}$.

Table 5 shows the relationship between different aspects of the HG and MoCA test.

The variable Alternating Trail Making was in a moderate, positive correlation with RFD_{\max_NDo} ($\rho = 0.327, p < 0.01$), and small, positive correlations with F_{\max_NDo} ($\rho = 0.244, p < 0.05$), $I_{\text{imp}F50\%_NDo}$ ($\rho = 0.226, p < 0.05$), F_{\max_Do} ($\rho = 0.282, p < 0.01$), RFD_{\max_Do} ($\rho = 0.272, p < 0.01$), and $I_{\text{imp}F50\%_Do}$ ($\rho = 0.276, p < .01$), with higher values of the Alternating Trail Making variable associated with higher values of the mentioned variables. Similarly, the variable Visuoconstructional Skills, Clock Hands was in a small, positive correlation with $I_{\text{imp}F50\%_Do}$ ($\rho = 0.251, p < 0.05$), with higher values of the Visuoconstructional Skills, Clock Hands variable associated with higher values of the $I_{\text{imp}F50\%_Do}$.

Finally, there were small, positive correlations between the Attention: Backward Digit Span and $tF_{\max 50\%_Do}$ ($\rho = 0.264, p < 0.01$) and $I_{\text{imp}F50\%_Do}$ ($\rho = 0.216, p < 0.05$), with high values of Attention: Backward Digit Span associated with higher values of both $tF_{\max 50\%_Do}$ and $I_{\text{imp}F50\%_Do}$.

Discussion

The idea of this study was to examine the physical characteristics presented by HGS and the quality of cognitive ability in elderly persons. There were significantly more women than men among the participants in this study for several reasons: women were represented in greater numbers than men at the institutions where we conducted the research, more women agreed to participate in the research, and more women met the inclusion criteria.

Our results indicate that muscle function, including strength, explosiveness, and endurance, correlates with different qualities of cognitive ability. An important finding from this study shows that higher values of muscle force (F_{\max}) correlate with better scores on the Categorical fluency test in the total sample of subjects. Second, these results indicate that there is a correlation between certain abilities achieved on the Moca test, such as Alternating Trail Making, Visuoconstructional Skills (Clock, Hand), and attention (Backward Digit Span) with maximum power (F_{\max}) and force development rate (RFD). The next important finding of this study is that endurance ($tF_{\max 50\%}$ and $I_{\text{imp}F50\%}$) was better in cognitively more competent (MoCA) subjects. We did not find a correlation between the results of the Go/ NoGo test and different aspects of HG.

It is well-known that a decline in muscle mass of 1%–2% per year and muscle strength begins in the fourth decade of life, accelerating beyond the fiftieth year of life²². In addition to the reduced muscle strength, changes in RFD capacity were observed in young people, especially the male. These changes are related to the early stage of contraction that the authors associate with the rate of muscle activation. The results indicate that muscle force F_{\max} and RFD have common mechanisms that depend on neuromuscular changes associated with healthy aging. In this study, we explored a connection between HGS and categorical verbal fluency. Research on specific cognitive abilities has shown that attention maintenance, speed of information processing, verbal short-term and long-term memory, as well as the level of vocabulary development are the abilities that underlie verbal fluency²³. Our findings indicate that a stronger HG (F_{\max}) was associated with better performance on the Categorical fluency test in our subjects. We find similar results in a recent study examining HGS and cognitive ability in elderly cancer survivors²⁴. Studies examining brain activity while performing semantic categorical fluency tests have revealed activity in the left dorsolateral prefrontal gyrus and left parahippocampal gyrus²⁵. In addition, activity was detected in the left ventrolateral, dorsolateral and medial regions of the frontal lobes as well as in the left inferior temporal lobe²⁶. Activity in bilateral frontal and temporal regions of the brain has also been reported^{26, 27}. Besides, it was found that HG activates the primary motor area, supplementary motor areas, and premotor cortex²⁸, which are part of the frontal lobe.

Since it is known that executive functions have been seen as regulated by the prefrontal regions of the frontal lobes and that Alternating Trail Making has been considered an indicator of multiple aspects of executive functions, this may explain the positive correlation between handgrip parameters and Alternating Trail Making in our subjects. According to some authors, the rate of force development (the ability to rapidly develop the muscle force) is a suitable parameter for assessing the explosive power of the elderly²⁹. Thus, the RFD parameter is correlated with many activities performed daily³⁰. Then, it is sensitive to detect acute and chronic changes in neuromuscular function³¹. Similarly, RFD can be a sign of changes in nervous system functioning, since RFD is principally dependent on the discharge rate of the motor units that are currently recruited, as well as on changes in the recruitment order characteristics³². These changes in the functioning of the nervous system can also be associated with the onset of depressive symptoms. We hypothesized that subjects with depression signs would have changes in some motor characteristics, particularly in F_{\max} and RFD. Our expectations were based on the following facts: atrophy of the hippocampus and other brain regions associated with depression³³; aging process is known to be associated with oxidative stress and inflammation³⁴, which may affect neuromuscular function as well as the onset of depressive symptoms; neurodegenerative and functional changes in dopamine striatal system associated with aging³⁵, that are connected with the onset of depressive symptoms³⁶.

and with changes in motor function³⁷. However, a correlation between depression and muscle strength characteristics measured by the HG test in the total group of our subjects was not found. We only noted that higher values of the Geriatric Depression Scale score (G score) were associated with lower values of RFD max in the subgroup of our female subjects.

The association of muscular endurance with cognitive performance in the elderly has been examined both through the impact of training and through cardiorespiratory fitness levels (VO_{2max}). Thus, Ozkaya et al.³⁸ conclude that strength training and endurance training may have facilitating effects on early information processing and cognition. Hayes et al.³⁹ found a positive relationship between cardiorespiratory (CRF) ability and executive functions. Wendell et al.⁴⁰ extend these findings to other cognitive functions. This association is probably supported by the positive influence of VO_{2max} on functional and structural changes of the frontoparietal region in the elderly⁴¹. It is noteworthy that more and more evidence points to the relationship between cognition and muscle strength and endurance. Based on this growing evidence, resistance exercises that contribute to the preservation and augmentation of muscle strength and endurance can initiate beneficial neurobiological processes and may be crucial for healthy aging involving brain and cognition preservation. Similar to the above-mentioned findings, our results indicate that endurance is associated with cognitive ability. However, in our study, we examined the connection in two different ways. First, we tested endurance through the HGS test and measured endurance at 50% of the max force of subjects, and then calculated the force impulse. Secondly, many studies have connected global mental status (e.g., Mini-Mental State Exam), but we included multiple aspects of cognitive ability using MoCA, Go/No-Go, categorical and verbal fluency.

Limitations

Our study has demonstrated that HGS is associated with some aspects of cognitive functioning. In addition to some strong points of this study, there are also several limitations. First, there was a gender ratio discrepancy in the composition of the study sample. Second, data on certain factors known to influence strength was not collected. Anthropometric data, as a precise indicator of type and level of physical activity, are one example. The collection of such data was beyond the scope and resources of our exploratory study. Additionally, prospective studies are needed to make the causal inference reliable.

Conclusion

We found that better cognitive performance was associated with better muscle strength and a slower decline in muscle strength. It seems possible that impaired cognitive control of movement affects muscle function and strength in elderly subjects. Therefore, we also confirm, that HGS can be a useful tool in geriatric practice in monitoring not only physical, but also cognitive function status and decline. These findings emphasize the need for increased awareness about it in clinical practice.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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Diagnosis of bacterial vaginosis: comparison of Nugent's and novel microscopic method

Dijagnoza bakterijske vaginoze: poređenje Nugent-ove i nove mikroskopske metode

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Abstract

Background/Aim. Bacterial vaginosis (BV) is a common cause of vaginal discomfort in women. The aim of this study was to compare Nugent's scoring system and novel microscopy method, introduced in our laboratory and used in BV diagnosis. **Methods.** This study included 705 pregnant and asymptomatic women between 24 and 28 weeks of pregnancy. The degree of agreement between methods was determined by the *kappa* (κ) index. The sensitivity, specificity, positive and negative predictive value of the novel microscopy method was compared to Nugent's score as standard. **Results.** Based on the scoring system of both methods, Nugent's and novel microscopy method, BV was diagnosed in 21% and 25% of women, respectively. Despite the disparities among diagnostic criteria, which mainly concerned classification of intermediary samples, the degree of agreement between categories, determined by κ index, was satisfactory: Nugent's vs. novel microscopy method ($\kappa = 0.68$; good agreement), and Nugent's vs. novel microscopy method without intermediary results ($\kappa = 0.83$; very good agreement). We also

demonstrated that compared to Nugent's method, as the golden standard, the novel microscopy method had high sensitivity and specificity (ranging from 75%–99.3%) and positive and negative predictive values (ranging from 88.8%–99.5%). Our method is based on a relative number of bacterial morphotypes, either rod forms ($\geq 1.5 \mu\text{m}$, *Lactobacilli*) or non-rod forms ($< 1.5 \mu\text{m}$, bacterial vaginosis associated bacteria) under 200 \times magnification, which extends the surface of examined preparation, but without prolongation of observer's working time. **Conclusion.** The novel microscopy method in diagnosing BV corresponded well with Nugent's scoring system which allows it to be an alternative method in diagnosing BV. Furthermore, the novel microscopy method is based on a relative number of bacterial morphotypes that appeared to be flexible and can be reorganized in the way to categorize findings into only two groups: normal and BV, which makes it comparable to dichotomous Amsel's clinical criterion.

Key words: diagnosis; microscopy; vaginal smears; vaginosis, bacterial.

Apstrakt

Uvod/Cilj. Bakterijska vaginoza (BV) je čest uzrok vaginalne nelagodnosti kod žena. Cilj ovog istraživanja bio je poređenje Nugent-ovog sistema vrednovanja mikroskopskih preparata i nove mikroskopske metode koju smo uveli u našoj laboratoriji radi dijagnoze BV. **Metode.** Istraživanjem je obuhvaćeno 705 asimptomatskih trudnica između 24. i 28. nedelje trudnoće. Stepenn slaganja između metoda je određivan *kappa* (κ) indeksom. Senzitivnost, specifičnost, pozitivna i negativna prediktivna vrednost nove mikroskopske metode su poređene sa Nugent-ovom metodom kao

standardom. **Rezultati.** Na osnovu sistema vrednovanja obe metode, po Nugent-u i nove mikroskopske metode, BV je dijagnostikovana kod 21%, i 25% žena, redom. Bez obzira na razlike između dijagnostičkih kriterijuma, koje su se uglavnom odnosile na klasifikaciju intermedijarnih rezultata, stepen slaganja između kategorija, određen *kappa* indeksom, bio je zadovoljavajući: Nugent-ov i novi mikroskopski metod su pokazali dobro slaganje ($\kappa = 0,68$), dok su Nugent-ov i novi mikroskopski metod bez intermedijarnih rezultata, pokazali veoma dobro slaganje ($\kappa = 0,83$). Takođe, pokazali smo da je u poređenju sa Nugent-ovom metodom, kao zlatnim standardom, nova mikroskopska metoda imala

visoku senzitivnost i specifičnost (od 75% do 99,3%), kao i dobru pozitivnu i negativnu prediktivnu vrednost (od 88,8% do 99,5%). Naša metoda je bazirana na relativnom broju bakterijskih morfotipova, bilo štapićastih formi ($> 1,5 \mu\text{m}$, *lactobacilli*), ili neštapićastih formi ($< 1,5 \mu\text{m}$, bakterije udružene sa BV) pod $200\times$ uvećanjem, što povećava površinu preparata koji se pregleda, ali bez produžavanja vremena za koje posmatrač pregleda preparat. **Zaključak.** Nova mikroskopska metoda se dobro podudarila sa Nugent-ovim sistemom skorovanja ukazujući na to da se može koristiti

kao alternativna mikroskopska metoda u dijagnostici BV. Novi mikroskopski metod je baziran na relativnom broju bakterijskih morfotipova i pokazao se fleksibilnim u smislu reorganizovanja tako da se sve kategorije uzoraka klasifikuju u samo dve grupe: normalan nalaz i BV, što ga čini komparabilnim dihotomnom kliničkom kriterijumu po Amsel-u.

Ključne reči:
dijagnoza; mikroskopija; vaginalni brisevi; vaginoza, bakterijska.

Introduction

The main constituents of a healthy vaginal microbiome are *lactobacilli*. The protective role of lactobacilli is reflected in their ability to antagonize with other bacteria for adherence to the vaginal epithelium as well as to synthesize antimicrobials (hydrogen peroxide, lactic acid, bacteriocins) which suppress the growth of pathogenic microbes¹⁻³. Any decrease in the number of *lactobacilli* can result in disturbance of vaginal microflora and subsequent development of bacterial vaginosis (BV). The composition of BV is complex. Molecular analysis has shown that BV is not a monobacterial disorder but can be caused by many microbes such as *Gardnerella vaginalis*, *Prevotella spp*, *Atopobium spp*, *Mobiluncus spp*, *Sneathia sanguinegens*⁴. Quite often, BV can be asymptomatic, which can make this disorder insidious in regard that it can cause obstetric and gynaecological complications without warning. Some of the consequences of BV can be premature birth, or increased risk to encounter additional infection (*Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, HSV2, and HIV)⁵⁻⁷.

This disorder can be diagnosed under various criteria (clinical or microscopic) introduced by Amsel et al.⁸, Nugent et al.⁹, Ison and Hay¹⁰, and Verhelst¹¹ (the first two of four are widely accepted as "golden" standards in BV diagnosing, clinical and microscopic, respectively). The method of Amsel et al.⁸ was mainly based on clinical findings and properties of vaginal discharge. According to the Amsel's criterion, a patient is positive for BV when 3 of 4 criteria are present (vaginal pH above 4.5, "milk-like" white-grayish vaginal discharge, positive whiff test, and clue cells on microscopic examination). Nugent et al.⁹ categorize the microscopic findings of Gram-stained vaginal smears by quantification of some of the present morphotypes, *Lactobacillus*, *Gardnerella-Bacteroides*, and *Mobiluncus* into: normal, intermediary, and BV. The Ison/Hay system is based on the observation of Gram stains to estimate the qualitative ratios of the observed morphotypes rather than the exact number of bacteria. In order to obtain a more precise classification, two additional categories have been introduced to Ison/Hay criteria, group 0 – without bacteria, and group IV – with a large amount of Gram-positive cocci¹⁰. Further modification by Verhelst et al.¹¹, using culture and molecular identification of vaginal microbiota, introduced even more categories, subdividing

grade I samples in several additional categories: Ia, Ib, Iab, I-like, I-PMN, regarding the relative concentration of Gram-positive rods (*lactobacilli*) and BV-associated bacterial morphotypes^{11,12}.

Although widely used, all of these methods mentioned above had certain insufficiencies. The method of Nugent et al.⁹ categorizes the smears by quantification of bacterial morphotypes, which demands noticeable time and skill of an observer (experienced microbiologist). Additionally, the Nugent-scoring system includes only three bacterial morphotypes and, therefore, it may not match the heterogeneity and complexity of the vaginal microflora. Albeit that Ison and Hay¹⁰ and Verhelst et al.¹¹ had overcome some deficiencies of the method of Nugent et al.⁹ by introducing qualitative assessment of vaginal smears, their method is still based on observation of small slide area (under $1,000\times$ magnification). Observing 5–20 fields of view under the $1,000\times$ magnification the actual scanned surface makes only a tiny fraction of the slide surface, thus being a source of sampling error^{13,14}.

In regard to overcoming some insufficiencies of previously mentioned criteria: time-consuming, a complicated numerical summing with narrow intervals, a need for experienced personnel, a demand for standardizing surface of the microscopic field of view, and evaluation of only three bacterial morphotypes, we established a novel method of microscopic examination of Gram-stained vaginal smears based on qualitative examination of preparations under $200\times$ magnification¹⁵. The categorization system of our method refers to six groups: three normal and three BV, which can make an easier comparison of microscopic method and dichotomous clinical assessment of samples such as the method of Amsel et al.⁸. To test its value, we compared our method to the already established Nugent's method.

Methods

Study population and design

This prospective study comprised of 705 pregnant and asymptomatic women between 24 and 28 weeks of pregnancy, seen during regularly planned appointments at the Military Medical Academy, Belgrade, from 2012 to 2014. Patients younger than 18 and older than 40 years, patients with multiple pregnancies, anomalies of the uterus,

cervical conization, or patients with previous preterm delivery were excluded from this study. Patients who were under any kind of therapy two weeks before the examination, as well as patients who had sexual intercourse a week before the appointment, were also excluded from the study. The institutional Ethics Committee approved the study protocol, and all study subjects agreed to participate through written informed consent.

Sampling and data collection

The specimens were prepared under standard ethical and laboratory protocols. After clinical examination, vaginal samples were collected by inserting a sterile dacron-tipped swab into the vagina. The swab was rolled round through 360 degrees against the vaginal wall at the mid-portion of the vault and carefully withdrawn to prevent contamination. Swabs were then smeared on a plain glass slide and air-dried at room temperature. The slides were Gram-stained and categorized according to Nugent's criteria (viewed under immersion, 1,000× magnification) and novel method of microscopic examination (viewed under immersion, 200× magnification), which will be further denoted here as the criterion of Nenadić et al.¹⁵.

Analysis of data

Nugent scoring system implies categorization of Gram-stained smears into three groups regarding morphotypes of bacteria under microscope 1,000× magnification. Morphotypes are scored by their presence/absence as the average number seen per oil immersion field (5–20 fields)⁹. For example, if more than 30 *Lactobacilli* are recognized in the visual field, the score is 0; if no *Lactobacilli* are detected, the score will be 4 points. If *Gardnerella*-like bacteria are absent, the score is 0; if more than 30 are observed, the score will be 4. The presence of other microorganisms, such as *Mobiluncus*, can add additional 2 points. According to the final score, all findings are designated as follows: I-normal (0-3), II-intermediate (4-6), and III-bacterial vaginosis (7-10). The scoring system of Nenadić et al.¹⁵ is based on the examination of Gram-stained vaginal smears under 200× magnification and their categorization depending on the presence of either rod forms (RFs) or non-rod forms (NRFs). The shortest length still observable as a rod at the 200× magnification is 1.5 µm. Based on this fact, under 200× magnification, there are no obstacles to recognizing the predominance of either RFs (> 1.5 µm, *Lactobacilli*) or NRFs (< 1.5 µm, bacterial vaginosis associated bacteria). The number of RFs and NRFs was estimated semi-quantitatively in the following way: numerous bacteria, covering the most of slide surface between, around, and over epithelial cells, were labelled as “full”; bacterial forms rare or absent between, but found mostly around and on epithelial cells were designated as “mid”; the absence of bacterial forms with only rare elements seen around and on epithelial cells were termed as “empty”. According to the predominance of either RFs or NRFs, each of these three categories was

additionally subdivided into a normal (N) and bacterial vaginosis (BV) subgroup, respectively. In this way, all slides were categorized into 6 groups. Three out of those six were normal: normal full – NF, normal mid – NM, and normal null – NN. The other three were bacterial vaginosis varieties: BV full – BVF, BV mid – BVM, and BV null – BVN. For the purpose of the study, Nugent's score was taken to be the gold standard.

With the aim to compare our results with Nugent's as “golden” standard, we grouped our findings into the following groups: six groups by novel microscopy method (NMM): NF, NM, NN, BVF, BVM, BVN, three groups by NMM (NF and NM were considered as normal, NN and BVN as intermediate, and BVM and BVF as BV), and two groups by NMM (N-normal: NF+NM+NN, and BV-bacterial vaginosis: BVF+ BVM+BVN).

Statistical analysis

Complete statistical analysis was conducted with commercially available statistical software SPSS v17.0. Variables were presented as frequencies of individual parameters (categories), and the statistical significance of differences was evaluated using the χ^2 test. The degree of agreement between categories (scale of measurement) was determined by the *kappa* index. Sensitivity and specificity were calculated in an ordinary manner. Statistically, a significant difference was evaluated on a minimal level $p < 0.05$.

Results

Based on NMM and Nugent's scoring, bacterial vaginosis was diagnosed in 25% and 21% of women, respectively. The normal finding was observed in 75% of women by NMM, and 63% by Nugent, while 16% of patients were classified as intermediate under Nugent.

According to the χ^2 test association has been found between NMM and Nugent et al.⁹ categorization ($\chi^2 = 669.800$; $df = 10$; $p < 0.001$). When we observed the group with normal findings, the best association has been found between groups with the intermediary result by Nugent et al.⁹ and NF (normal full by NMM, 96.0%), and between intermediary group by Nugent et al.⁹ and NM (normal mid by NMM, 80%) (Figure 1). The group with the intermediary result by Nugent et al.⁹ was in a significant association with BVN (bacterial vaginosis normal, 57%) and NN (normal null, 44%). BV group has shown the best association with BVF (bacterial vaginosis full, 99%) and BVM (bacterial vaginosis mid, 78%). It can be observed that around half of patients from Nugent's intermediary group were grouped as NMM's NULL groups (hypocellular: NN+BVN).

In Figure 2, it can be seen that the intermediary group under NMM was formed by adding NN (normal null) to BVN (bacterial vaginosis null), considering that the majority of intermediary patients by Nugent et al.⁹ were contained within these groups (Figure 1). It was shown that the best association

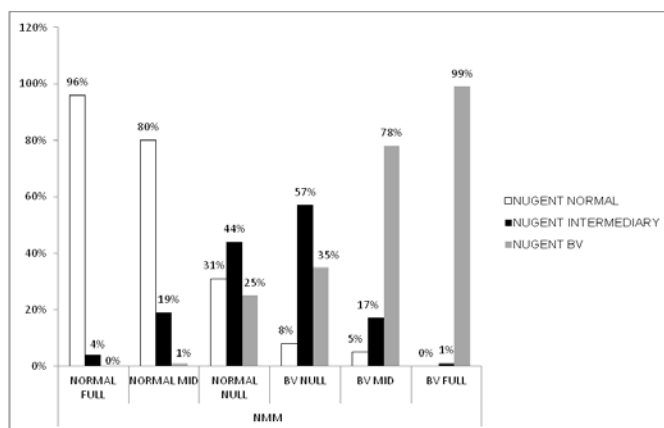


Fig. 1 – Comparison of six groups of results classified by the novel microscopy method (NMM) and Nugent’s criteria. BV – bacterial vaginosis.

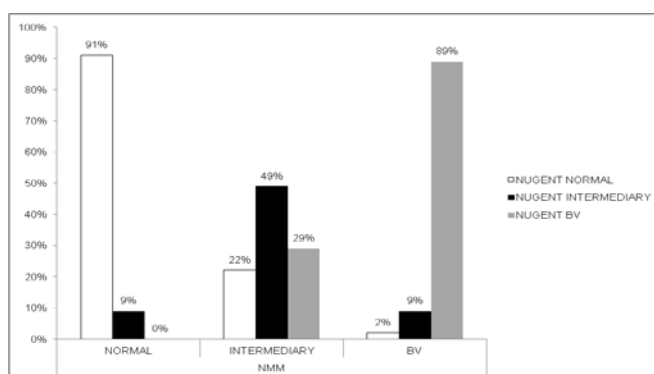


Fig. 2 – Comparison of results classified by the novel microscopy method (NMM) into 3 groups [normal, intermediary, and bacterial vaginosis (BV)] and Nugent’s criteria. The intermediary group of NMM was formed by adding normal null (NN) to the BV null group of NMM.

($\chi^2 = 634.442$; $df = 4$; $p < 0.001$) was found between patients with normal finding (91%) and those with bacterial vaginosis (89%) (Figure 2). On the other hand, the weakest association was observed in patients with the intermediary result (49%).

When we observe results presented in Figure 3, the best association was found in groups with normal finding (82%)

and groups categorized as BV (74%) ($\chi^2 = 437.40$; $df = 2$; $p < 0.001$). Twenty-two percent of the intermediary group determined by the method of Nugent et al.⁹ was categorized as BV according to NMM, while 14% of intermediary findings determined by the method of Nugent et al.⁹ was classified as NMM's normal group.

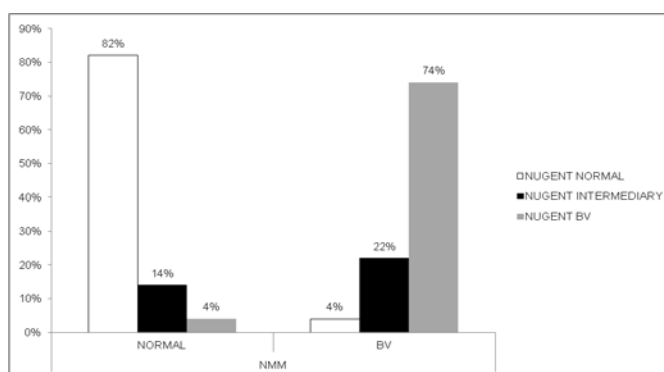


Fig. 3 – Comparative results of categorization by the novel microscopy method (NMM) and the Nugent’s method. NMM reorganized results into two groups: patients with normal findings and bacterial vaginosis (BV).

The group with normal findings, under NMM, was formed by summarizing all groups with normal findings: NN (normal null) +NM (normal mid) +NF (normal full).

Table 1 shows mutual agreement of overall results from our study under Nugent's criteria and NMM presented through the kappa index ($\kappa = 0.68$; $p < 0.001$, good agreement) (Table 1) and mutual agreement of results classified under NMM and Nugent's criteria, when IMD (intermediary) group was excluded (Table 1). The greatest discrepancy was observed among IMD results. Almost half of IMD cases, according to Nugent et al.⁹, 41/113 (36.3%) and 11/113 (9.7%), were placed into the normal and BV group by NMM, respectively. Furthermore, 35/148 (23.6%) of BV findings determined by the method of Nenadić et al.¹⁵ were designated as IMD, while 26/444 (5.9%) of normal cases determined by the method of Nugent et al.⁹ were grouped as IMD, according to NMM. Moreover, three normal cases determined by the method of Nugent et al.⁹ were classified as BV by NMM, and two BV according to the method of Nugent et al.⁹ were categorized as normal according to the method of Nenadić et al.¹⁵. On the whole, Nugent's and NMM criteria diverged in 118/705 (16.7%) of cases. Finally, when the results were analyzed after removal of the IMD group (Table 1), an increase in agreement among two different criteria was observed (the kappa index rose from 0.68 to 0.83).

Sensitivity, specificity, positive and negative predictive value of NMM, compared to the Nugent's score as standard, is given in Table 2. The intermediary score, grade II, was

considered either positive, negative, or excluded. In the case when the intermediary score was considered negative/normal, the sensitivity and specificity of the Nenadić et al.¹⁵ criterion was lower, but still high, with high positive and negative predictive values. When IMD and BV samples were analyzed as one group, the sensitivity of NMM increased from 75.0% to 94.6% without significant loss of specificity. The positive and negative predictive values remained high. Finally, when we excluded IMD findings from our analysis, we found that 111 of 113 BV samples according to the method of Nugent et al.⁹ and three of 419 N samples according to this method met positive NMM criteria for BV. Thus the sensitivity and specificity of NMM were very high, (98.2% and 99.3%, respectively), as well as positive and negative predictive values (97.4% and 99.5%, respectively).

Discussion

The human vaginal microbiome is very important for the health of women. It can be changed by hormonal status (it is not the same before puberty, during the reproductive period, or among menopausal women), certain sexual behavior, and it varies according to ethnic affiliation. Nevertheless, although BV may appear at any age, it is the most frequent in the reproductive period. The

Table 1

Mutual agreement of overall results classified by the Nugent's method and novel microscopy method (NMM) presented through the Kappa index (κ), and mutual agreement of the Nugent's method and NMM, when intermediary (IMD) findings were excluded from the Nugent's categorization presented through the Kappa index (κ) as well

Nugent's method	NMM			NMM without IMD	
	N	IMD	BV	N	BV
N	415	26	3	416	3
IMD	41	61	11		
BV	2	35	111	2	111
	$\kappa^7 = 0.68, p < 0.001$			$\kappa^7 = 0.83, p < 0.001$	

N – normal finding; BV – bacterial vaginosis.

Table 2

Sensitivity (SN), specificity (SP), positive and negative predictive values (PPV and NPV, respectively) of the novel microscopy method (NMM), compared to the Nugent's method as standard, in the next order: when intermediary (IMD) findings were added to normal (N) Nugent's findings, when IMD findings were added to the bacterial vaginosis (BV) findings determined by the Nugent's method, and when Nugent's IMD findings were excluded from data analysis

Nugent's method	NNM						
	N	BV	Total	SN (%)	SP (%)	PPV (%)	NPV (%)
IMD considered N							
IMD/N	543	14	557				
BV	37	111	148	75.0	97.5	88.8	93.6
Total	580	125	705				
IMD considered BV							
N	416	29	445				
BV/IMD	43	217	260	94.6	93.5	89.5	96.7
Total	459	246	705				
IMD excluded							
N	416	3	419				
BV	2	111	113	98.2	99.3	97.4	99.5
Total	418	114	532				

most striking event in shifting of healthy vaginal environment towards BV is the substitution of dominant *Lactobacilli* by a mixture of mainly anaerobic bacteria such as *Gardnerella vaginalis*, *Atopobium vaginae*, and *Prevotella spp* ^{16–18}. Adequate diagnosis of BV is demanding, and choosing the right method for its diagnosis requires a review of hardly explicable results such as intermediary results ¹⁹. Although there are many criteria and mutually comparable scoring systems, it is not convincing that they will always classify the same category of patients. As is well known, demonstration of infectious agent existence is often a basic criterion in diagnosing the infective disease. This is not the case with BV since the real cause of the disorder is not yet defined. Thus, the patient must meet clinical or laboratory criteria which do not consider the presence or quantity of a specific bacterium. It is important to keep this in mind when comparing different diagnostic methods. BV does not evolve from a commonly defined bacterial infection caused by one agent but can rather be compared to consequences caused by anaerobic mixed flora in other parts of the organism. Diagnosis based on diagnostic criteria is actually the weighting of criteria to provide the best possible agreement between the criteria and the presence of BV. It is important that the examiner, whether a clinician or laboratory technician, is well trained and able to evaluate the clinical adequacy of the diverse methods available for BV diagnosis.

Clinically, BV is usually diagnosed by physical examination, pH of vaginal discharge, whiff test, and presence of clue cells which represents the diagnostic system proposed by Amsel et al. ⁸ in the early 1980s, the Nugent's criterion is the method mostly used for diagnosing BV, and it is considered to be the golden standard among microscopic methods ¹³. However, its score intervals are very narrow, differing in only a few bacteria, and the observed number of bacterial morphotypes may vary depending on the examiner. The homogeneity and thickness of the specimen may be influenced by the way of spreading the sample on the glass slide ¹⁴. To avoid demanding counting of bacterial morphotypes, a qualitative microscopic examination was introduced by Ison and Hay ¹⁰ and Verhels et al. ¹¹. These methods give an advantage in saving the observer's time and more precise differentiation of *Lactobacillus* morphotypes, but on the other hand, they examine small microscopy fields, which can influence the results (because of unequally scattered smears over the slides).

In our institutions, clinical examinations, as well as microscopy, are in routine use in the diagnosis of BV, but often there is neither sufficient time nor expertise available to practice the quantitative scoring systems. Therefore, the main goal of our study was to validate simpler grading schemes for microscopic diagnosis of BV, previously described by Nenadić et al. ¹⁵ (novel microscopy method), against the established reference method introduced by Nugent et al. ⁹.

Comparing the novel microscopy method with the reference method by Nugent et al. ⁹, we also demonstrated

high sensitivity, specificity, positive and negative predictive values, and kappa indexes for the novel microscopy method. Generally, results of the present study indicated that both methods, that of Nugent et al. ⁹ and novel microscopy method are factually similar and nominate our qualitative assessment of the vaginal microbial flora as an alternative method in diagnosing BV.

What is more, the novel microscopy method has several advantages compared to other methods of microscopic diagnosis of BV. The principal difference between previous methods and the novel microscopy method is that previously established criteria use microscopic observation under 1,000× magnification, while the novel method is based on slide examination under 200× magnification. It is obvious that examining Gram-stained samples under 200× magnification comprises a much greater area than under 1,000× magnification. Actually, according to Nugent et al. ⁹ (1,000× magnification), we observe 5–20 fields from a total of 17,143 fields, while when viewed under 200×, according to novel microscopy method, we monitor 100–150 fields from a total 686 fields ¹⁵. Besides observing a bigger surface, analysis can be done in 5–10 min, and we do not need to include burdensome counting of individual bacteria like under Nugent's criteria.

However, regardless of the microscopic method used for diagnosing BV, for an accurate diagnosis of the disease, it is necessary to evaluate the clinical aspects and clinical adequacy of diverse methods available. Besides various methods currently used, clinicians still have difficulties deciding for patients about patients with BV that should be treated. What makes this decision even more difficult are discrepancies in the classification of intermediary findings. It has been shown that the composition of intermediary flora is divided among *Lactobacilli* and bacteria associated with BV, which is the main reason why the intermediary “phase” is considered the “transient phase” between the healthy vaginal microbiome and BV ^{20, 21}. From our study, we could indirectly assume that most of the patients with intermediary findings, according to Nugent et al. ⁹, actually belonged to the group with a low number of bacterial forms. A possible explanation for these “illogical” results lies in the narrow intervals in Nugent's categorization criterion. For example, in the original Nugent's criterion, counting is performed on 5–10 visual fields under the magnification 1,000x, notified as an interval on an ordinary scale (in the range from 0–1,000,000 bacteria *per* visual field). Evaluation of bacterial numbers in intervals is carried out assuming that the number of bacteria from 1–30, counted on part of the visual field, can be used for approximate bacterial number estimation on the entire visual field. What was illogical in Nugent's categorization is that patients with 4 or fewer bacterial forms were assigned as 0, 1, or 2 points, while patients with the bacterial number above 4 and above 30 were assigned with 3 and 4 points, respectively. Therefore, 0 points will be given only to those patients with the finding of 0 bacterial numbers on 5 observed visual fields. Accordingly, if we imagine a finding that is “clean” under the method by Ison and Hay ¹⁰ and if we did not find any *Lactobacillus* on 5 visual fields, the

patient would receive 4 points. If we also observed *G. vaginalis* in the latter patient case, with an average number of > 4 *per* field, this finding would be assigned with 3 additional points (total of 7 points) and categorized as BV. There is a possibility in reobservation of aforementioned Gram-stained smear to count bacteria slightly different with the average number of exactly 4 bacterial forms *per* visual field. In this case, a patient will be assigned 2 points instead of 3 points and categorized as an intermediary group by Nugent's criteria. Taking all previously mentioned into consideration, we can conclude that cases with small bacterial numbers could be tricky for observers, which is furthermore complicated by narrow diagnostic criteria. Given that a low number of bacteria does not mean the absence of disorder (inflammation and potential risk of miscarriage), we should pay more attention to these groups: "clean" under methods by Ison and Hay¹⁰, intermediary according to Nugent et al.⁹, and NN and BVN under the novel microscopy method. The reasons could be various, but we will try, on practical examples, to discuss some of them.

In the repeated observation of preparations, probability of analyzing the same 10 visual fields is almost nonexistent. During the first examination, we can see small bacterial numbers and categorize patients as intermediary results. While we observe the same sample again, there is a possibility to assign preparation with 7 points (BV), which means – the lesser the cellularity, the greater the chance to misinterpret the finding through repeated observation. If we involve additional observers, the likelihood of different preparation "reading" can become even higher. It is important to stress another yet observed rule from our study: homogeneity of preparation is proportional to cellularity. For example, according to the novel microscopy method, the highest homogeneity is noted in patients with BVF and NF. According to our investigation, the probability of finding BVN areas in BVF preparations was very low (under 5%), while the possibility of finding BVN areas in BVM preparations was slightly higher (10%–15%). However, in our opinion, the Nugent's criterion has two crucial advantages concerning other diagnostic criteria: first, as we said before, it is well established and widely used criterion because of its simplicity (golden standard); and second, Nugent et al.⁹ have an intermediary group (compared to Amsel et al.⁸). We cannot diminish the significance of the intermediary group without an explanation.

In accordance with presented findings from our study, we have clearly shown that tested methods are reliable in diagnosing extreme categories, either BV negative or positive,

but the problem arises in the classification of the intermediary group, the most difficult to interpret. As we have shown, the difference in the percentage of IMD patients is significant (16% according to Nugent et al.⁹). Besides the fact that the largest number of disagreements was observed in IMD samples, the kappa value was not low because differences occurred between successive categories but not between extreme categories. Therefore, excluding IMD patients from our analysis, we found nearly perfect agreement between tested criteria, with very high sensitivity, specificity, positive and negative predictive values. By shifting criteria, in order to conjoin IMD findings to normal or BV, both sensitivity and specificity have been decreased, confirming that IMD samples truly represent an intermediary status between normal and BV.

These findings suggest that the Nugent's method seems to recognize a higher number of positive cases compared to other methods for scoring Gram-stained samples. Moreover, other studies indicated that, compared to the method by Amsel et al.⁸, the Nugent's criterion can overestimate the real prevalence of BV and may even interpret healthy individuals to be diseased^{22–24}. Nevertheless, measuring agreement between two sets of criteria usually take one set as a working definition of the disease, but it is unable to determine superiority between them because of the basic differences between these two methods. What further complicates the problem of BV diagnosis is that the Amsel's method, mainly based on clinical findings, is dichotomous, having only two categories, whereas microscopic methods allow assessment of variation in vaginal microflora as a continuum and have three or more categories. Therefore, it is of great importance to provide the best possible agreement between clinical and/or laboratory criteria and the presence of BV. In line with that, to improve clinical adequacy of BV diagnosis revised set of criteria that combines clinical and microbiological parameters is needed.

Conclusion

The novel microscopy method scoring system seems to constitute a good classification method, as it allows the microscopist to formulate an impression based on the relative numbers of RFs and NRFs morphotypes while the influence of the surface area and bacterial density are lessened. Furthermore, the novel microscopy method is flexible and can be reorganized in the way to categorize findings into only two groups: normal and BV, the fact that may have important clinical implications.

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Comparative histochemical and morphometric analysis of muscle fibers of the *psaos* muscle in individuals of both genders with aging

Usporedna histohemijska i morfometrijska analiza mišićnih vlakana psoasnog mišića kod osoba oba pola tokom starenja

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Abstract

Background/Aim. There is a reduction of the *psaos* muscle with aging, and histopathological analysis (HPA) on *postmortem* material also shows its significant changes with advancing age. The aim of this study was to determine the presence and distribution of types I and II muscle fibers in the human *psaos* muscle in individuals of different ages and genders. **Methods.** The material consisted of tissue samples of the right *iliopsoas* muscle taken from 30 adult cadavers (18 males and 12 females), aged from 30 to 90 years, divided into three age groups. The material was obtained from the Institute of Forensic Medicine, Faculty of Medicine, University of Niš, Serbia. Hematoxylin and eosin (H&E) staining was used in the HPA of muscle cells. **Results.** The values of astereological parameters (area, perimeter, and Feret's diameter) of type I and type II muscle fibers were greater in male cases in comparison with female ones, although with no statistically significant difference. Based on the histochemical and morphometric analysis, it was concluded that, after 70 years of life, a loss of type II muscle fibers occurred, which was more conspicuous in female cases. **Conclusion.** During aging, the loss of type II muscle fibers, as well as the atrophy of type I and type II muscle fibers, demonstrate similar dynamics in both genders.

Key words:

age factors; histological techniques; muscle atrophy; muscle, skeletal; muscle fibers, skeletal; *psaos* muscles; gender factors.

Apstrakt

Uvod/Cilj. Veličina psoasnog mišića smanjuje se sa starenjem i histopatološka analiza (HPA) postmortalnog materijala takođe pokazuje značajne promene u njemu u zavisnosti od godina života. Cilj istraživanja bio je da se utvrdi prisustvo i distribucija mišićnih vlakana tipa I i II psoasnog mišića kod osoba različitih starosnih kategorija i različitog pola. **Metode.** Materijal su činili tkivni uzorci desnog bedrenoslabinog mišića 30 odraslih kadavera (18 muških i 12 ženskih), starosti od 30 do 90 godina, raspoređenih u tri starosne grupe. Materijal je dobijen sa Instituta za sudsku medicinu, Medicinskog fakulteta Univerziteta u Nišu, Srbija. Hematoksilin-eozin (H&E) bojenje je korišćeno u HPA mišićnih ćelija. **Rezultati.** Vrednosti astereoloških parametara (area, perimetar i Feretov dijametar) mišićnih vlakana tipa I i tipa II bile su veće kod kadavera muškog pola u poređenju sa kadaverima ženskog pola, ali bez statistički značajne razlike. Na osnovu histohemijske i morfometrijske analize zaključeno je da nakon navršene 70. godine života dolazi do gubitka mišićnih vlakana tipa II, što je kod kadavera ženskog pola bilo izraženije. **Zaključak.** Sa starenjem, gubitak mišićnih vlakana tipa II, kao i atrofija mišićnih vlakana tipa I i tipa II pokazuju sličnu dinamiku kod oba pola.

Ključne reči:

životno doba, faktor; histološke tehnike; mišići, atrofija; mišići, skeletni; mišići, skeletni, vlakna; mišići, slabinski; pol, faktor.

Introduction

The *iliopsoas* muscle, [*musculus (m.) iliopsoas*], belongs to the group of inner hip muscles. It consists of the *iliacus (m. iliacus)* and *psaos* muscles (*m. psaos*), and it functions as the chief flexor of the hip joint.

This muscle is specific since it represents a sort of connection between the trunk and lower extremity¹, and since it attaches to the vertebrae, it is responsible together with other muscles for the upright posture and ambulation of humans. Some of the studies in which the authors used computerized tomography imaging to examine the transversal section of

the *psaos* muscle reported a reduction of its size with aging, and histopathological analysis (HPA) of the muscle on *post-mortem* material also showed significant changes in the muscle with advancing age². Imamura et al.³ had similar results in 1983 studying the *quadriceps femoris* and plantar flexors of the foot. Morphometric analysis of the muscle was performed mostly on animal models^{4,5}.

Skeletal muscles consist of connective tissue sheaths enveloping them from the outside (*epimysium*) and numerous fascicles, enveloped and separated one from another by a connective tissue layer called *perimysium*. Muscle fascicles consist of a large number of myofibrils containing contractile proteins, actin, and myosin, arranged in a parallel fashion^{6,7}.

A muscle fiber is a multinuclear, syncytial unit shaped like an elongated, narrow and slender cylinder. The nuclei are subcapsular, and there are 4–6 of them per cell on the transversal section⁸.

Red muscles, with a greater mitochondrial and lipid content and greater capillary density, are by function intended to maintain posture or to be engaged during longer activities. The color of red muscles is the consequence of a relatively higher content of myoglobin compared to white muscles which contain fewer mitochondria but abundant glycogen, which makes them better suited for anaerobic respiration and sudden and occasional contractions⁸.

Human muscles contain red and white muscle fibers arranged in a typical, combined mosaic pattern, resembling a chessboard. Depending on anatomical localization and function, the proportions of type I and type II muscle fibers vary but type II fibers are, nevertheless, predominant with 60%–65%, compared to 35%–40% for type I fibers⁹. Type II muscle fibers are darker in color, while type I fibers are of a paler color^{9,10}.

In the earliest studies investigating the difference between type I and II muscle fibers between genders¹⁰, it was concluded that some of the fibers are larger in men. Type II muscle fibers are usually larger in men compared to type I fibers, in contrast to women in whom type I fibers have an equal or greater diameter compared to type II muscle fibers.

In 1970, in the study by Brooke and Kaiser¹⁰, who investigated biceps muscles, conclusions that are still valid in general were drawn about the gender difference in skeletal muscles. The investigation of the *vastus externus* muscle by Lexell et al.¹¹ in 1988 and Kobayashi¹² in 1991 did not demonstrate any significant differences in the diameter between type I and type II muscle fibers in men and women. As for the *biceps brachii*, a much higher percentage of type II fibers is present in men, while in women, the percentages of both types are about equal. In contrast, the prevalence of type I and II fibers of the *vastus externus* muscle is approximately the same in both genders¹³.

It is thought that the established difference in the size of muscle fibers between men and women is determined by the fact that men are relatively higher and heavier than women, with a larger muscle mass, and are more physically active. Androgenous hormones also play a role in the size of muscle fibers^{14,15}. The difference between muscle fibers in men and women depends on the examined muscles as well¹⁶.

The effects of physical exercise and training on the muscle system have been investigated in numerous studies^{13,17}. The results of these studies are in general contradictory, but some general principles do exist. It is evident that physical exercise and training of any kind increase the diameter of muscle fibers. In essence, anaerobic activities lead to hypertrophy of type II muscle fibers, which is frequently seen in sprinters. In long-distance runners, in whom aerobic metabolism is more significant, type I muscle fibers are usually larger.

Power training, for instance, weightlifting, produces significant hypertrophy of type II fibers and lesser (if any) hypertrophy of type I muscle fibers¹⁸. It is well known that sprinters in general have a greater number of type II muscle fibers compared to sedentary controls, and long-distance runners have a greater number of type I fibers compared to untrained individuals.

Many authors believe that these two groups of runners have a genetically determined composition of muscles as to muscle fiber types and that muscle fiber type conversion is negligible if it occurs at all¹⁷.

In the process of aging, starting from the sixth decade and after 70 years of age, skeletal muscles structurally and functionally change so that after 75 years of age, the power of muscles is reduced by 30–50%. The reason behind this reduction is the reduction of muscle fiber diameter. At 75 years of age, the diameter is reduced by 80% compared to the age of 25 years. Since the power of contraction is not linearly associated with muscle fiber diameter but is proportional to the surface of muscle fiber transversal section, a diameter that is 80% of the normal diameter produces a 60% loss of muscle strength (contraction force)¹⁹.

Due to reduced elasticity, flexibility, and joint diseases of different intensity, older individuals are less active, which is associated with the loss of muscle volume and contraction force. This is supported by the fact that aging individuals have selective atrophy of type II muscle fibers¹⁹. The effects of poorer nutrition in the elderly have not been sufficiently studied, although it is well known that cachexia is associated with the atrophy of type II muscle fibers²⁰.

Based on the above-mentioned, there may be an association between aging and loss of muscle mass in both genders. Given the possible relationship between the process of aging and muscle fiber changes in that process in both genders, the aim of this study was as follows: to detect the presence and distribution of types I and II muscle fibers in the human *psaos* muscle of individuals of different ages and genders using the hematoxylin and eosin (H&E) method and to determine their morphological characteristics using immunohistochemical analysis and monoclonal antibody against myosin; to quantify the presence of the dynamics of types I and II muscle fibers in the human *psaos* muscle in both genders during aging, measuring volume density of the fibers, by way of stereological methods in the sections stained immunohistochemically and by the use of a monoclonal antibody against myosin; to quantify the changes in size and shape of muscle fibers in the human *psaos* muscle in both genders during aging, measuring the area, perimeter, and Feret's diameter.

Methods

The study was conducted at the Institute of Anatomy, Institute of Histology and Embryology, Institute of Pathological Anatomy, and Institute of Forensic Medicine, which represent the teaching and scientific bases of the Faculty of Medicine, University of Niš, Serbia.

The study material consisted of tissue samples of the right *iliopsoas* muscle taken from 30 adult cadavers (18 male, 12 female), aged 30 to 90 years, autopsied at the Institute of Forensic Medicine, Faculty of Medicine in Niš, in the period from January to April 2013. The study was conducted abiding by the ethical norms regulating the use of cadaveric material in biomedical research by the Ethics Committee of the University of Niš Faculty of Medicine (Decision no. 01-9337-18). Autopsy findings did not indicate the presence of any pathological changes or traumatic damage to the right *iliopsoas* muscle. The cadavers were divided into three age groups: first (I), with cases aged 30–49 years ($n = 10$); second (II), with cases aged 50–69 years ($n = 10$); and third (III), with cases aged 70 years and above ($n = 10$).

The samples of the right *psaos* major muscle sized 5×5.5 mm were taken utilizing the incision perpendicular to the muscle at the level of the mid-distance between the upper border of the twelfth thoracic vertebra (T12) and lower border of the fifth lumbar vertebra (L5).

Histological analysis

Histological analysis, as well as the identification of possible changes of the muscle fibers of the right *iliopsoas* muscle during aging, was based on light microscopy-based assessment of their properties. The tissue of the *psaos* muscle was fixed in 10% buffered formalin during the next 24 hours. The obtained paraffin molds of the *psaos* major muscle were used to obtain up to $5 \mu\text{m}$ thick tissue sections. We used classical H&E and Periodic Acid-Schiff (PAS) methods to identify the basic structures of the *psaos* muscle. The stained histological sections were then analyzed using light microscopy under $4\times$, $10\times$, and $40\times$ magnification. Digital images of the analyzed sections were obtained using a 1.3-megapixel digital camera.

Immunohistochemical analysis

Using immunohistochemical analysis, we established the presence of cells with a positive reaction to applied immunohistochemical markers. The ultravision LP-HRP polymer (Cat. No. TL-125HL) detection technique using a monoclonal antibody against myosin (anti-Myosin, Skeletal Muscle, Clone, MYSN02, Ready to Use, Thermo Scientific Lab Vision, Ca, 1:320) was used in analysis of muscle fibers type II.

Morphometric analysis of muscle fibers

Morphometric analysis of muscle fibers of the *psaos* major muscle was performed in 10 randomly selected visual

fields per each analyzed case (270 visual fields in total for 27 analyzed cases). Stereological analysis of type I and type II muscle fibers in the analyzed visual fields was performed by measuring their volume density using a multipurpose test system, M168. Astereological analysis of type I and type II muscle fibers was performed by measuring their area (A_{MI} and A_{MII}), perimeter (B_{MI} and B_{MII}), and Feret's diameter ($D_{FM I}$ and $D_{FM II}$) of the profile of transversally sectioned fibers.

Statistical analysis

Results are presented as mean values and standard deviation (SD). Independent samples *t*-tests were used to test statistical differences between two samples. The normality of the distribution was validated by the Kolmogorov-Smirnov test. Data were analyzed using SPSS 16.0 (Statistical package for the social sciences, version 16.0, SPSS Inc, Chicago, IL, USA).

Results

The morphological analysis involved histological analysis of transversal sections of the *psaos* muscle tissue stained with H&E and analysis of transversal sections of the *psaos* muscle tissue stained using the immunohistochemical ultravision LP-HRP polymer detection technique.

Classical fascicular structure of skeletal muscles was seen in transversal sections of *m. psaos*, stained with H&E, in all age groups. Connective tissue sheaths within the muscle had usual organization and contents; in the more abundant connective tissue of the *perimysium*, there were nerve elements, arterioles, and venules, while more tender connective tissue of the *endomysium* contained capillaries and rare cells with slender projections enveloping individual muscle cells.

Identification of type II muscle fibers in transversal sections of the *psaos* muscle was performed using the anti-MYSN02 antibody, and immunopositivity was seen as a brown, fine-grained reaction of the sarcoplasm.

In the first age group, MYSN02-immunopositive type II muscle fibers were found in groups, rarely as individual fibers, and between them, there were less numerous type I muscle fibers that did not show a positive reaction. In transversal sections of the muscle, type I and II fibers had an irregular, polygonal shape, with sharper angles seen with type II fibers. Both muscle fiber types were of similar diameter and with typically elongated or oval nuclei.

In the second age group, MYSN02-immunopositive type II muscle fibers were approximately equally prevalent as nonstained type I fibers. Type II muscle fibers were mostly polygonal, with sharp angles, in contrast to type I fibers which were more oval. In some transversal sections of the muscle, individual type II muscle fibers had a characteristic lamellar arrangement of myofibrils in the sarcoplasm, while in most type II muscle fibers immunopositivity manifested as a fine-grained reaction in the sarcoplasm.

In the third age group, MYSN02-immunopositive type II muscle fibers showed polymorphism of shape and thickness –

the cells were oval and with a smaller diameter compared to the nonstained type I muscle fibers. Immunopositive muscle fibers did not demonstrate regular distribution in the form of isolated groups such as in younger age groups; instead, they were irregularly distributed between the nonstained type I fibers.

In all studied age groups, a smaller number of MYSN02-immunopositive type II muscle fibers was seen in female cases, compared to male ones (Figures 1 a–f).

The Kolmogorov-Smirnov tests showed that data were

normally distributed. The average age of female cases was statistically significantly higher compared to male cases, ($T = 2$; $SS = 25$; $p = 0.01$) (Figure 2), which had a significant influence on the interpretation of observed gender-related differences when morphometric parameters of the *psoas* muscle fibers were concerned.

The average values of morphometric parameters of type I and type II muscle fibers in male and female cases are shown in Table 1.

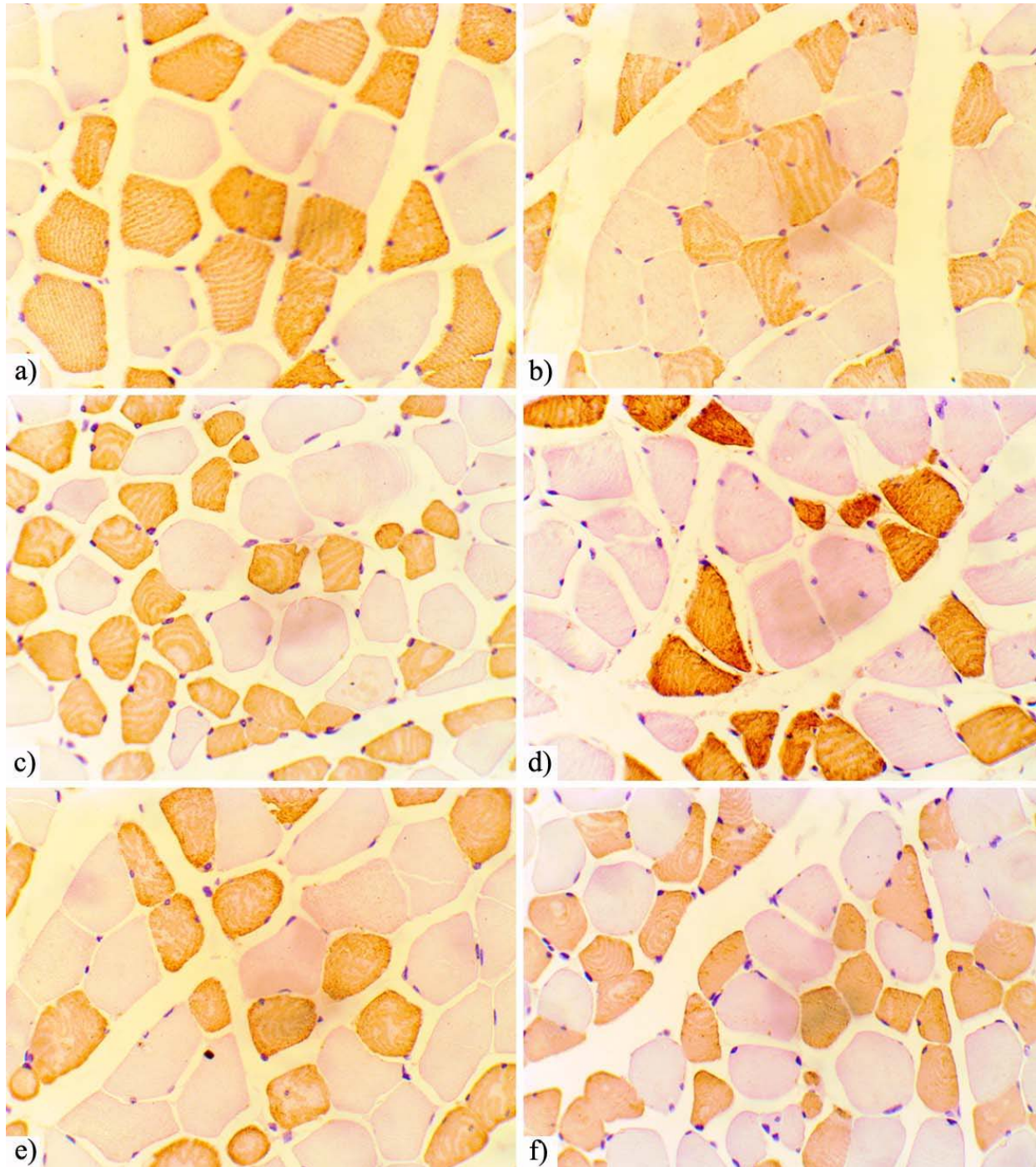


Fig. 1 – MYSN02-immunopositivity in transverse sections of the *musculus psoas*, by age groups and gender: a) a man aged 31 years; b) a woman aged 35 years; c) a man aged 52 years; d) a woman aged 56 years; e) a man aged 73 years; f) a woman aged 75 years. A reduction of the number and thickness of immunopositive type II muscle fibers can be seen with aging, predominantly in men, and a reduced number of type II muscle fibers in women, compared to men (LP-HRP, $\times 400$).

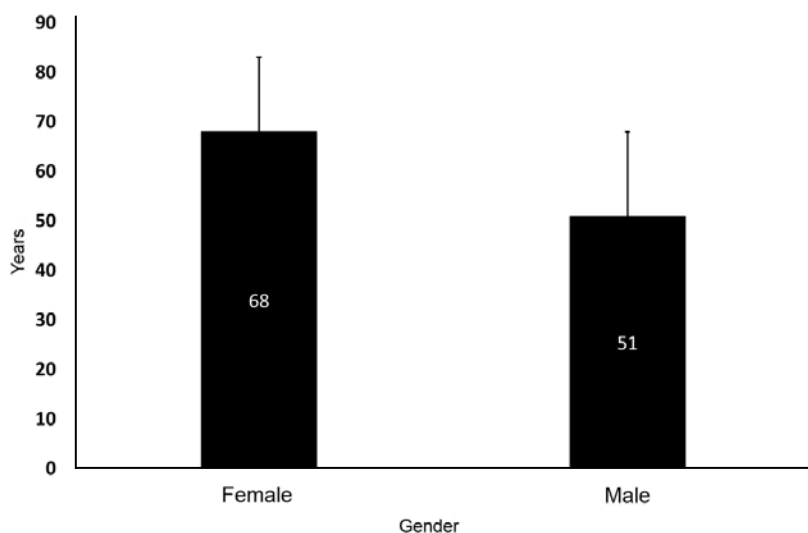


Fig. 2 – Average age of male and female cases.

Table 1

Average values of morphometric parameters of type I and type II muscle fibers in male (n = 14) and female (n = 13) cases

Parameter	Mean ± SD
V _{vI} (%)	42.57 ± 5.34
female	41.93 ± 8.28
male	42.57 ± 5.34
V _{vII} (%)	25.00 ± 5.63
female	30.54 ± 6.67
male	25.00 ± 5.63
AM _I (μm ²)	1,112.88 ± 234.29
female	1,318.85 ± 493.91
male	1,112.88 ± 234.29
BM _I (μm)	126.69 ± 14.83
female	136.34 ± 26.43
male	126.69 ± 14.83
D _{FM_I} (μm)	47.82 ± 6.16
female	51.50 ± 10.00
male	47.82 ± 6.16
AM _{II} (μm ²)	743.25 ± 223.62
female	931.14 ± 423.21
male	743.25 ± 223.62
BM _{II} (μm)	104.84 ± 17.43
female	117.58 ± 28.03
male	104.84 ± 17.43
D _{FM_{II}} (μm)	40.20 ± 7.06
female	45.27 ± 10.97
male	40.20 ± 7.06

V_{vI} – volume density of type I muscle fibers; V_{vII} – volume density of type II muscle fibers; AM_I – area of type I muscle fibers; BM_I – perimeter of type I muscle fibers; D_{FM_I} – Feret's diameter of type I muscle fibers; AM_{II} – area of type II muscle fibers; BM_{II} – perimeter of type II muscle fibers; D_{FM_{II}} – Feret's diameter of type II muscle fibers; SD – standard deviation.

The results of the *t*-test showed that there is no statistically significant difference ($p > 0.05$) between the genders regarding given parameters (average area, perimeter, and Feret's diameter of type I and II muscle fibers). The values in the male group were slightly higher than in the female group (Figure 3), suggesting a tendency for higher values in males, but future studies are needed to confirm that.

Finally, average volume density of type II muscle fibers was statistically significantly greater in male cases ($T = 2.34$; $SS = 25$; $p = 0.028$) (Figure 4). Nevertheless, since female cases were statistically significantly older than male cases, we could not decisively tell whether the observed difference was the consequence of aging or gender-related differences.

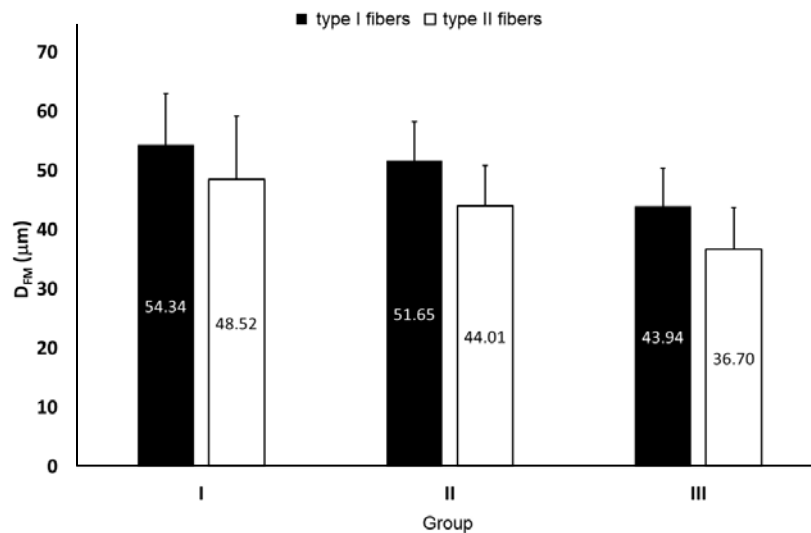


Fig. 3 – Average Feret's diameter of type I and type II muscle fibers (D_{FM}) in the analyzed age groups: I – aged 30–49 years; II – aged 50–69 years; III – more and equal to 70 years.

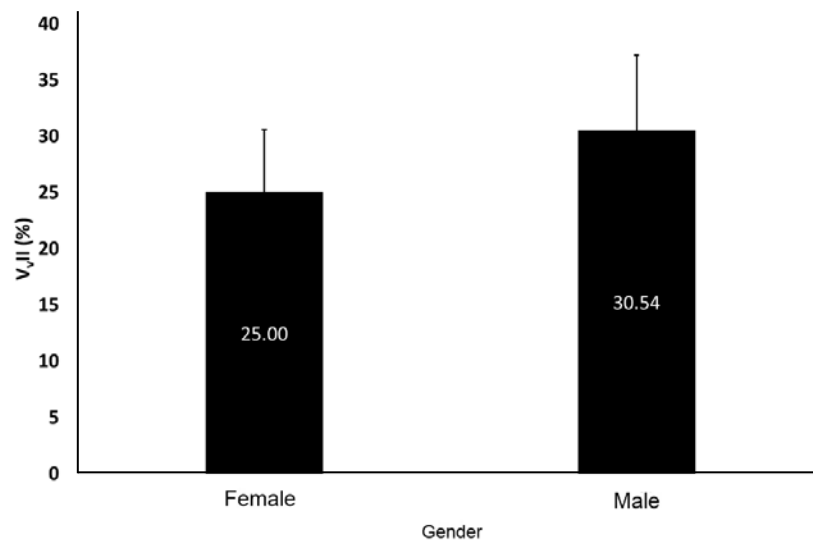


Fig. 4 – Average volume density of type II muscle fibers ($V_{v,II}$) in male and female cases.

Discussion

The results of our study represent a morphological and morphometric analysis of changes affecting the fibers of the *psaos* muscle during aging in cases of different ages and of both genders.

Morphological analysis of type I and type II muscle fibers of the *psaos* muscle demonstrated that in more advanced ages, changes affecting muscle fibers occurred, especially the type II ones. These changes were quantified in morphometric analysis, and after that statistically analyzed, which provided an insight into their prevalence in cases of different ages, monitoring of their dynamics, and analysis of their interrelatedness during the process of aging.

M. psaos major, the object of our study, is a lower extremity muscle. Its origin is complex and involves lateral parts

of the trunk and corresponding intervertebral discs from the twelfth thoracic to the fifth lumbar vertebra. Distally, the muscle joins the *iliacus* muscle forming the *iliopsoas* muscle, which attaches with its terminal tendon to the trochanter minor of the femur. The *m. psaos major* has a flexion role and is involved, together with adjacent muscles, in external rotation and adduction of the hip²¹. The *psaos* muscle, therefore, has an important dynamic and active postural function and belongs to lower extremity muscles which are important for everyday activities, such as walking, climbing the stairs, getting up from a chair, etc. Detection of age-related changes affecting the *psaos* muscle is thus of vital importance in the preservation of mobility and prevention of disability.

Arbanas et al.²¹ have immunohistochemically and morphometrically analyzed the samples of the *psaos* muscle from 15 men aged 18 to 35 years in order to study the

composition of muscle fibers. The age of this group of cases partly matches the age of the first group of our cases. The authors reported that the *psaos* muscle was mainly composed of type II muscle fibers (60%) – fast-twitching, glycolytic, and undergoing fatigue more quickly compared to type I fibers (40%) – slow-twitching, oxidative, and able to resist fatigue for longer periods of time. Similar to our own findings, they established that type I muscle fibers were characterized by a significantly greater transversal section area compared to type II fibers. Based on the composition of the *psaos* major muscle, Arbanas et al.²¹ concluded that this muscle had complex dynamic and postural functions. The results of our morphometric analysis revealed slightly greater volume density values of type I muscle fibers compared to type II fibers of the *psaos* muscle. This could indicate a possible predominance of type I fibers in our study. However, volume density is a stereological parameter, the value of which is influenced by the number and area of the analyzed structure (in this case, a corresponding muscle fiber type). The fact that type I fibers have a significantly greater area of the transversal section compared to type II fibers and that the prevalence of type I fibers was approximately the same as the prevalence of type II fibers can account for a slightly higher value of their volume density compared to type II muscle fibers in our first age group. Our results, therefore, demonstrate a similar prevalence of type I and type II muscle fibers in our first (youngest) age group, which agrees with the results obtained by Arbanas et al.²¹.

Histological changes in the skeletal muscles in older individuals are reflected in the reduction of muscle mass with simultaneous increase of fatty and connective tissue, as seen in our study as well. The size of type II muscle fibers is reduced, while the size of type I fibers remains unaffected. The reduction in the size of muscle fibers with advancing age can be attributed to the loss of myosin heavy chains^{20,22}. Moreover, the accumulation of "ring-like" and "torn" muscle fibers can be seen, then the accumulation of lipofuscin and non-myelinated rod-like structures, as well as the reduction of the number of blood vessels. Neuromuscular damage involves the increase of the size of the motor unit and the reduction of the number of motor neurons in the anterior horns of spinal cord gray matter. Furthermore, the process of aging is associated with the reduced production of new muscle fibers, as a consequence of the reduced activity of myosatellite cells. At the cellular level, muscle alterations associated with aging involve proliferation of the sarcoplasmic reticulum and T-tubular system and disorganization of sarcomeres, myofilaments, and Z-lines²³.

A significantly greater loss of type II muscle fibers compared to type I fibers can be explained by changes in the neuromuscular system associated with aging, as well as the reduction of number and function of myosatellite cells as myogenic stem-cells which may differentiate into new muscle fibers^{24,25}.

In our study, the obtained values of stereological parameters of type I and type II muscle fibers were greater in male cases compared to female ones, but these differences were not statistically significant. In addition, the average volume density of type I muscle fibers was greater in female cases, but again the difference did not reach statistical significance. In contrast, the average volume density of type II muscle fibers was statistically significantly greater in male cases. In the literature, there is information about a different distribution of the type I and type II muscle fibers in different genders¹⁰. It was emphasized that in men both muscle mass and muscle fiber size was greater than in women. Individual muscle fibers are larger in men than in women. Type II muscle fibers are usually larger in men, but type I muscle fibers are of equal or greater diameter in women. It was thought that the gender difference was the consequence of greater height and weight of men compared to women, with greater muscle mass and more physically active^{26,27}. The impact of male sex hormones cannot be neglected either. Androgenous hormones have an impact on muscle fiber size in men; it is well known that testosterone therapy leads to muscle hypertrophy¹⁵. Bennington and Krupp¹⁶ believed that some of the observed gender-related differences depended on the muscle which was analyzed. For instance, the *biceps brachii* muscle in men contains a markedly higher percentage of type II muscle fibers, while in women, this muscle contains similar percentages of these two muscle fiber types. In our study, we also observed certain differences in some of the muscle fiber parameters between male and female cases. However, with the exception of the volume density of type II muscle fibers, other differences in most of the parameters were not statistically significant. Since female cases were statistically significantly older than male cases, we could not decide with certainty whether the observed difference in the content of type II muscle fibers was the natural consequence of aging or if it was gender-related.

Conclusion

Based on the above elaborations of other authors' and our own study, the following conclusions may be drawn: the loss of type II muscle fibers is associated with a continual (and of similar intensity) atrophy of type I and type II muscle fibers in both men and women. The loss of type II muscle fibers, as well as the atrophy of type I and type II muscle fibers, demonstrate similar dynamics in both genders during aging. The values of stereological parameters (area, perimeter, and Feret's diameter) of type I and type II muscle fibers are higher in male than in female cases but without any statistically significant difference. The average volume density of type I muscle fibers is greater in female cases compared to male ones but with no any statistically significant difference.

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Diffusion-weighted magnetic resonance imaging in evaluating malignant lymph node invasion in patients with female genital neoplasms

Magnetonrezonantna sekvenca difuzionog kretanja u proceni metastatske invazije limfnih čvorova kod malignih tumora ženskih polnih organa

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Abstract

Background/Aim. Functional imaging, including diffusion-weighted magnetic resonance imaging (DWI MRI) and apparent diffusion coefficient (ADC) map, provides promising results in discrimination benign from malignant pelvic and inguinal lymph nodes in patients with gynecological malignancies. The aim of the study was to assess diagnostic performances of DWI in differentiation between benign and malignant pelvic and inguinal lymph nodes in patients with gynecological malignancies. **Methods.** The prospective clinical study was conducted at the Clinical Center of Vojvodina, Serbia, from 2013 to 2016, comprising 80 patients with malignant gynecological tumors. Preoperatively, all patients underwent MRI examination, followed by standard surgical treatment with complete pelvic and/or inguinal lymphadenectomy. A combination of ADC value criteria and size-based criteria yields MRI the following diagnostic performances in discrimination between benign and malignant lymph nodes: sensitivity 95%, specificity 92%, overall accuracy 92.5%, positive predictive value 46%, and negative predictive value 99.6%. Histopathological examination of surgically removed material and lymph nodes separated in

pelvic and inguinal anatomic groups was performed after the surgery. **Results.** A total of 2,320 lymph nodes were mapped and histopathologically examined in 80 patients included in the study. Metastases in lymph nodes were histopathologically confirmed in 28 (35%) patients. Measured ADC values were significantly lower in metastatic (mean \pm standard deviation (SD), ADC: $0.8725 \times 10^{-3} \pm 0.0125 \times 10^{-3} \text{ mm}^2/\text{s}$) than benign lymph nodes (mean \pm SD, ADC: $1.116 \times 10^{-3} \pm 0.1848 \times 10^{-3} \text{ mm}^2/\text{s}$; $p = 0.001$). If ADC value of $0.860 \times 10^{-3} \text{ mm}^2/\text{s}$ was determined as a cut-off value for discrimination between benign and malignant lymph nodes, DWI sensitivity was 89%, specificity 85%, and overall accuracy was 86%, positive predictive values 30%, and negative predictive value 99%. **Conclusion.** DWI MRI sequence is a fast, simple, non-invasive method that aids significantly to MRI diagnostic performances in discrimination between benign and malignant pelvic and inguinal lymph nodes.

Key words: diagnosis, differential; diffusion magnetic resonance imaging; genital neoplasms, female; lymph nodes; lymphatic metastasis; magnetic resonance imaging.

Apstrakt

Uvod/Cilj. Funkcionalna radiološka dijagnostika, uključujući i magnetonrezonantnu sekvencu difuzionog kretanja (*diffusion-weighted magnetic resonance imaging* – DWIMRI) i iz nje izvedenu mapu očiglednog koeficijenta difuzije – *apparent diffusion coefficient* (ADC), daju obećavajuće rezultate u mogućnosti razlikovanja benignih od maligno izmenjenih limfnih čvorova male karlice i ingvinuma kod bolesnica sa malignim tumorima ženskih polnih organa. Cilj rada bio je da se procene dijagnostičke osobine DWI u razlikovanju benigno- od maligno-izmenjenih pelvičnih i ingvinalnih lim-

fnih čvorova kod bolesnica sa ginekološkim malignim oboljenjima. **Metode.** Prospektivnom kliničkom studijom, sprovedenom u Kliničkom centru Vojvodine, u periodu od 2013. do 2016. godine, obuhvaćeno je 80 bolesnica sa malignim tumorima ženskih polnih organa. Svim bolesnicama je preoperativno učinjen magnetonrezonantni pregled male karlice, uz naknadno sprovedeno standardno hirurško lečenje po protokolu hirurškog lečenja za dato maligno ginekološko oboljenje sa karličnom i/ili ingvinalnom limfadenektomijom. Na osnovu definisanog modela koji kombinuje kriterijum ADC vrednosti sa kriterijumom veličine, MRI ima sledeće dijagnostičke performanse za

razlikovanje maligno- od benigno- izmenjenih limfnih čvorova: senzitivnost od 95%, specifičnost od 92%, sveukupnu tačnost od 92,5%, pozitivnu prediktivnu vrednost od 46% i negativnu prediktivnu vrednost od 99,6%. Postoperativno je izvršena patohistološka analiza hirurški uklonjenih materijala i limfnih čvorova razdvojenih po anatomskim grupama u karlici i ingvinalnoj regiji. **Rezultati.** Ukupno 2 320 limfnih čvorova mapirano je i patohistološki pregledano kod 80 bolesnika. Metastaze u limfnim čvorovima patohistološki su verifikovane kod 28 (35%) bolesnika. Izmerena ADC vrednost bila je značajno niža kod metastatski izmenjenih limfnih čvorova [srednja vrednost (SV) \pm standardna devijacija (SD), ADC: $0,8725 \times 10^{-3} \text{ mm}^2/\text{s} \pm 0,0125 \times 10^{-3} \text{ mm}^2/\text{s}$] u poređenju sa limfnim čvorovima koji nisu bili metastatski izmenjeni (SV \pm SD, ADC: $1,116 \times 10^{-3} \text{ mm}^2/\text{s} \pm 0,1848 \times 10^{-3} \text{ mm}^2/\text{s}$; $p = 0,001$). Za ADC vrednost od $0,860 \times 10^{-3}$

mm^2/s , kao kritičnu vrednost za razlikovanje metastatskih od limfnih čvorova koji nisu bili metastatski izmenjeni, senzitivnost DWI MR iznosila je 89%, specifičnost 85%, ukupna tačnost 86%, pozitivna prediktivna vrednost 30%, a negativna prediktivna vrednost 99%. **Zaključak.** Magnetno-rezonantna sekvenca difuzionog kretanja je brza, jednostavna, neinvazivna metoda koja značajno doprinosi dijagnostičkim mogućnostima magnetne rezonance u razlikovanju benigno- od maligno-izmenjenih limfnih čvorova male karlice i ingvinuma.

Ključne reči:

dijagnoza, diferencijalna; magnetna rezonanca, difuziona; polni organi, ženski; limfne žlezde; neoplazme, limfna metastaza; magnetna rezonanca, snimanje.

Introduction

Malignant gynecological tumors are among the leading causes of morbidity and mortality among women, both in Serbia and worldwide ^{1,2}. The presence of lymph node metastases has a significant impact on tumor staging, as well as treatment planning and prognosis. When evaluating lymphatic metastases by radiological studies, it is very important to be familiar with the potential localization of metastases, the probability of metastases in particular groups of lymph nodes depending on the primary tumor, and with the impact that the lymphatic metastases have on the stage and treatment of the disease.

The most common typical lymphatic pathways of malignant gynecological tumors spread are superficial inguinal, pelvic, and paraaortic pathways.

Imaging is an integral part of the strategy in planning optimal treatment of gynecological cancer ³. Computed tomography (CT) and magnetic resonance imaging (MRI) are the golden standard of imaging nodal status in these patients, mostly relying on short lymph node axis diameter as discriminating criteria. However, both modalities have low sensitivity in detecting pelvic lymph node metastases ⁴. The combination of functional and morphological imaging yields better precision in the detection of lymph node involvement ⁵.

Due to technical development, diffusion-weighted imaging (DWI) and derived apparent diffusion coefficient (ADC) have been refined and shortened and thus incorporated in routine MRI protocols. Restriction of water mole-

cules' diffusion is directly proportional to tissue cellularity ⁶. Such restriction of diffusion is primarily seen in malignant tumors, hypercellular metastases, and fibrosis, where a higher number of cells with an intact cellular wall is present compared to normal tissue ⁷.

The aim of the study was to determine the diagnostic performance of DWI in detecting pelvic and inguinal lymph nodes in patients with malignant gynecological tumors.

Methods

The prospective clinical study was conducted from 2013 to 2016 at the Center for Radiology, Clinic for Gynecology and Obstetrics and Department of Pathology, Clinical Center of Vojvodina in Novi Sad, Serbia and comprised 80 patients with malignant vulvar, vaginal, cervical, uterine, and ovarian tumors.

Staging of the disease was based on the histopathological assessment of complete surgical specimen, including examination of removed lymph nodes, based on the International Federation of Gynecology and Obstetrics (FIGO) classification.

Preoperatively, all patients underwent pelvic MRI at the Center for Radiology, Clinical Center of Vojvodina within 30 days prior to the surgery. All examinations were performed on a 1.5 T General Electric Signa HDx machine using a phased array coil and routine protocols for pelvic MRI (Table 1).

Table 1

Routine protocol for pelvic magnetic resonance imaging (MRI)

Sequence	T1 weighted	T2 weighted	T2 weighted	Diffusion weighted
Type	fast spin echo	fast spin echo	fast spin echo	spin echo planar imaging
Plane	axial	axial	sagittal	axial
TR [ms]	460	2,640	3,560	10,760
TE [ms]	12.9	99.3	103.9	78.3
Slice thickness [mm]	5	5	5	5
FOV [cm]	30 \times 30	30 \times 30	33 \times 33	30 \times 30
Matrix	320 \times 224	320 \times 224	384 \times 256	82 \times 128
NEX	4	4	2	8
b value [s/mm ²]	–	–	–	800; 1,200

TR – repetition time; TE – time to echo; FOV – field-of-view; NEX – number of excitations.

Based on a defined model which combines criteria of ADC values with size criteria, MRI has the following diagnostic performances in discriminating malignant and benign lymph nodes: sensitivity 95%, specificity 92%, overall accuracy 92.5%, positive predictive value (PPV) 46%, and negative predictive value (NPV) 99.6%. Depending on the type of the tumor, additional sequences were added, including thin-sliced T2W sequences perpendicular to the long axis of the cervix or uterine body, axial T1W tomograms with fat saturation, coronal T2W as well as biplanar and dynamic postcontrast tomograms. A corresponding ADC map was calculated for each patient. Qualitative analysis of signal intensity on DWI and quantitative analysis of ADC values were performed using the lowest measured ADC value. Using the receiver operating characteristic (ROC) curve analysis, the following threshold values were used for determining metastatic invasion of lymph nodes: short axis (S) > 7.75 mm; long axis (L) > 10 mm; L/S ratio ≤ 1.23 and minimal ADC $\leq 0.860 \times 10^{-3} \text{ mm}^2/\text{s}$.

All patients underwent standard surgical treatment depending on the type and stage of the tumor, including pelvic and/or inguinal lymphadenectomy.

Postoperatively, histopathological analysis of the surgical specimen was performed, with special emphasis on the lymph node group and position.

Statistical analysis was performed using Statsoft Statistica 10 software package (StatSoft, Inc. Tulsa, Oklahoma, USA).

Results

The study comprised 80 women, aged 32–79 years [mean age 57.35, standard deviation (SD) ± 9.99], with histologically verified gynecological tumor: 3 (3.75%) patients with vulvar, 1 (1.25%) patient with vaginal, 32 (40%) cervical, 30 (37.5%) with the uterine body, and 14 (17.5%) with malignant ovarian tumor.

A total of 2,320 lymph nodes were mapped and histologically examined in 80 patients. Lymph nodes metastases were verified in 28 (35%) patients.

In 28 (35%) patients with positive lymph nodes, 152 (27.28%) out of 557 lymph nodes were metastatic (Figure 1).

Pelvic lymph nodes metastases were observed in 2 (7.14%) patients with vulvar cancer, 11 (39.28%) patients with cervical cancer, 9 (32.14%) patients with uterine body tumors, and 6 (21.42%) patients with malignant ovarian tumors (Figure 2).

The distribution of metastases in relation to pelvic lymph nodes is shown in Table 2.

The period between preoperative pelvic MRI and surgery was 1–22 days (mean \pm SD: 10.48 ± 2.7).

There was no statistically significant difference between the S of of lymph nodes with metastatic invasion (mean \pm SD: 8.3 ± 5.4 mm, range 4.5–30 mm) and lymph nodes with no metastatic invasion (mean \pm SD: 6.3 ± 1.5 mm, range: 4.5–9.6 mm; $p = 0.191$).

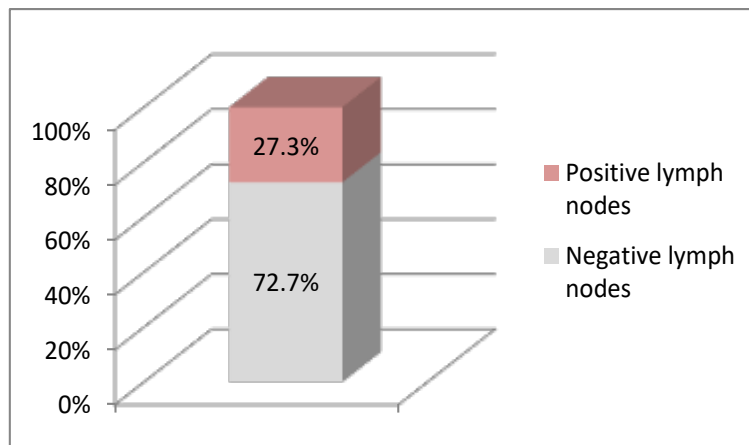


Fig. 1 – Ratio of positive and negative lymph nodes in 28 patients with verified metastases in pelvic lymph nodes.

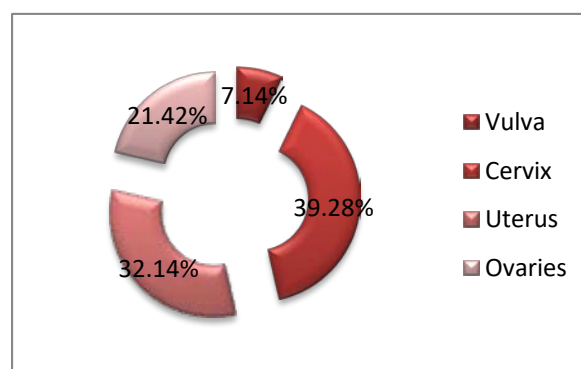


Fig. 2 – Distribution of positive lymph nodes in relation to primary tumor localization.

Table 2**Distribution of metastases in relation to pelvic lymph nodes**

Tumor localization	Group of pelvic lymph nodes						total
	parametrial	obturator	external iliac	internal iliac	common iliac	inguinal	
Vulva	0	0	0	0	0	8 (5.26)	8 (5.26)
Cervix	8 (5.26)	23 (15.13)	16 (10.52)	13 (8.55)	5 (3.28)	0	65 (42.77)
Uterine body	0	14 (9.21)	16 (10.53)	14 (9.21)	2 (1.31)	0	46 (30.26)
Ovary	0	11 (7.23)	8 (5.26)	9 (5.92)	5 (3.28)	0	33 (21.71)
Total	8 (5.26)	48 (31.58)	40 (26.32)	36 (23.68)	12 (7.90)	8 (5.26)	152 (100)

All values are expressed as number (percentage).

There was no statistically significant difference between the L of lymph nodes with metastatic invasion (mean \pm SD: 12.6 ± 6.1 mm, range: 8–35 mm) and lymph nodes with no metastatic invasion (mean \pm SD: 11.3 ± 2.6 mm, range: 7.1–17.9 mm; $p = 0.419$).

Measured ADC value was significantly lower in metastatic lymph nodes (mean \pm SD: $0.8725 \times 10^{-3} \pm 0.0125 \times 10^{-3}$ mm²/s) compared to lymph nodes with no metastatic invasion (mean \pm SD: $1.116 \times 10^{-3} \pm 0.1848 \times 10^{-3}$ mm²/s; $p = 0.001$) (Figures 3–5).

The area under the ROC curve for differentiation between metastatic and benign lymph nodes was presented with a value of 0.901 [standard error 0.022; 95 % confidence interval (CI), 0.857–0.937] (Figure 6). ADC value of 0.860×10^{-3} mm²/s was derived from the ROC curve as a threshold value for distinction between metastatic and be-

nign lymph nodes (Figure 7). In other words, the lymph node with ADC value $\leq 0.860 \times 10^{-3}$ mm²/s was regarded as metastatic.

With the calculated threshold value from the ROC curve, lesion-based sensitivity was 89%, specificity 85%, and overall accuracy was 86%.

MRI performances were significantly better for minimal ADC values compared to all size-based criteria ($p = 0.001$ for minimal ADC value compared to all other criteria). Since the area under the ROC curve was larger for minimal ADC values than for all other criteria, minimal ADC value was used as a representative index for differentiation between metastatic and non-metastatic lymph nodes. The comparison of diagnostic performance of MRI based on ADC values and size criteria is shown in Table 3.

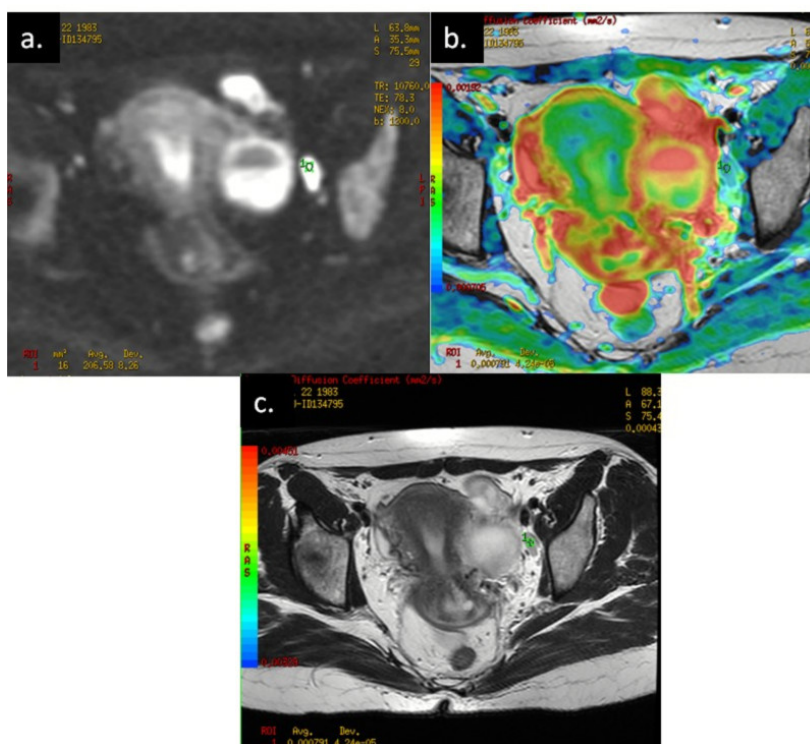


Fig. 3 – A 33-year-old-patient with serous cystadenocarcinoma of the left ovary, the International Federation Gynecology and Obstetrics (FIGO) stage IIIC. Metastatic enlarged external iliac lymph node with restricted diffusion: a) axial high b-value diffusion-weighted imaging (DWI); b) fusion of apparent diffusion coefficient (ADC) map and axial T2W; c) axial T2W.

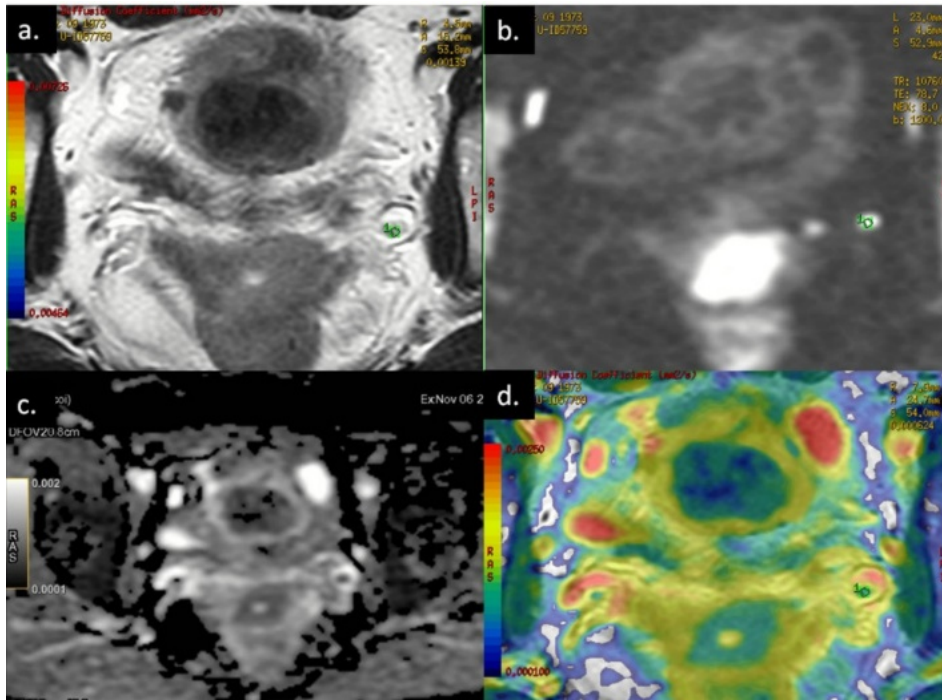


Fig. 4 – A 43-year-old-patient with squamocellular cervical cancer, FIGO IIB stage. Parametrial lymph node sized 5 mm, with restricted diffusion and histological confirmation of metastatic involvement: a) axial T2W; b) axial high b-value DWI; c) ADC map; d) fusion of ADC map and axial T2W. For abbreviations see Figure 3.

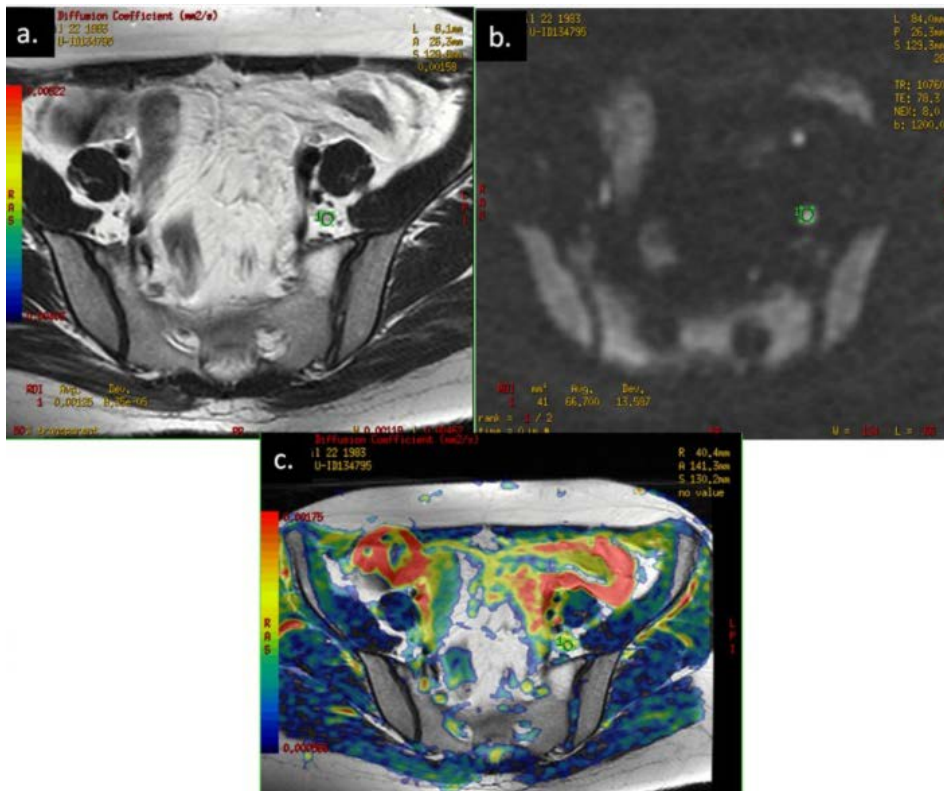


Fig. 5 – A 63-year-old patient with endometrial cancer FIGO IC stage. Normal-sized left common iliac lymph node, with high signal intensity on DWI, but with high ADC values ($1.25 \times 10^{-3} \text{ mm}^2/\text{s}$) and histopathology confirmed reactive lymph node: a) axial T2W; b) axial high b-value DWI; c) fusion of ADC map and axial T2W. For abbreviations see Figure 3.

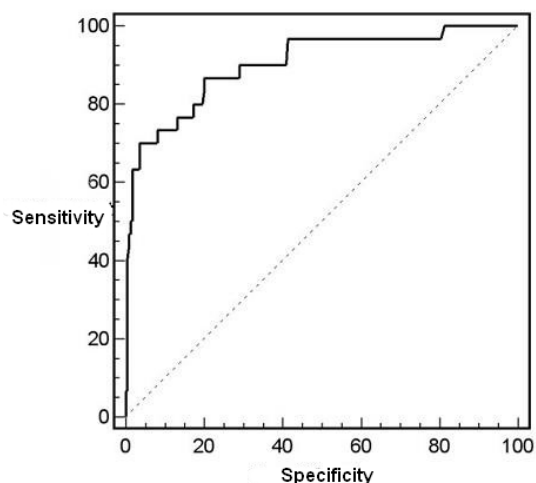


Fig. 6 – Receiver operating characteristic (ROC) curve for apparent diffusion coefficient (ADC) values in discriminating between malignant and benign lymph nodes.

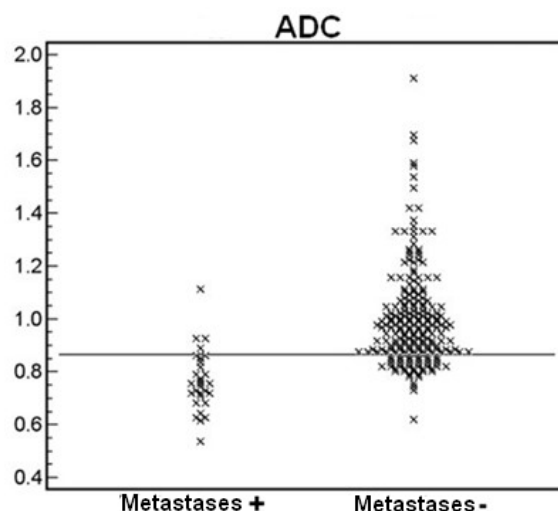


Fig. 7 – Apparent diffusion coefficient (ADC) values of metastatic and benign lymph nodes.

Table 3

Comparison of diagnostic performance of MRI based on ADC values and size criteria

Parameter (%)	Minimal ADC	Short-axis (S) diameter	Long-axis (L) diameter	Long to short axis criteria (L/S ratio)
Sensitivity	89	55	73	52
Specificity	85	74	66	73
PPV	30	13	13	12
NPV	99	96	97	96
Overall accuracy	86	73	67	72

MRI – magnetic resonance imaging; ADC – apparent diffusion coefficient; PPV – positive predictive value; NPV – negative predictive value.

ROC curves of different predictive models which combine the following criteria: ADC values alone, in combination with size criteria and size criteria alone, are shown in Figure 8. A model which combines ADC and size criteria is more accurate in detecting lymph node metastases compared to the other two models.

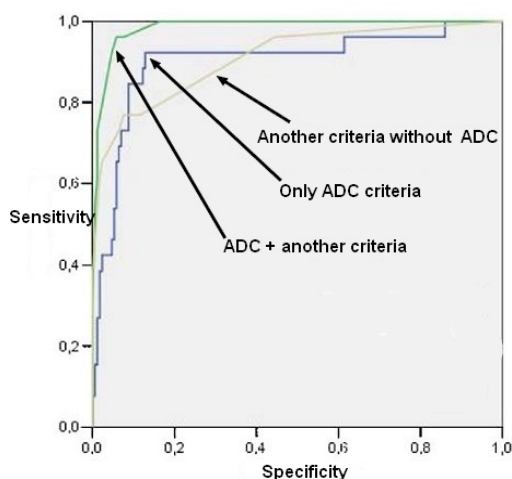


Fig. 8 – A receiver operating characteristic (ROC) curves of different predictive models which include apparent diffusion coefficient (ADC) values and size criteria, alone or in combination.

Discussion

Conventional imaging methods cannot discriminate normal from metastatic lymph nodes with satisfactory precision, especially when normal in size. Therefore, numerous studies have been conducted worldwide searching for diagnostic methods or a combination of methods that could discriminate benign from malignant lymph nodes, being minimally invasive, cheap, easily available, and simple. The method which satisfies all these criteria and yields promising results is DWI.

Up to date, several studies have been done analyzing the role of DWI in discriminating benign from malignant lymph nodes⁸⁻¹³. However, the conclusions of these studies differ a lot, leading to the need for more research on the topic.

Traditional lymph node size criterion used in clinical practice defines lymph nodes with short axis larger than 10 mm as probable metastases. However, researchers showed that this criterion is imprecise¹⁴⁻¹⁸, which was confirmed in our study. A high number of false-negative results is explained by a high percentage of positive lymph nodes smaller than 10 mm on histopathological examination and microscopic metastases that are beyond the possibility of recognition by conventional imaging modalities.

Our study confirmed that ADC values of metastatic lymph nodes (mean ± SD: $0.8725 \times 10^{-3} \text{ mm}^2/\text{s} \pm 0.0125 \times$

$10^{-3} \text{ mm}^2/\text{s}$) were statistically significantly lower than benign (mean \pm SD: $1.116 \times 10^{-3} \text{ mm}^2/\text{s} \pm 0.1848 \times 10^{-3} \text{ mm}^2/\text{s}$; $p = 0.001$), which is in concordance with the results of other similar studies^{10, 11, 19}.

In our study, diagnostic performances of MRI were significantly better for minimal ADC value compared to all size-based criteria ($p = 0.001$ for minimal ADC value compared to all other criteria), which is similar to the results of other authors¹².

We analyzed the correlation of ADC values and different size criteria on conventional T2W tomograms with histopathological reports as a reference standard. It was shown that minimal ADC values were significantly lower in metastatic lymph nodes compared to benign ones. The sensitivity, specificity, and overall accuracy of DWI, *ie*, minimal ADC values, in the detection of positive lymph nodes, were significantly higher compared to size criteria (S and L, S to L ratio). These results suggest that DWI can enhance visualization and detection of metastatic lymph nodes with a short diameter above 5 mm in patients with malignant gynecological tumors. Good diagnostic performance of DWI is based on its ability to obtain functional information regarding active microstructural changes within a lymph node, which occur before alterations of size. Thus, diagnosis based on ADC criteria overcomes the main limitation of size-based criteria in detecting metastases in normal-sized lymph nodes.

The S on MRI is routinely used for detecting metastases in lymph nodes. Although the first studies indicated a promising detection^{18, 20}, two recent prospective studies that compared S of 9 mm or 10 mm in correlation with histopathological findings showed low sensitivity of 30% and 36%, despite high specificity of 93% and 97%^{21, 22}. Besides low accuracy, another problem with S criteria is the lack of consensus on optimal “cut-off” value for discriminating metastatic from benign lymph nodes. Although most authors used a S diameter of 10 mm as a “cut-off” value, some advocated the size of 9 mm or 8 mm^{19, 22, 23}. As the S diameter of many metastatic lymph nodes can range from 8 mm to 10 mm, even the smallest (1 mm) changes in size criteria can lead to significant changes in sensitivity and specificity. Some authors examined the L diameter of the lymph node or shape by calculating the L/S ratio based on which they assessed the lymph node status^{4, 24, 25}. Our results, which show unsatisfactory diagnostic accuracy for these two criteria, indicate that these strategies can be used with limitations.

Studies with strict “lesion-by-lesion” comparison of imaging and histopathological findings showed poor sensi-

tivity of CT (24%), MRI (29%), and even position emission tomography (PET)-CT (58%)²⁶. Such limited sensitivity is mainly based on inaccurate detection of small metastatic lymph nodes based on size criteria, which is imprecise^{12, 27, 28}. Moreover, even the standard uptake value of PET-CT is unsatisfactory when metastatic lymph nodes are of small dimensions^{22, 29, 30}. Regarding these limitations, ADC values are favorable compared to CT, conventional MRI, and PET-CT because ADC measurements are relatively independent of the size of the lesions as long as the surface of the lymph node allows precise region of interest (ROI) placement.

Since the area under the ROC curve was higher for minimal ADC value compared to all size criteria, minimal ADC value was used as a representative index for differentiation metastatic from benign lymph nodes. We compared ROC curves of different predictive models that include the following criteria: ADC value alone, in combination with size criteria, and size criteria alone. The model combining ADC value and size criteria is more efficient in detecting malignant lymph nodes compared to the other two models. The combination of ADC value and size criteria improves the sensitivity of MRI from 89% to 95%, specificity from 85% to 92%, overall accuracy from 86% to 92.5%, PPV from 30% to 46%, and NPV from 99% to 99.6%. The model comprising only ADC values criteria has equally good diagnostic performances as the model that includes other MRI criteria.

Conclusion

Size criterion, widely used in clinical radiology for discrimination between malignant and benign lymph nodes, is insufficiently precise. The study confirmed a high positive correlation between preoperative assessment of metastatic invasion of pelvic and inguinal lymph nodes in patients with malignant genital tumors by DWI and postoperative histopathological examination. Using a cut-off ADC value of $0.860 \times 10^{-3} \text{ mm}^2/\text{s}$, the sensitivity of MRI DWI in detecting metastatic pelvic lymph nodes was 89%, and specificity 85%. The combination of ADC values and morphological criteria is the most precise predictor of metastatic infiltration of pelvic lymph nodes in patients with malignant genital tumors. DWI sequence is a fast, simple, non-invasive method that has a significant contribution to the diagnostic capability of MRI in the distinction between benign and malignant pelvic lymph nodes.

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Intraosseous epidermal inclusion cyst of the right ring finger for 40 years

Epidermalna inkluziona cista u kosti desnog domalog prsta tokom 40 godina

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Yong-Huang and Jie-Feng Huang made equal contributions, and are co-correspondence author.

Abstract

Introduction. Intraosseous epidermal inclusion cysts (IEpC) are benign bone lesions lined with squamous epithelium. Finger phalanges are the second most common site of predilection after the skull. **Case report.** We presented a case of a typical IEpC at the distal phalanx of the right ring finger following a remote history of a crush injury to the finger (40 years earlier). The patient experienced painful enlargement and progressive swelling of that finger during the previous month. On physical examination, the finger showed typical “clubbing” with local tenderness. X-ray showed bone destruction, and magnetic resonance imaging (MRI) revealed abnormal signals in bone tissue in the distal phalanx of the right ring finger. The patient underwent distal phalanx amputation of the right ring finger. The diagnosis of IEpC was histopathologically confirmed. At follow-up 2 years later, the stump healed well and without recurrence. **Conclusion.** IEpC with a history of up to 40 years is very rare, and although the patient presents with a typical “clubbing” finger, the diagnosis was eventually confirmed by surgery and pathology.

Key words:

amputation; bone cysts; crush injuries; epidermal cyst; fingers; hand.

Apstrakt

Uvod. Intraosealne epidermalne inkluzione ciste (IEpC) su benigne lezije kostiju obložene skvamoznim epitelom. Posle lobanje, falange prstiju su drugo najčešće predilekciono mesto za pojavu ovih lezija. **Prikaz bolesnika.** Prikazan je slučaj tipične IEpC na distalnoj falangi desnog domalog prsta koja je nastala posle povrede prsta (mehanizmom nagnječenja – *crush*), 40 godina ranije. Pacijent se žalio na bolno uvećanje i progresivan otok tog prsta, tokom prethodnih mesec dana. Objektivnim pregledom, prst je imao karakterističan „maljičast” izgled uz lokalnu osetljivost. Radiografskim pregledom viđena je destrukcija kosti, a magnetnom rezonancom abnormalni signali u tkivu kosti u distalnoj falangi desnog domalog prsta. Urađena je amputacija distalne falange desnog domalog prsta. Dijagnoza IEpC potvrđena je histopatološkim ispitivanjem. Praćenjem pacijenta, dve godine kasnije, nađeno je da je patrljak dobro zarastao, bez recidiva. **Zaključak.** Pojava IEpC sa istorijom povredivanja do 40 godina je veoma retka pojava. Bez obzira što prst pacijenta ima tipičan „maljičast” izgled, konačna dijagnoza se potvrđuje hirurškim i patološkim nalazom.

Ključne reči:

amputacija; kost, ciste; povrede, kraš; cista, epidermalna; prsti; ruka.

Introduction

Intraosseous epidermal inclusion cysts (IEpC) are benign bone lesions lined with squamous epithelium. The skull is the most common site, mainly involving the parietal and temporal bones. Finger phalanges are the second most common site of predilection, followed by maxilla, mandible, temporomandibular joint, vertebrae, tibia, and femur¹. Although the etiology of these tumors is not fully understood, it is believed that they occur due to cortical rupture of the bone leading to the proliferation of epidermal cells in the bone matrix¹⁻³.

We presented a case of a typical IEpC at the distal phalanx of the right ring finger following a remote history of a crush injury to the finger. The article was approved by the Hospital Ethics Committee. The patient gave informed consent for submitting his pictures for publication.

Case report

A 56-year-old man was presented to the hospital with a 1-month history of painful enlargement and progressive swelling of the distal phalanx of the right ring finger. During this period, he did not receive any treatment, nor did he take relevant drugs, such as anti-inflammatory and analgesic drugs.

The patient reported a history of finger injury without fracture 40 years ago. It got healed, but the swelling was slowly increasing in size. The patient did not go to the doctor for treatment because the finger showed no symptoms such as pain and febrile except slight swelling over the years. He had no symptoms or personal history of rheumatoid arthritis.

On physical examination, the finger showed a typical "clubbing" (Figures 1–3) with local tenderness. X-ray

showed bone destruction, and magnetic resonance imaging (MRI) revealed abnormal signals in bone tissue in the distal phalanx of the right ring finger (Figures 2 and 3). The patient underwent distal phalanx amputation of the right ring finger. During the operation, we found that the swelling part was well wrapped, and it had a milky soft-tissue appearance. Histopathological examination revealed an IEpC (Figure 4). The wound healed well 2 weeks after the operation, and the stitches were removed. Two years later at follow-up, the stump healed well and without recurrence.



Fig. 1 – The right ring finger showed obvious “clubbing” finger.



Fig. 2 – X-ray showed bone destruction in distal phalanx of the right ring finger.



Fig. 3 – Magnetic resonance imaging (MRI) revealed abnormal signals in bone tissue in distal phalanx of the right ring finger [figures b and c are contrast-enhanced MRI; the arrows indicate the typical cystic region].

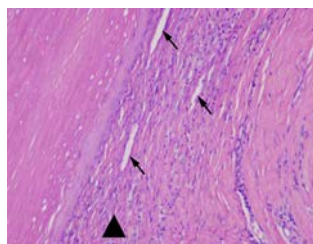


Fig. 4 – Histopathological examination revealed intraosseous epidermal inclusion cyst (hematoxylin and eosin staining, ×200). (“▲” Squamous epithelium; “→” The stratum corneum thickens and separates).

Discussion

IEpC are usually seen in subcutaneous tissue, however, intraosseous IEpC is a rare benign lesion⁴. Finger phalanges are the second most common site of predilection after the skull¹. The most common age range is 25–50 for men and less for women^{1, 3–5}. Phalangeal cysts can result from any type of injury caused by traumatic implantation of epidermal fragments into the bone or from the migration of nail bed fragments into the phalangeal.

The typical manifestation of IEpC in a finger is “clubbing”. According to relevant reports of epidermal cyst^{4, 6–8}, our patient's “clubbed finger” is the most typical, and the patient's 40-year history is very rare. The imaging findings of IEpC are typically well-defined osteolytic lesions with or without soft tissue swelling. This is different from infection or metastasis, which would be a poorly defined osteolytic lesion¹. The differential diagnoses of phalangeal lesions are broad and include inflammatory (i.e., chronic infection), as well as benign and malignant processes. Distally-based erosive cystic lesions

include those secondary to tophaceous gout or a localized giant cell tumor of the tendon sheath, giant cell reparative granuloma, simple bone cyst, aneurysmal bone cyst, osteoid osteoma, and epidermoid inclusion cyst⁴. The usual treatment is curettage and removal of the cyst capsule³. Recurrence can occur if the excision was incomplete^{1, 3, 4}. Bone grafting is rarely indicated. In our case, with the bone mass of the distal phalanx destroyed by the cyst, the decision was made to perform an amputation.

Conclusion

IEpC with a history of up to 40 years is very rare, and although the patient presents with a typical “clubbed finger”, the diagnosis was eventually confirmed by surgery and pathology.

Conflict of interest

The authors declare that they have no conflict of interest. There is no funding source.

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Melanoma and squamous cell carcinoma developing on a burn scar

Melanom i skvamocelularni karcinom kože nastali na ožiljku od opekotine

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Abstract

Introduction. Development of malignancy in chronic burn scars is described in 2% of cases, with cutaneous squamous cell carcinoma (cSCC) being the most frequent. It develops several years after the burn injury, as a consequence of malignant transformation in chronically inflamed tissue. Melanoma in a burn scar is, however, quite rare; in several cases, a synchronous or metachronous appearance of melanoma and cSCC was noted. Based on the previous rare reports, melanoma and cSCC can concurrently arise on thermal burn scars, as well as on the areas treated with skin grafts. **Case report.** We reported a case of a 67-year-old male who was accidentally scalded by boiling water at the age of 14. The patient subsequently developed melanoma at the age of 60, according to clinical and dermoscopic examination at the site of the burn scar, and after excision and histopathological analysis, the diagnosis of melanoma was confirmed (superficial spreading melanoma, Breslow 0.87 mm, with ulceration, pT1b). Complete surgical excision was done, and the diagnosis was confirmed with histopathological analysis. After the diagnosis of melanoma, regular follow-up every six months was scheduled, and three years later, cSCC was suspected and confirmed after surgical excision and histopathologic analysis. **Conclusion.** This case highlights the importance of long-term dermatological follow-up after burn injuries and early detection and treatment of skin cancer that can lead to better outcomes in these patients.

Key words:

carcinoma, squamous cell; early diagnosis; diagnosis, differential; histological techniques; melanoma; burns; cicatrix.

Apstrakt

Uvod. Maligni tumori kože nastali na terenu ožiljaka od opekotina opisani su kod 2% bolesnika, a planocelularni karcinom kože (karcinom skvamoznih ćelija kože, *cutaneous squamous cell carcinoma* – cSCC) je najčešći. On se javlja nekoliko godina posle opekotine kao posledica maligne transformacije ćelija u hronično inflamiranom tkivu. Razvoj melanoma na ožiljku od opekotine je veoma redak; opisano je nekoliko slučajeva sinhronog ili metahronog pojavljivanja melanoma i cSCC. Melanom i cSCC mogu se istovremeno pojaviti na ožiljku od opekotine, kao i na regiji sa autotransplantatom kože. **Prikaz bolesnika.** Prikazali smo muškarca starog 67 godina koji je imao opekotinu od ključale vode u 14. Godini života. U 60. godini života, na mestu ožiljka od opekotine, kliničkim i dermoskopskim pregledom posumnjano je na melanom, a nakon ekscizije i histopatološke analize potvrđena je sumnja i postavljena dijagnoza melanoma (površinsko šireći, *Breslow* 0,87 mm, sa ulceracijom, pT1b). Nakon postavljene dijagnoze bolesnik je redovno kontrolisan svakih šest meseci. Tokom treće godine praćenja, zbog sumnje na cSCC, učinjena je kompletna hirurška ekscizija i dijagnoza cSCC je potvrđena histopatološkom analizom. **Zaključak.** Prikazani slučaj naglašava važnost dugotrajnog dermatološkog praćenja osoba nakon opekotina kože, i ranu dijagnozu i odgovarajuće hirurško lečenje nastalih malignih karcinoma kože jer se na taj način poboljšava ishod lečenja ovih bolesnika.

Ključne reči:

karcinom, planocelularni; dijagnoza, rana; dijagnoza, diferencijalna; histološke tehnike; melanom; opekotine; ožiljak.

Introduction

About 2% of chronic burn scars develop into malignant tumors many years after the burn occurrence¹. Cutaneous squamous cell carcinoma (cSCC) is the most frequent cancer that arises on the affected skin², even though in the general population, this form of a malignant tumor is less common than basal cell carcinoma. Available empirical evidence suggests a mean time latency of 23 to 37 years³. However, not many cases of melanoma on thermal burn scars are reported in pertinent literature^{1, 2, 4-7}. They nonetheless suggest that melanoma (MM) and cSCC can concurrently arise on thermal burn scars, as well as on the areas treated with skin grafts^{2, 6}.

Case report

We reported a case of a 67-year-old male who suffered burns after being accidentally scalded by boiling water at the

age of 14. The first symptoms appeared 46 years after the burn when the patient noticed painless pigmented and crusty lesions in the middle of the burn scar that were clinically indicative of melanoma.

Clinical examination of his right lumbar region revealed an asymmetric pigmented flat lesion exceeding 3 cm in diameter that was brown and black in color, with irregular borders, according to the ABCDE rule. It was accompanied by one tumefaction covered in brown crusts on the surface of the surrounding skin (Figure 1). After clinical examination, wide surgical excision and local flap reconstruction were performed [Figures 2 a) and b)].

Histopathological diagnosis of one lesion was ulcerated superficial spreading melanoma (SSM) in the scar tissue (Breslow 0.85 mm, Clark III, pT1b), whereas the other lesion was diagnosed as lentiginous melanocytic hyperplasia (LMH). Final pathological findings confirmed negative excision margins, which extended 1 cm into the surrounding tissue, in accordance with the European guidelines (Figures 3 a–d).



Fig. 1 – Two painless black lesions in the middle of the burn scar, developing 46 years after the burn.



Fig. 2 – a) Planning and b) performing wide surgical excision with local flap reconstruction.

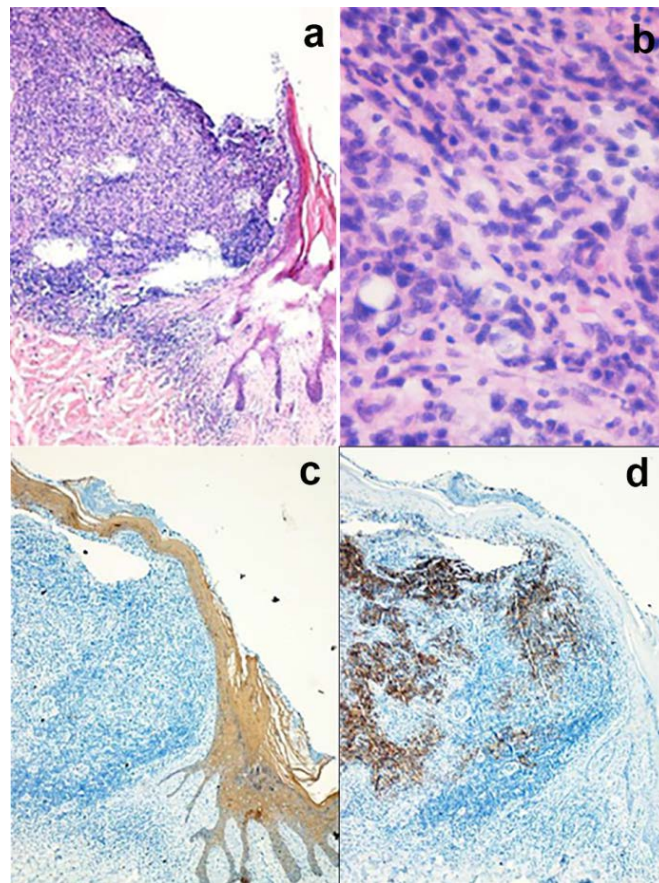


Fig. 3 – Histopathologic analysis showing:
a) Ulcerated superficial spreading melanoma (SSM) in a scar tissue (Breslow 0.85 mm, Clark III, pT1b) [hematoxylin and eosin (HE), $\times 10$]; b) atypical melanocytes (HE, $\times 10$); c) pan-cytokeratin antibody staining ($\times 10$); d) HMB-45 antibody staining ($\times 10$).

At the three-year follow-up, an area of persistent erythema and desquamation was observed on the lumbar region, which was clinically suspicious of malignant transformation [Figures 4 a) and b)]. Consequently, wide surgical excision with skin auto-

transplantation was performed (Figure 5) and intraepithelial cSCC with clear margins was histologically confirmed (Figure 6). The patient attends regular follow-ups and remains free of the disease until today (six years after the intervention).

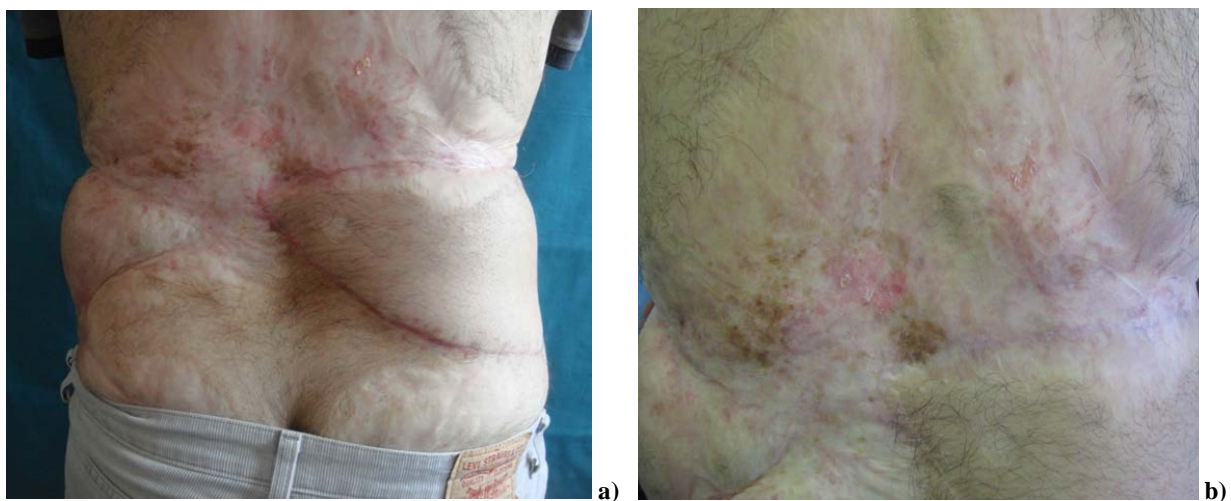


Fig. 4 – a) and b): during the follow-up, 3 years later, area of persistent erythema and desquamation was observed on the lumbar region.



Fig. 5 – Wide surgical excision with skin autotransplantation was performed.

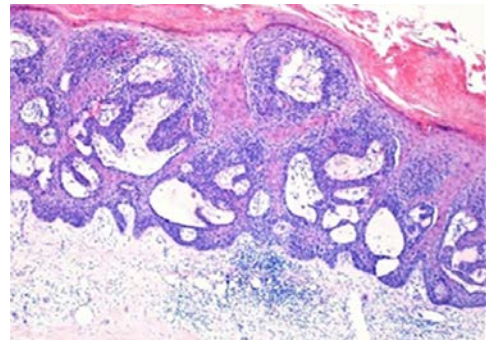


Fig. 6 – Histopathologic analysis showed intraepithelial squamous cell carcinoma with clear margins [hematoxylin and eosin (HE), $\times 5$].

Discussion

In the presented patient, the burn resulted in the development of extensive scarring of the skin on his back, which subsequently led to the emergence of two different malignant tumors in the burn scar region. Melanoma occurred 46 years after the burn, followed by a squamous cell carcinoma 3 years later.

There are fewer epidemiological data available on the risk of skin cancer arising in scars but is described to occur in 2% of patients. A review by Wallingford et al. ⁸ has identified a major gap in scientific knowledge regarding the incidence of scar neoplasms, despite a significant number of case reports. Although burn patients are not at higher risk of developing skin cancers in general, a modest increase in the prevalence of cSCC at sites of past burn injuries cannot be excluded, nor can excess risk in longer study follow-up periods be disregarded.

In the study conducted by Sadeh Fazeli et al. ⁹, cSCC was the most common diagnosis in association with chronic cutaneous inflammation and burns, which was well-differentiated most of the time. Moreover, several cases of concurrent melanoma and sarcoma have been reported. Koh et al. ¹⁰ described an extremely rare, aggressive variant of cSCC with extreme acantholysis, pseudoangiosarcomatous SCC developing on a burn scar. In this report, the authors emphasized the importance of establishing a diagnosis based on histological and immunohistochemical examination.

The development of melanoma on a burn scar is relatively rare; in several cases, a synchronous or metachronous appearance of melanoma and cSCC was reported ^{2, 11, 12}. In one report, multiple melanomas developed at the site of a burn scar, and in another, rare desmoplastic melanoma with regional lymph node metastases was described ^{13, 14}.

In the largest review, 23 cases of melanoma and 5 cases with concomitant presence of cSCC and melanoma were de-

scribed, and in the majority of patients, skin cancer developed after a long period from the age of burn occurrence to the melanoma diagnosis (41 ± 26 years in the 23 malignant melanoma only cases, and 48 ± 13 years in the 5 cases involving both cSCC and MM) ⁸. Furthermore, in another report, the average latency time from burn to melanoma diagnosis was 45 years ¹. Our patient developed melanoma 46 years after burn injury, which was followed by a diagnosis of cSCC three years later. A very long latency period was reported by Uchida et al. ⁷, who described a case of a 78-year-old Japanese female with malignant melanoma that developed on a thermal burn scar after more than 70 years. This suggests the need for a long-term dermatological follow-up of all burn patients.

The mortality rate associated with cSCC is about 1% ¹⁵, while in skin cancers developing in a scar, it can be as high as 21–38% based on previous literature, median survival after scar cancer diagnosis was reported to be 25 months, while 5-year survival rate varies from 52% to 80% ². In patients with involvement of regional lymph nodes, median survival was 16 months, increasing to 66 months for those without lymph node metastases ³. Our patient is on regular follow-ups and is disease-free during the six-year follow-up.

Self-skin examination and regular clinical and dermoscopic follow-up should be recommended to patients with burn injuries for an early diagnosis of skin cancer to avoid complications and poor outcome in advanced disease. Initial early grafting for a deep burn wound and proper scar care is also advised ¹⁶.

Conclusion

Periodical and very long follow-up of thermal burn scars is important for early diagnosis of malignant transformation and timely and adequate treatment in order to improve the outcome in these patients.

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Therapeutic approach to the iatrogenic invasive mole – A report of two cases

Terapijski pristup jatrogeno nastaloj invazivnoj moli – prikaz dva slučaja

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Abstract

Introduction. Invasive mole, a form of gestational trophoblastic neoplasia (GTN), is defined as penetration of molar tissue into the myometrium and/or presence of extra-uterine metastases. An invasive mole arising from a complete hydatidiform mole is more common than an invasive mole arising from the partial hydatidiform mole. Dilatation and uterine evacuation and/or curettage (D&E/C) is the first step in managing molar pregnancy. Uterine perforation is the most serious complication of this procedure. A less common one is the false passage. **Case report.** The first case report describes a 47-year-old woman who was referred to our Clinic under the suspicion of GTN, with elevated serum beta human chorionic gonadotropin (beta hCG) levels. Intraoperatively, devitalized ovular tissue arising from the uterine perforation was observed. Histopathological exam (HPE) of tissue obtained from hysterectomy confirmed an invasive mole as a result of uterine perforation made during D&E/C. The second patient, a 32-year-old woman with vaginal bleeding, nausea, and high levels of serum beta hCG levels was admitted to our Clinic. After four D&E/C, and persistently high levels of serum beta hCG levels, explorative laparotomy has been performed. A false passage created during D&E/C with necrotic and molar tissue was observed. The partial hydatidiform mole was confirmed by the HPE. **Conclusion.** This is the second reported case in the literature of postmolar GTN as a result of iatrogenic perforation of the uterus, and also the first described case of postmolar GTN arising from a false passage, created as an iatrogenic complication of D&E/C. A national survey of iatrogenic events during the treatment of gestational trophoblastic diseases is needed since these events could completely change the therapeutic strategies in managing these diseases.

Key words:

diagnosis; gynecologic surgical procedures; histological techniques; iatrogenic disease; postoperative complications; trophoblastic neoplasms; uterine perforation.

Apstrakt

Uvod. Invazivnu molu, oblik gestacijskih trofoblastnih neoplazmi (GTN), karakteriše penetracija molarnog tkiva u miometrijum i/ili prisustvo udaljenih metastaza. Invazivna mola nastala nakon kompletne mole je češća od invazivne mole nastale od parcijalne hidatiformne mole. Kiretaža materične šupljine sa evakuacijom sadržaja sukcijom je prvi korak u tretmanu molarne trudnoće. Perforacija uterusa je najopasnija komplikacija te procedure, dok je „lažni put“ (*false passage*) dosta ređa komplikacija. **Prikaz bolesnika.** Prvi prikaz opisuje bolesnicu staru 47 godina koja je primljena u našu Kliniku sa povišenim vrednostima serumskog beta humanog horionskog gonadotropina (beta hCG)-a pod sumnjom na GTN. Intraoperativno je uočena perforacija u predelu levog roga uterusa kroz koju je prominiralo ovularno tkivo. Histopatološkom analizom (HPA) nakon histerektomije potvrđena je invazivna mola nastala kao posledica jatrogene perforacije uterusa uzrokovane kiretažom. Drugi slučaj predstavlja bolesnicu staru 32 godine koja je primljena u Kliniku zbog krvarenja *ex utero*, sa mučninom i povišenim vrednostima beta hCG. S obzirom na perzistirajuće povišene vrednosti beta hCG-a i nakon četiri kiretaže, odlučeno je da se izvrši eksplorativna laparotomija. Intraoperativno je uočen „lažni put“ nastao kao komplikacija kiretaže, sa nekrotičnim i molarnim tkivom. HPA tkiva potvrdila je invazivnu molu nastalu od parcijalne mole, kao posledica pomenute jatrogene komplikacije. **Zaključak.** Prvi slučaj prikazan u ovom radu predstavlja drugi u literaturi opisani slučaj postmolarne GTN nastale kao rezultat jatrogene perforacije uterusa načinjene tokom kiretaže. Drugi slučaj opisan u ovom radu je prvi u literaturi opisan slučaj postmolarne GTN nastale stvaranjem „lažnog puta“ kao jatrogene komplikacije kiretaže materične šupljine. Smatramo da je nacionalni registar jatrogenih komplikacija nastalih tokom lečenja gestacijskih trofoblastnih bolesti neophodan, budući da te komplikacije mogu značajno uticati na terapijske protokole u lečenju ovih bolesti.

Ključne reči:

dijagnoza; hirurgija, ginekološka, procedure; histološke tehnike; jatrogena bolest; postoperativne komplikacije; neoplazme, trofoblastne; materica, perforacija.

Introduction

Invasive mole, a form of gestational trophoblastic neoplasia (GTN), is defined as penetration of molar tissue into the myometrium and/or presence of extrauterine metastases. The myometrial invasion occurs via direct tissue extension or through venous channels^{1, 2}. Malignant alterations occur in 15%–20% of complete hydatidiform mole, while transformation into postmolar GTN from partial hydatidiform mole happens in less than 5% of cases¹.

Diagnosis of the invasive mole is almost exclusively based on clinical findings, while the histopathological exam usually shows the existence of edematous villi and proliferative trophoblasts in the myometrial tissue^{1, 3}. Distant metastases are found rarely, mostly in the lungs, vagina, and brain².

Numerous reports recommend dilatation and uterine evacuation and/or curettage to be performed as the first step in managing molar pregnancy. Dilatation and evacuation and/or curettage are regarded as safe procedures but not without potential complications, the most serious of them being uterine perforation. Other complications include false passage, cervical or vaginal lacerations, and endometritis⁴.

We presented two cases of invasive mole with signs of the destruction of uterine tissue possibly arising after iatrogenic dilatation and evacuation/curettage complications.

Case report

Case 1

A 47-year-old woman was referred to Gynecology and Obstetrics Clinic “Narodni Front” under the suspicion of invasive mole, with elevated serum beta human chorionic gonadotropin (beta hCG) levels, without

symptoms of vaginal bleeding or abdominal pain.

The patient had one failed assisted reproduction attempt five months before hospitalization. Two months prior to admission to the Clinic, the patient was diagnosed with pregnancy with a visualized gestational sac on ultrasound exam with mean sac diameter estimated for 5 gestational weeks, levels of serum beta hCG > 10,000 mIU/mL, and a suspicious change in the uterine fundus projection. After two weeks, the ultrasonographic exam raised suspicion for molar pregnancy, and the serum beta hCG level was 72,889 mIU/mL. Instrumental revision of the uterine cavity was performed, which showed chorionic villi with the elements of the hydatidiform transformation on the histopathological exam (HPE). Since the levels of serum beta hCG failed to drop, the second uterine curettage was performed. Histopathological exam of the obtained tissue samples showed decidual fragments along with atypical cytotrophoblasts and syncytiotrophoblasts. The last serum beta hCG level before admission was 80,500 mIU/mL. Ultrasonographic exam performed at the Clinic showed the large field of the destruction of the corpus of the uterus, without residual tissue at the uterine cavity, and without theca-lutein ovarian cysts. The decision was to perform laparoscopic hysterectomy with conservation of the adnexa.

Intraoperatively, a softened uterus with devitalized ovular tissue arising from the uterine perforation was observed (Figure 1A). To avoid dissemination of the neoplastic tissue, a conversion into the transversal laparotomy and subsequent hysterectomy with conservation of both ovaries were performed. Results from the histopathological analysis (HPA) of the uterus (Figure 1B) revealed chorionic villi with hydrops degeneration in the superficial myometrium with a myometrial invasion of atypical cytotrophoblasts, syncytiotrophoblasts, and intermedial trophoblasts (Figure 2A), along with the extensive vascular myometrial invasion of atypical trophoblasts (Figure 2B).



Fig. 1 – A) Devitalized ovular tissue arising from the uterine perforation (laparoscopic view); B) Myometrial destruction in the region of the left uterine cornu and the left uterine wall (sample gained after hysterectomy).

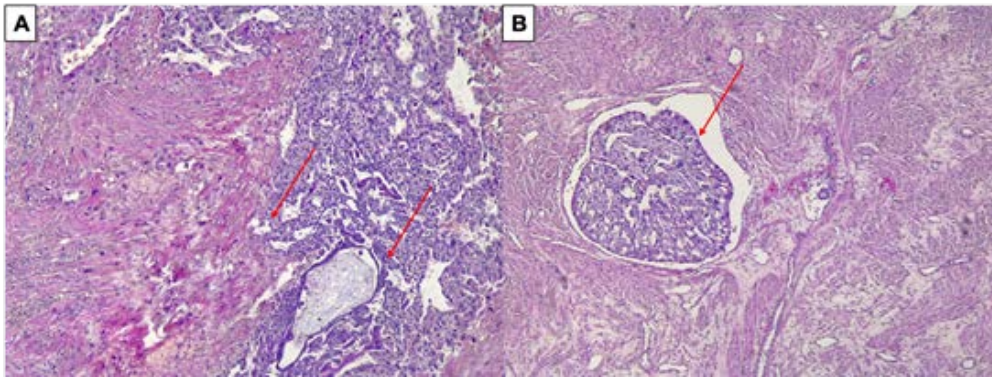


Fig. 2 – A) Chorionic villus (left arrow) with hydrops degeneration in the superficial myometrium and myometrial invasion of atypical cytotrophoblasts, syncytiotrophoblasts, and intermedial trophoblasts (right arrow) [hematoxylin and eosin staining (HE), $\times 5$]; B) Vascular myometrial invasion of atypical trophoblasts (arrow) (HE, $\times 5$).

Case II

A 32-year-old woman was admitted to Gynecology and Obstetrics Clinic “Narodni Front” with signs of vaginal bleeding and nausea after uterus dilatation and evacuation and two curettages.

The patient was diagnosed with pregnancy two months before admission to the Clinic. After presenting with vaginal bleeding and ultrasonographic signs of missed abortion, a dilatation and suction curettage was performed. In the following two weeks, serum levels of beta hCG remained high. After another episode of vaginal bleeding, the first recurettage was performed. The HPA of the obtained tissue showed chorionic villi and circumferent proliferation of atypical trophoblasts. A week after the first recurettage, the beta hCG serum level was 10,880 mIU/mL. The second recurettage was performed and the HPA of the tissue revealed irregular secretory endometrium and decidual tissue with intermediate trophoblasts of the placental site. Immediate serum beta hCG levels after the intervention were 90,000 mIU/mL and 131,100 mIU/mL. The patient underwent the third recurettage and was administrated with one cycle of chemotherapy [methotrexate (MTX) and folinic acid (FA) – MTX+FA]. The radiographic exam of the chest and head showed the absence of metastatic changes. Since

the levels of serum beta hCG failed to drop after one cycle of chemotherapy (73,361 mIU/mL), and the ultrasound exam revealed the field of the tissue destruction in the region of the left uterine cornu a decision for the hysterectomy has been made.

The procedure revealed a lividly colored surface in the regions of the fundus, left cornu, and anterior wall. After incision, necrotic and molar tissue was evacuated from the left cornu. A false passage, going through the left lateral uterine wall to the vesicouterine fold was revealed. Evacuation of necrotic and molar tissue from the false passage was also performed (Figure 3A). The obtained samples were sent to the HPA, and the uterine incision was managed by simple interrupted stitches (Figure 3B). Another hemostatic suture was placed in the posterior uterine wall.

The HPA of the sample obtained from the left uterine cornu revealed partial hydatidiform mole (Figures 4A and 4B), and the analysis of the sample from false passage confirmed the presence of chorionic villi and mildly atypical cytotrophoblasts, syncytiotrophoblasts, and intermedial trophoblasts in the myometrial layer without vascular invasion.

Levels of serum beta hCG saw a significant decline in the days following the procedure.

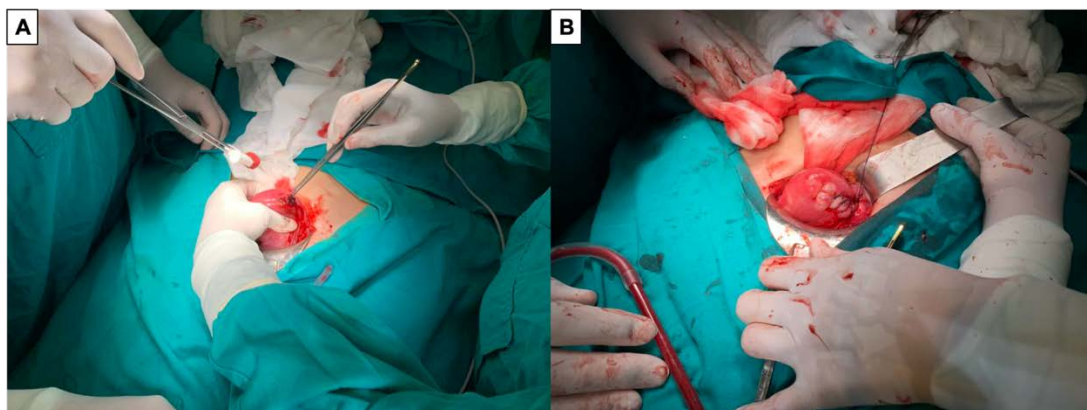


Fig. 3 – A) Evacuation of necrotic and molar tissue from the false passage; B) Uterine incision managed by simple interrupted stitches.

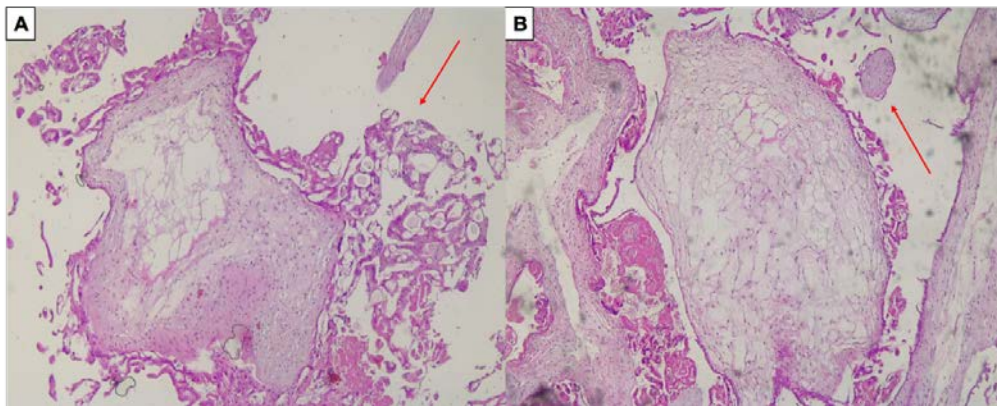


Fig. 4 – A) Chorionic villus surrounded by circumferential mildly atypical trophoblasts (arrow) [hematoxylin and eosin staining (HE), $\times 10$]; B) Chorionic villus with circumferential mildly atypical trophoblasts and normal chorionic villi (arrow), confirming the diagnosis of partial hydatidiform mole (HE, $\times 10$).

Discussion

To the best of our knowledge, this is only the second reported case in the literature of postmolar GTN as a result of iatrogenic perforation of the uterus, and also the first described case of postmolar GTN arising from a false passage, created as an iatrogenic complication of uterine curettage.

Gestational trophoblastic diseases (GTD) are a group of diseases that originate from abnormal placental tissue and include complete and partial hydatidiform mole, along with malignant forms, named gestational trophoblastic neoplasia: invasive mole, choriocarcinoma, placental site trophoblastic tumor, and epithelioid trophoblastic tumor⁵. The newest guidelines have expanded the GTN group with atypical placental site nodule because of its possible coexistence and/or progression into the placental site trophoblastic tumor, and epithelioid trophoblastic tumor⁶.

The exact incidence of GTD varies mostly from geographical location, race, socioeconomic factors, and nutritional factors^{6, 7}. Reports from the literature acknowledge choriocarcinoma and invasive mole as the most frequent forms of GTN, while the incidence of placental site trophoblastic tumor and the epithelioid trophoblastic tumor is around 0.2–2% of the GTN cases^{1, 6}.

An invasive mole arising from a complete hydatidiform mole is more common than an invasive mole arising from the partial hydatidiform mole. As far as we are aware, our case of invasive mole originating from the created false passage is the first reported case of invasive mole arising from the iatrogenic event in the management of partial hydatidiform mole.

According to the literature, serum beta hCG levels before evacuation $> 100,000$ mIU/mL are one of the prognostic markers for the risk of development of postmolar GTN¹. Along with serum beta hCG levels, other prognostic markers include uterine enlargement and theca lutein cyst with > 6 cm in their diameter¹. Both of our patients had pre-evacuation serum beta hCG levels $< 100,000$ mIU/mL,

which could favor the iatrogenic nature of GTN. Moreover, theca lutein cysts were not present in both patients.

Regardless of the size of the uterus, suction evacuation of molar tissue followed by a sharp curettage is the preferred first step in the management of patients with suspected GTD who wish to preserve their fertility. This should be done immediately after the hydatidiform mole is diagnosed⁶.

On the other hand, every patient with suspected or diagnosed GTN should be evaluated and assessed through the International Federation of Gynecology and Obstetrics (FIGO) and modified World Health Organization (WHO) scoring system^{3, 6}. In the majority of the cases, patients with GTN develop a low-risk disease³. The favorable treatment method for these patients is usually monotherapy. An invasive mole is an almost completely curable disease due to its high sensitivity to chemotherapy.

There is still an ongoing debate in the scientific community whether hysterectomy could prevent the progression from hydatidiform mole to GTN since reports are suggesting the potential dissemination of molar tissue intraoperatively⁸. Nevertheless, a hysterectomy is still the treatment of choice in most of the medical centers worldwide for older patients (aged 40 or more) with GTD who no longer require fertility⁸.

A second curettage approximately seven days after the first curettage was introduced as a therapeutic approach for patients with GTD almost four decades ago^{9, 10}. A recent study found that the routine second curettage might increase the risk for the development of GTN⁹. Moreover, some researchers advocate that the usage of oxytocin during or after the curettage might lead to trophoblastic embolism in the lungs⁹. On the other hand, some studies suggest that the second curettage could be seen as an alternative to the initial chemotherapy for patients with low-risk disease¹¹.

There are insufficient data regarding the incidence of uterine perforation after dilatation and curettage in patients with GTD. Furthermore, reviews from the literature impose caution when performing this procedure because, in these cases, the uterus is often softened and the perforation is possible².

We have presented a case of invasive mole caused by uterine perforation. In our opinion, the uterine evacuation was performed inadequately on the already softened and morphologically transformed uterus. This provided a path for the trophoblasts into the myometrium. A conversion from laparoscopy to laparotomy was done to avoid possible dissemination of trophoblastic tissue. A hysterectomy was done primarily because two-thirds of the uterine wall was destructed by an invasive mole.

Even though the patient from our second case underwent four curettages, serum levels of beta hCG were still elevated. Only diagnostic laparoscopy revealed performed false passage, which caused the penetration of trophoblasts into the myometrium. Even though the false passage is one of the possible complications of uterine curettage, its incidence is relatively low and its consequences are not life-threatening. The false passage is usually formed during the initial dilatation of the cervix. This is why numerous guidelines suggest that the dilatation should be performed by an expert, as gently as possible and with caution.

Conclusion

Dilatation and evacuation are still the treatment of choice in patients with GTD who wish to preserve their

fertility. Uterine perforation, arguably the most serious complication of this procedure, besides life-threatening consequences, could be the base of the iatrogenic cause of GTN.

We also presented the first case of an invasive mole arising from the false passage, a rare yet another possible complication of dilatation and curettage. This underlines the importance of uterine evacuation in these patients to be done cautiously and performed by an expert. Lastly, we think that a national survey of iatrogenic events during the treatment of GTD is needed since these complications could easily be overlooked. Moreover, these events could completely change the therapeutic strategies, and, instead of the simple procedure, the patient could require chemotherapy treatment or even hysterectomy.

Disclosure statement

The authors declare no conflicts of interest.

Acknowledgement

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Minimally invasive treatment of transorbital penetrating injury of skull base and cavernous sinus – A case report

Minimalno invazivno lečenje penetrantne transorbitalne povrede baze lobanje i kavernoznog sinusa

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Abstract

Introduction. A transorbital intracranial injury with a foreign body can be a very complex and controversial therapeutic problem. The orbit's content is susceptible to penetrating trauma, and neurovascular skull base structures are at high risk from injury. There are some traditional cranial surgical approaches and more recently reported different endoscopic approaches for treating this kind of injury. **Case report.** We presented a case of a 30-year-old male who had an accident at work when a piece of wood hit him in his head and entered through the medial aspect of his left orbit with skull base and cavernous sinus injury. Rapid and complete radiological and clinical assessments were performed, and the patient was treated in a minimally invasive manner. The foreign body was manually extracted with an endoscopic and endovascular team ready to treat adverse events. No postoperative complications were reported, and visual acuity increased at the one-month follow-up. **Conclusion.** Penetrating wounds of the orbit represent a challenge that requires a multidisciplinary assessment and well-organized management. Combined endoscopic minimally invasive approaches should be considered during the treatment of this kind of injury.

Key words:

decompression, surgical; head injuries, penetrating; minimally invasive surgical procedures; neurosurgical procedures.

Apstrakt

Uvod. Transorbitalna intrakranijalna povreda stranim telom predstavlja veoma složen i kontroverzan terapijski problem. Sadržaj očne duplje je podložan penetrantnim povredama, a postoji i veliki rizik od povreda neurovaskularnih struktura baze lobanje. Postoje tradicionalni transkranijalni hirurški pristupi, ali se u skorije vreme sve više koriste različiti endoskopski pristupi pri tretiranju tih povreda. **Prikaz bolesnika.** Prikazan je tridesetogodišnji muškarac koji je, na radnom mestu, zadobio penetrantnu povredu leve očne duplje komadom drveta što je dovelo do povrede baze lobanje i kavernoznog sinusa. Posle kliničkog pregleda, brze i kompletne radiološke dijagnostike i sprovedene procene, pacijent je zbrinut minimalno invazivnom metodom. Strano telo je ručno izvađeno uz pripravan endoskopski i endovaskularni tim za lečenje mogućeg pogoršanja stanja i komplikacija. Do komplikacija nije došlo, a na kontrolnom pregledu mesec dana po otpustu ustanovljeno je poboljšanje oštrine vida pacijenta. **Zaključak.** Penetrantne povrede očne duplje predstavljaju izazov koji zahteva multidisciplinarni i dobro organizovan pristup lečenju. Trebalo bi uvek razmotriti kombinovni, minimalno invazivni endoskopski tretman ovakvih povreda.

Ključne reči:

dekompresija, hirurška; glava, penetrantne povrede; hirurgija, minimalno invazivne procedure; neurohirurške procedure.

Introduction

Transorbital penetrating injuries present a significant threat not only to orbital but also major skull base vascu-

lar structures and cranial nerves. This type of injury represents a small portion of all head injuries; however, they make up 24% of penetrating head injuries in adults and 45% in children ^{1, 2}. The penetrating injury and foreign

body of the orbit can be followed by traumatic optic neuropathy presented by partial or complete loss of visual function. The extent of cerebral and skull base injuries is related mainly to the size, shape, trajectory, and velocity of the penetrating object and orbital bone anatomy. Some injuries may be occult with a smaller foreign object and entry wound, and the foreign body itself may be composed of different materials.

Computed tomography (CT) is most frequently used to assess head injuries, mainly because of the need for rapid diagnosis and potential metal foreign material in this kind of trauma^{3,4}. The local physical, neurological, and ophthalmic examination must be performed before and after any treatment.

The aims of the treatment are to remove the foreign body while preserving the orbital content. Reconstruction of the skull base relationships, if required, is mandatory with as little secondary injury as possible⁵. Recovery and the functional outcome depend on many factors, the time elapsed after the injury being one of the most important⁶. The range of potential delayed intracranial complications from penetrating orbital injuries is rather broad and includes meningitis, encephalitis, pyogenic abscess, cerebrospinal fluid leakage, traumatic pseudoaneurysm, or carotid-cavernous fistula⁷.

We reported successful extraction of wooden foreign body from the orbit with skull base and cavernous sinus injury.

Case report

A 30-year-old male was admitted to the Emergency Department after an accidental work injury when a piece of wood hit him in his head, in the region of the superior eyelid. It entered into the medial aspect of his left orbit and became wedged with a massive part visible outside (Figure 1). He was admitted within one hour after the injury, completely conscious, with a Glasgow Coma Scale score of 15/15. Vision, light reflex, as well as all bulbomotor functions in the left eye were lost. No cornea, lens, or posterior segment damage of the eyeball was present. The numbness into the ipsilateral V1 region was present, and the rest of the cranial nerves were normal. Other than the entry wound, there were no signs of injury or bleeding. A non-contrast-enhanced CT scan revealed a hypodense foreign body localized along the axis of the orbit, between the superior rectus, medial rectus muscles, and the optic nerve. The tip of this piece of wood penetrated the skull base and entered into the anterior part of the cavernous sinus (Figure 2). A CT angiogram showed no carotid injury, but there were some air bubbles into the cavernous sinus itself.

The multidisciplinary team proposed manual extraction of the foreign body in general anesthesia, with the tip of it gently detached from the cavernous sinus and pointed away – towards the lateral and caudal side during the extraction process. The immediate postoperative course was uneventful, and the patient was placed on high-dose steroids for optic

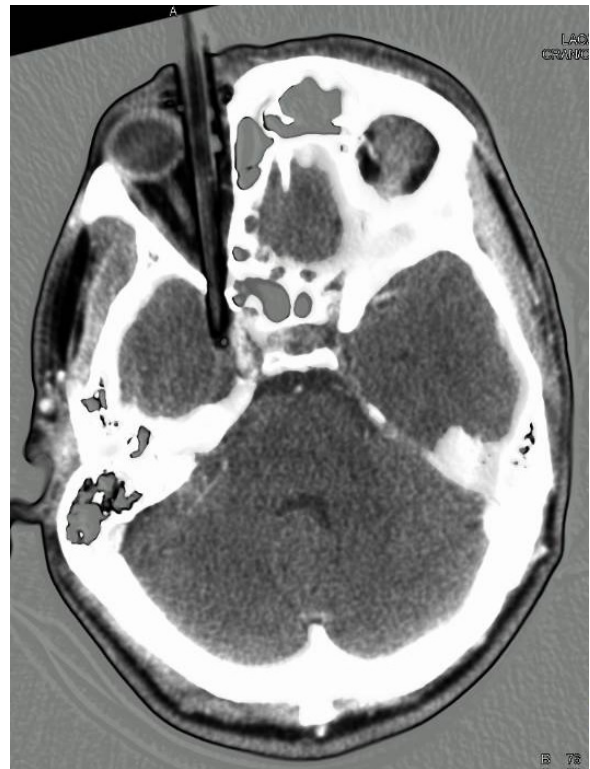


Fig. 1 – Computed tomography reconstruction of patient initial appearance with wooden foreign body penetrated into the left orbit (anatomical view).



Fig. 2 – Preoperative axial computed tomography scan reveals tip of the foreign body (asterisk) in the anterior part of cavernous sinus with some air bubbles consequently in it (arrow).

nerve protection as well as prophylactic antibiotics for three weeks. A control CT scan revealed no significant bleeding and secondary injury (Figure 3). The patient was discharged home three weeks after the surgery. The left pupil remained

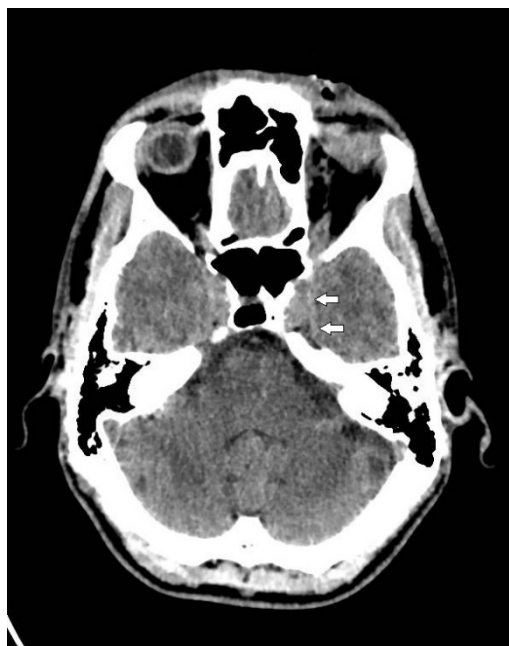


Fig. 3 – Postoperative axial computed tomography scan demonstrates complete decompression of the orbit and no additional intracranial hemorrhage in the region of cavernous sinus (arrows).

nonreactive to light, and facial numbness resolved completely. Visual acuity improved, and all bulbomotors regained their function at the one-month follow-up with a good local cosmetic result.

Discussion

Penetrating transorbital injury always represents a complex and controversial therapeutic problem that requires a thorough patient condition assessment. These injuries often lead to severe consequences, and complications can occur if appropriate surgical intervention is not performed. Traumatic optic neuropathy is one of the most important ophthalmic

emergencies, and recovery success depends on the rapid treatment of optic nerve injury and decompression within 8 hours^{8,9}. Standard skull and facial radiographs are not sufficient, and CT studies should include dedicated axial, coronal, and sagittal images. Fractures can be absent when the natural skull base foramina are transversed. The role of angiography is not to be underestimated before the treatment because it is essential to assess the extent of the injury and rule out carotid dissection, traumatic pseudoaneurysm, cavernous sinus thrombosis, or carotid-cavernous fistula¹⁰.

Some traditional and rather extensive cranial approaches to posterior orbit have been described in the literature, and in the last decade, the role of endoscopy in treating the anterior cranial fossa and skull base pathology is continuously evolving^{11,12}. Our multidisciplinary team from neurosurgery, interventional neuroradiology, anesthesiology, maxillofacial surgery, and ophthalmology planned the best and the least invasive way to extract the foreign body¹³. There were two main solutions to this problem. The first is pterional craniotomy and direct microsurgical control of the cavernous sinus and potential carotid injury during extraction. The second is manual extraction itself and, if needed, subsequent endoscopic examination with local tamponade of the wound. Angio suite was prepared for fast access and endovascular carotid control in case of uncontrolled bleeding during an actual treatment^{14,15}. The endoscope inserted through the wound or additional surgical incision offers good exposure of the orbit and all anterior skull base region, with the possibility of angled vision to important neurovascular structures¹⁶.

Conclusion

Surgical removal of penetrating orbital foreign bodies is a classic example of an interdisciplinary therapeutic approach, where the best outcome is usually the result of rapid intervention and assessing the best approach. Combined endoscopic minimally invasive approaches should be considered during the treatment of this kind of injury.

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Introduction. After the introductory notes, the aim of the article should be stated in brief (the reasons for the study or observation), only significant data from the literature, but not extensive, detailed consideration of the subject, nor data or conclusions from the work being reported.

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Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

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

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. *The Washington Manual of Medical Therapeutics*, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

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

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

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Primeri referenci:

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

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

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

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

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

Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u levom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

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