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SADRŽAJ / CONTENTS

Alexander Porfiryevich Borodin (Novemeber 12, 1833 – February 27, 1887), a famous Russian composer was also a well-known scientist. His research in toxicology, organic chemistry and biochemistry had a great influence on the synthesis of many pharmacologically active substances used in modern medicine (see pages 233–6).

Aleksandar Porfirijevič Borodin (12. novembar 1833 – 27. februar 1887), čuveni ruski kompozitor bio je i dobro poznati naučnik. Njegova istraživanja u oblasti toksikologije, organske hemije i biohemije imala su velik uticaj na sintezu mnogih farmakološki aktivnih supstancija koje su našle primenu u modernoj medicini (vidi str. 233–6).

PRELIMINARY REPORT

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A preview of the efficiency of systemic family therapy in treatment of children with posttraumatic stress disorder developed after car accident

Preliminarna procena efikasnosti sistemske porodične terapije u lečenju dece sa posttraumatskim stresnim poremećajem izazvanim saobraćajnom nezgodom

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Abstract

Background/Aim. Traumatic stress refers to physical and emotional reactions caused by events which represent a life threat or a disturbance of physical and phychological integrity of a child, as well as their parents or gaerdians. Car accidents are the main cause of posttraumatic stress disorder (PTSD) in children. The aim of this study was to preview clinical efficiency of systemic family therapy (SFT) as therapy intervention in treatment of children with posttraumatic stress disorder (PTSD) traumatized in car accident under identical circumstances of exposure. We pointed out the importance of specific family factors (family cohesion and adaptability, emotional reaction of the parents) on PTSD clinical outcome. **Methods.** The sample of this clinical observational study included 7-sixth grade pupiles – 5 boys and 2 girls, aged 13. All of the pupils were involved in car accident with one death. Two groups were formed – one group included three children who were involved in 8 SFT sessions together with their families. The second group included 4 children who received an antidepressant sertraline in the period of three months. **Results.** Two months after the car accident, before the beginning of the therapy, all of the children were the members of rigidly enmeshed family systems, considering the high average cohesion scores and the low average adaptability scores on the FACES III. Three months after the received therapy, having evaluated the results of the therapeutic approaches, we established that the adaptability scores of the families included in the SFT were higher than the scores of the families of the children who received pharmacotherapy with one boy still meeting the criteria for PTSD. **Conclusion.** Systemic family therapy was efficient in the treatment of children with PTSD, traumatized in car accident. Therapy efficiency was higher when both parents and children were included in SFT than in the case when they were not included in the family therapy. The change in the functioning of the family systems was not accidental or simply time-dependant, but it depended on the therapy which was applied and the increased level of family adaptability as the main risk factor of retraumatization.

Key words:
stress disorders, post-traumatic; child; family; accidents, traffic; questionnaires; therapeutics.

Apstrakt

Uvod/Cilj. Traumatski stres obuhvata fizičke i emocionalne reakcije na događaje opasne po život koje remete fizički i psihološki integritet dece, kao i njihovih roditelja ili staratelja. Saobraćajne nezgode predstavljaju glavni uzrok posttraumatskog stresnog poremećaja (*posttraumatic stress disorder* - PTSD) kod dece. Cilj rada bio je da se prikaže klinička efikasnost sistemske porodične terapije (*systemic family therapy* – SFT) kod dece sa PTSD, traumatizovane u saobraćajnoj nezgodi u istovetnim okolnostima. Želeli smo da naglasimo i značaj reagovanja porodice na akutnu traumatizaciju dece, kao i značaj specifičnih porodičnih faktora na moguću retraumatizaciju dece. **Metode.** Uzorak je sačinjavalo sedam učenika VI razreda osnovne škole, pet dečaka i dve devojčice, uzrasta 13 godina. Svi učenici bili su učesnici saobraćajne nezgode sa jednim smrtnim ishodom. Formirane su dve grupe: jednu grupu činilo je troje dece, koja su zajedno sa porodicama bila uključena u osam sesija SFT, a drugu grupu činilo je četvoro dece koja su lečena antidepresivom sertralinom tokom tri meseca. **Rezultati.** Dva meseca nakon saobraćajne nezgode, pre započinjanja terapije, sva deca iz istraživanja bila su članovi rigidno umreženih porodica. Tri meseca nakon primenjene terapije, ocenjivanjem rezultata primenjenih terapijskih pristupa, klinički, ali i testovno, uočeno je da je stepen

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adaptibilnosti porodica uključenih u SFT bio viši u poređenju sa porodicama dece uključenih u farmakoterapijsko lečenje. **Zaključak.** Sistemska porodična terapija pokazala se efikasnom u lečenju i prevenciji retraumatizacije dece sa PTSD, traumatizovane u saobraćajnoj nezgodi. Rizik od retraumatizacije bio je manji uključenjem dece i roditelja u SFT nego što je to bio slučaj sa decom čiji roditelji nisu bili uključeni u porodičnu terapiju. Smatramo da promena funkcionisanja porodičnih sistema nije bila slučajna i da je zavisila od primenjene terapije i povećanja nivoa porodične adaptabilnosti kao mogućeg glavnog faktora rizika od retraumatizacije.

Ključne reči:
stresni poremećaji, posttraumatski; deca; porodica; udesi; upitnici; lečenje.

Introduction

Traumatic stress refers to physical and emotional reactions caused by events which represent to threat a life or disturbance of physical or psychological integrity of a child or a person of critical importance to the child. The term "retraumatization" is used to denote reactivation of a trauma and to describe a mild and passing or marked and permanent increase in posttraumatic sress dysorder (PTSD) symptoms [1]. In both classifications of mental disorders, in all revisions, the criteria for diagnosing PTSD in adults were the same to those in children and adolescents, except in the last revision of DSM IV classification.

Systemic family therapy (SFT) is, by definition, a therapeutic method designed to change nonfunctioning patterns of family interaction in stressful situations and transitional points in the family's life cycle. SFT does not focus on the cause, treatment of symptoms or diagnosing an individual disorder in identified patients.

Car accidents are the main cause of PTSD in children in industrialized countries [1]. Six months after the trauma, 25%–30% of the children who survived car accidents and up to 78%–82% of those who already met the criteria for acute stress disorder met the criteria for PTSD [2, 3]. It is apparent that a traumatic event is necessary, but insufficient to cause PTSD in conditions of equal exposure to trauma, *ie* that there are other, indirect, factors for appearance and continuation of PTSD, in both children and adults [4, 5]. The reactions of the child's environment (parents, the public) also represent a risk for secondary retraumatization [6]. The ability of parents and guardians' to control and manage their own emotions, as well as to be emotionally available to the child after the trauma, represents the most important measure for the degree of psychological disturbances the child will experience after the trauma and the most important protective factor in retraumatization [1, 7, 8]. An elevated degree of anxiety and neuroticism, as well as the existence of other mental problems represent important individual predisposing factors and increase the vulnerability to traumatic experiences [9].

It has been shown that in adults, unlike in children, personal belief that they would experience death during an incident or the presence of a traumatic death or body mutilation of another person represents a high risk factor for the development of psychopathology [10]. In some of the described cases, an indirect traumatic event, such as realizing that the child had been exposed to trauma, was enough to develop a traumatic reaction in the form of peritraumatic stress disorder in parents [5, 11]. An inadequate emotional and social support longitudinally increases emotional and social isolation of adults, as well as the intensity of PTSD symptoms [12].

The key symptoms of PTSD have been classified into 3 groups: re-experiencing, avoidance and hyperarousal. In children and adolescents, the symptoms may vary or be incompletely manifested due to the way children manifest symptoms of re-experiencing or emotion towards a traumatic event [13]. It is typical that the symptoms show a tendency towards grouping around the signal of re-experiencing while children and adolescents attempt to avoid an emotional experience of the trauma, which leads to a series of signs showing an increase in psychological arousal [4]. That is why PTSD may be undiagnosed or misdiagnosed as depression, generalized anxiety, or a mixed conduct and emotional disorder leading to omitting the required therapeutic interventions [1, 2]. The fifth edition of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM-V) is going to propose special criteria for PTSD in preschool and school children, as well as in adolescents. The introduction of a new entity – Developmental Trauma Disorder is also going to be proposed [14].

So far, no psychotherapeutic approach to treating PTSD developed after a car accident has shown superiority, but multiple studies have emphasized the efficiency of trauma focused cognitive-behavioral therapy (TF-CBT) [7, 10]. The record of TF-CBT efficiency has been well supported by data in the literature [10], but the data about TF-CBT superiority over SFT is very limited. On the other hand, the data of SFT influence on war veterans suffering from PTSD is very well supported, especially those regarding the key importance of social and emotional support from the patient's closest environment, also affected by the disorder, but whose symptoms are significantly less present [15]. The efficiency of both monotherapeutic SFT and SFT in combination with other therapeutic methods (especially with cognitive-behavioral therapy has also been well documented [16, 17].

Recovery from PTSD includes integration and organization of the traumatic memory into a coherent content, as well as the establishment and maintenance of emotional control during repeated exposure to real or conditioned trauma signals. [10].

The aim of this clinical observational study was to show clinical efficiency of SFT as therapy intervention in treatment of children with PTSD, traumatized in a car accident under identical circumstances. We pointed out the importance of family reactions to acute traumatization of children, as well as the significance of these specific family factors (parents' emotional reaction, family cohesion and adaptability) on PTSD clinical outcome [18–20].

Methods

The sample of this observational study included of 7 pupils from the same, sixth grade elementary school class, 5 boys and 2 girls, aged 13. In May 2009, all of the pupils were involved in car accident. Out of 50 pupils who were on the bus in which one of their fellow pupils died, the seven previously mentioned children developed clinical symptoms of PTSD two months after the accident, and together with their parents seeked for psychiatric help. Other pupils were not available for research. None of the study subjects eyewitnessed the actual death of their classmate at a moment of bus crash.

They were diagnosed according to the semistructured diagnostic interview Kiddie-Sads-Present and Lifetima Version (K-SADS-PL) and according to DSM-IV-TR inclusion criteria, subsequntly based on their medical history, the medical history of their relatives, their psychological status and psychological testing [18, 19]. None of the seven children had any comorbid physical or mental disorder. No participant of the study renounced the applied therapeutic procedures.

Two groups of instruments were used for testing. The first group of instruments was used to determine the children's general and specific psychological functioning and to make a diagnosis (K-SADS-PL and DSM-IV-TR classifications). Psychological testing was performed using the WISK IQ test [21], while personality characteristics were reviewed using Eysenck's EPQ test [22].

The second group of tests was used to determine possible mediatory factors in maintaining symptoms in children. Relations within their families, their organization and communication were reviewed with a systemic interview of families and by using the FACES III questionnaire [23], while the emotional functioning of the parents was determined using the Beck's Anxiety Inventory – BAI, which the parents filled out during their first interview and three months later [24].

Taking into account basic needs of the patients, as well as therapy recommendations and ethical dilemmas, we provided our test subjects with nondirective supportive therapy (NST) for posttraumatic reactions and 8 sessions of individual, TF-CBT. The principles and techniques regarding TF-CBT have been well documented, as have those regarding NST.

Two groups of examinees were formed. One group was made up of 3 children who, along with their families, took part in 8 sessions of SFT, while the other group was made up of four children who were treated with an antidepressant selective serotonin re-uptake inhibitor (SSRI) sertraline (50–100 mg) in the period of three months, with a single dose taken each morning. The inclusion criteria for family therapy were negative attitudes of parents towards the use of drugs in children. This could be a problem in randomizing, but such attitude would certainly represent an inclusion limitation of those children in the medication treatment protocol.

The SFT included: direct conversation about the trauma-triggering event, psychoeducation about the family's reactions to the traumatic event and their skills to adaptation, the use of reframing and externalization techniques aided by "trauma narratives", challenging networking and overprotection, narrowing intrusiveness and triangulation, supporting attempts at solving the problem completely and independently.

Results

All the test subjects had symptoms of: re-experiencing (intrusive thoughts, images, scenes about the traumatic event, recurring nightmares with oneiric sequences of the accident); avoidance/inhibition (avoiding to talk about the accident, avoiding to ride the bus, not going on field trips or excursions, showing lack of motivation to study, avoiding contact with other children); hyperarousal (increased irritability and anger management problems, difficulty in focusing attention accompanied by hypervigilance).

Place of residence (country-town), IQ, personality characteristics (Table 1) did not influence the development of symptoms. The average values from Table 1 did not show statistically significant differences between the sexes.

Table 1

General characteristics of the children with posttraumatic stress dysorder after car accident

Sex	Number		Age	Place of residence		IQ	Applied therapy	
	n	%		Country	Town		NST + TFCBT + SSRI	NST + TFCBT + SFT
Male	5	71.5	13.5 ± 0.5	2	3	103 ± 8	3	2
Female	2	28.5	13.5 ± 0.3	1	1	100 ± 2	1	1
Total	7	100	13.5 ± 0.4	3	4	101.5 ± 5	4	3

NST – nondirective support therapy; TF- CBT –trauma-focused cognitive behavioral therapy; SSRI – selective serotonin re-uptake inhibitor (sertraline); SFT – systemic family therapy

Two months after the car accident and before the treatment, all the children from the research were the members of family systems which, at that point, were organized rigidly, taking into account the elevated average scores on the cohesion scale and low scores on the adaptability scale on FACES III (Figure 1).

After the evaluation of the therapy, in the group of children involved in SFT a decrease in the level of cohesion and increase in adaptability was noted in both groups, but the difference in the group of children involved in SFT was more noticeable.

Three months after applying the therapy, evaluating the results of the applied therapeutic approaches, it was noticed that the boy, involved both in TF-CBT and pharmacotherapy, still met the K-SADS-PL and DSM-IV criteria for PTSD. All of the mothers had significantly higher scores on the Becks

anxiety scale, as did most of the fathers (suspected peritraumatic stress syndrome/peritraumatic dissociation). After the evaluating of the parents' BAI scores, a significant difference between the first and the scores after the applied therapy was noted in both groups (Figure 2).

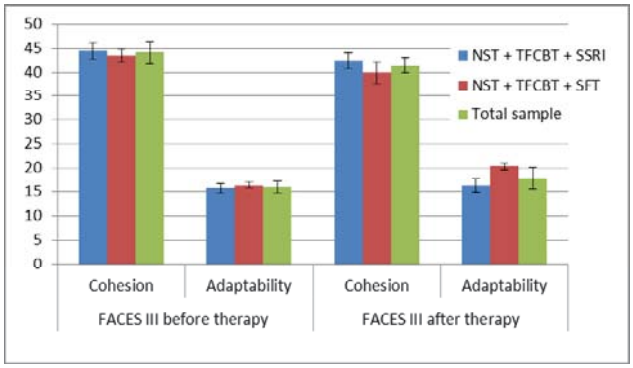

Fig. 1 – Scores on the FACES-III questionnaire before and after the applied therapy;
NST – nondirective support therapy; TF- CBT – trauma-focused cognitive behavioral therapy; SSRI – selective serotonin re-uptake inhibitor (sertraline); SFT – systemic family therapy

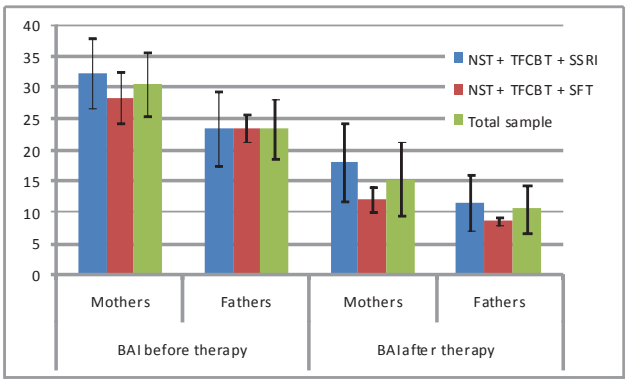

Fig. 2 – Scores on the Beck's Anxiety inventory (BAI) before and after the applied therapy;
NST – nondirective support therapy; TF-CBT – trauma-focused cognitive behavioral therapy; SSRI – selective serotonin re-uptake inhibitor (sertraline); SFT – systemic family therapy

Discussion

So far, there has been no "golden standard" for diagnosing or monitoring PTSD symptoms in children and adolescents and that is why we were guided by the recommendation that a clinical interview, an examination and a family's medical history were optimal in diagnosing PTSD in children [1, 13, 25–27].

It was shown that TF-CBT therapy, in combination with SSRIs and SFT, showed a favorable tendency towards the prevention of PTSD and reduction of anxiety and depression, which is in accordance with the findings of the National Institute for Health and Clinical Excellence [1, 24].

In this pilot clinical preview we showed that NST and TF-CBT, in combination with both therapies tested (SSRI and SFT) had a favorable tendency towards PTSD prevention, but that the combination of NST with TF-CBT and SFT led to a higher level of family adaptability than the combination of NST with TF-CBT and pharmacotherapy (SSRI).

The fact that the tested children were the members of family systems which were, at the time, rigidly organized, can be interpreted as an attempt of the family system to stabilize the chaos in disturbed family routines after an acute traumatic event and a powerful emotional response from the parents (elevated scores on BAI), which probably had a powerful negative effect on the development and maintenance of symptoms in children [28].

Three months after starting the therapy, the level of compensatory family cohesion was greatly reduced in both tested groups, but it was noted that the level of adaptability in the group of children and families included in SFT was considerably increased in comparison with the level of adaptability in the families of the children treated with pharmacotherapy. Our findings may, on one hand, signify a low base level of capacity for adaptability in family systems, which may be viewed as a significant risk factor, but it can also show the significance of family therapy in correction of this dimension, which was increased in children treated with SFT. The significance of the obtained results also lies in the fact that the positive change in family systems functioning is not random and time-dependent, but depends on the type of therapy applied. This is also backed up by the fact that the boy who was included in SSRI therapy, and whose parents, mother in particular, continued to show a high degree of anxiety reactions, still had symptoms of PTSD.

Data from the literature also show that high scores of a family system on the adaptability scale correlate with the harmful influence on the manifestation of different symptoms, which has been observed in abused children as well [7.] Therefore, it can be discussed that in both cases (increased and decreased family adaptability), the child is under greater risk of losing security (the problem of organized and predictable behavior of the environment) and identity ("is no longer the child that used to be before the trauma"), since, by the parents' behavior, the structure of family system is being compromised, age boundaries blurred, and routines and rules are not maintained, which conceptualizes the child as „traumatized". This places it in the position in which it mentally attenuates the traumatic event, which is made significantly more difficult by new interactions in the family. *

Our results show that without including family members in the therapy and the simultaneous adjustment of family functioning and the way it interacts with the child, the family continues to function according to dysfunctional patterns, which reinforce the position of the child as "the PTSD child", and that of the parents as "the parents of a PTSD child", in a situation when the disorder is not endogenous.

*One of the examined families reacted with the fall in adaptability and rise in cohesion to such an extent that the relatives came to console the parents in an atmosphere of grief at the loss, which objectively did not occur, as the boy in question survived the accident with no physical injuries.

Such systemic model of family dynamics is in accordance with other findings in the literature [29, 30]. Hence, we believe that the greatest efficiency of SFT is in the area of regulating the level of adaptability in family functioning. Thus, the findings of this study confirm the validity of inclusion of this type of therapy in preventing traumatization and retraumatization by changing the family's perception of the child from that of a "traumatized child" towards a new definition of "a child facing a psychological problem caused by an unpleasant experience", together with encouraging the parents to sustain reasonable discipline, family hierarchy and routines during the stressful period.

Conclusion

A combination of TF-CBT and SFT showed a higher clinical efficiency in the reduction of PTSD symptoms in comparison with a therapeutic approach which included TF-CBT and pharmacotherapy with SSRIs. SFT showed clinical and tested efficiency in regulating adaptability levels in family functioning by reducing them to functional levels in treatment of children with PTSD, traumatized in car accident under identical circumstances.

Still there is not enough clinical data about the efficiency or a combined SFT and TF-CBT in traumatized children for it to be practically applied, but it seems logical that research should continue in that direction, and not only in the field of acute traumatization. Such research would support the recommendation that mental care facilities should have trained staff to work on the prevention of retraumatization, not only with traumatized children, but also with their closest environment.

This study also showed the importance of parental emotional reactions to acute traumatization of children, as well as the significance of parental emotional support and the organization of family functioning as protective factors in preventing PTSD and retraumatization. According to our results, it seems reasonable for future researches to investigate family circumstances that lead children from the same traumatic context to develop PTSD or not.

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Clinical features, treatments and outcomes of influenza A (H1N1) 2009 among the hospitalized patients in the Clinic for Infectious Diseases in Novi Sad

Kliničke karakteristike, terapije i ishodi lečenja gripa A (H1N1) 2009. kod bolesnika koji su ležali u Klinici za infektivne bolesti u Novom Sadu

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Abstract

Background/Aim. Most infections caused by influenza A (H1N1) 2009 virus are presented by mild respiratory symptoms. However, some patients required admission to the intensive care unit (ICU). In this article we aimed to describe the clinical and laboratory characteristics of the patients with influenza A (H1N1) 2009, antiviral therapy use, the disease outcome and risk factors associated with the severe disease. **Methods.** The patients with the signs and simptoms of novel influenza A (H1N1) 2009, admitted to the Clinic for Infectious Disease in Novi Sad, were evaluated. The study included 293 patients hospitalized between October 2009 and February 2010. Basic demographic data, underlying medical conditions, clinical signs and symptoms, duration of the disease before the admission, laboratory tests, radiographic findings, treatment, and the final outcome (survived, died) were all noted. Factors associated with severe disease requiring ICU admission were determined by comparing the ICU cases with control groups of the patients admitted to the hospital but not to ICU. **Results.** The average age of the patients was 32.72 years. A total of 114 (38.9%) of the patients had an underlying medical condition. Asthma and chronic obstructive pulmonary disease were present in 44 (15.01%) of the patients, chronic cardiovascular diseases in 28 (9.56%), diabetes mellitus in 16 (5.46%), malignity in 15 (4.44%) of the patients and 11 (3.75%) of the patients were pregnant. Fever was registered in 282 (96.24%), myalgias in 119 (40.61%), headache in 48 (16.38%), cough in 240 (81.91%), sore throat in 25 (8.53%), runny nose and sneezing in 17 (5.8%) and dyspnea in 110 (37.54%) of the patients. A total of 192 (65.53%) had radiological findings that were consistent with pneumonia. A total of 154 (56.61%) of the patients received antiviral therapy within 48 h. A total of 280 (96.24%) patients were discharged and 13 (4.44%) were transferred to ICU. Fatal outcome was noticed in 2/13 (15.3%) ICU treated patients and 11/13 (84.7%) patients survived. The median time from the onset of illness to the initiation of antiviral treatment was 7.1 days for the patients admitted to ICU and 3.2 days for non-ICU patients ($p < 0.05$). Low blood oxygen saturation ($SaO_2 \leq 92\%$) was more common in ICU admitted patients, 10/13 (76,92%), compared to 28/280 (10%) non-ICU admitted ones ($p < 0.01$). Serum C-reactive protein (CRP) levels > 200 mg/L were noticed in 9/13 (69.23%) patients admitted to ICU and 85/280 (30.35%) patients who were not ($p < 0.05$). **Conclusion.** Most novel influenza A (H1N1) 2009 infections presented mild respiratory disease. Prompt antiviral therapy in patients with A (H1N1) virus infection seem to be the best approach to avoid serious form of the disease. Special attention should be payed to patients having low level of peripheral oxygen saturation and raised CRP serum level.

Key words:
influenza a virus, h_1n_1 subtype; serbia; diagnostic techniques and procedures; drug therapy; comorbidity; pneumonia; prognosis.

Apstrakt

Uvod/Cilj. Infekcije uzrokovane virusom gripa A (H1N1) u većini slučajeva manifestuju u blagom respiratornom simptomatologijom. Ipak, neki bolesnici zahtevaju hospitalizaciju u jedinicama intenzivne nege (*intensive care units* – ICU). Cilj rada bio je prikazivanje kliničkih i laboratorijskih karakteristika bolesnika sa gripom A (H1N1) 2009, primenjene antivirusne terapije, ishoda bolesti i faktora rizika od razvoja teškog oblika bolesti. **Metode.** Studijom su bili obuhvaćeni

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bolesnici sa znacima i simptomima pandemijskog gripa A (H1N1), hospitalizovani u Klinici za infektivne bolesti u Novom Sadu. Studijom je bilo obuhvaćeno 293 bolesnika hospitalizovanih u periodu od oktobra 2009. do februara 2010. godine. Analizirani su osnovni demografski podaci, osnovne bolesti, znaci i simptomi gripa, trajanje simptoma do hospitalizacije, laboratorijske analize, radiografski nalaz, terapija i ishod bolesti (preživeli, umrli). Definisanje faktora rizika od težeg oblika bolesti vršeno je upoređivanjem karakteristika ICU bolesnika sa karakteristikama bolesnika koji nisu zahtevali hospitalizaciju u ICU. **Rezultati.** Prosečna starost bolesnika bila je 32,72 godine. Osnovne bolesti imalo je 114 (38,9%) bolesnika. Astma i hronična opstruktivna bolest pluća registrovana je kod 44 (15,01%) bolesnika, hronična kardiovaskularna oboljenja imalo je 28 (9,56%), šećernu bolest 16 (5,46%), malignitet 15 (4,44%) bolesnika, a 11 (3,75%) bolesnika bile su trudnice. Povišena temperatura registrovana je kod 282 (96,24%), bolovi u mišićima kod 119 (40,61%), glavobolja kod 48 (16,38%), kašalj kod 240 (81,91%), gušobolja kod 25 (8,53%), kijavica kod 17 (5,8%) i dispnea kod 110 (37,54%) bolesnika. Radiološki verifikovanu pneumoniju imalo je 192 (65,53%) bolesnika. Antivirusna terapija kod 154 (56,61%) bolesnika primenjena je u prvih 48 časova bolesti. Sa klinike je otpušteno 280 (96,24%) bolesnika, a 13 (4,44%) bolesnika je premešteno u ICU. Smrtni ishod zabeležen je kod 2/13 (15,3%) bolesnika lečenih u ICU, dok je 11/13 (84,7%) preživelo. Prosečno vreme od pojave simptoma do primene antivirusne terapije iznosilo je 7,1 dana za bolesnike lečene u ICU, a 3,2 dana za bolesnike koji nisu bili hospitalizovani u ICU ($p < 0,05$). Hipoksemija sa saturacijom kiseonika $SaO_2 \leq$ 92% registrovana je kod 10/13 (76,92%) bolesnika lečenih u ICU, i kod 28/280 (10%) koji nisu premešteni u ICU ($p < 0,01$). Vrednosti C-reaktivnog proteina (CRP) u serumu > 200 mg/L zabeležena su kod 9/13 (69,23%) bolesnika u ICU i kod 85/280 (30,35%) bolesnika bez transfera u ICU ($p < 0,05$). **Zaključak.** Novi grip se ispoljava uglavnom kao blago respiratorno oboljenje. Rana primena antivirusne terapije može sprečiti razvoj težih formi oboljenja. Posebnu pažnju treba obratiti na bolesnike sa hipoksemijom i porastom vrednosti CRP u serumu.

Ključne reči:
grip a virus, podtip h_1n_1; srbija; dijagnostičke tehnike i procedure; lečenje lekovima; komorbiditet; pneumonija; prognoza.

Introduction

During the spring of 2009, a novel influenza A (H1N1) virus of swine origin caused human infection and acute respiratory illness in Mexico [1, 2]. After initially spreading among persons in the United States and Canada the virus spread globally, resulting in the first influenza pandemic since 1968 with circulation outside the usual influenza season in the Northern Hemisphere [3, 4]. By March 2010, almost all countries had reported cases, and more than 17,700 deaths among laboratory-confirmed cases had been reported to the World Health Organization (WHO) [5].

Compared with seasonal influenza, the number of hospitalizations, admission to intensive care units (ICU), and invasive life support were disproportionately high among children and young adults, whereas underlying medical conditions, especially pregnancy, immunosuppression, obesity, diabetes, cardiovascular and pulmonary disease were identified as risk factors for hospitalization of patients with pandemic influenza A (H1N1) 2009 [6–10]. Frequently reported complications have included pneumonia, bacterial coinfection and exacerbation of underlying medical conditions [11–13].

The first case of pandemic influenza A (H1N1) in Vojvodina was registered on June 24, 2009. At the end of October 2009, the mandatory outbreak investigation of acute respiratory illness detected new cases of pandemic influenza A (H1N1) among students who had returned from school-organised trips to Prague, Bratislava and Vienna. This was considered the beginning of pandemic influenza in the Autonomous Province of Vojvodina [14, 15].

Although most patients presented mild and self-limited symptoms with no sign of pulmonary involvement, some patients required admission to an ICU and received maximal life support measures. Therefore, the information on the clinical spectrum and risk factors for severe form of the disease, treatment and outcome of patients with pandemic influenza A (H1N1) 2009 is still collected [8, 16–18].

The aim of the study was to describe clinical and laboratory characteristics of the patients with pandemic influenza A (H1N1) 2009, antiviral therapy use, disease outcome and risk factors associated with severe disease requiring admission to intensive care unit (ICU).

Methods

We retrospectively studied 293 patients with confirmed or suspected novel influenza A (H1N1) 2009 hospitalized in the Clinic for Infectious Diseases, Clinical Center of Vojvodina, between October 2009 and February 2010. We classified patients according to case definitions (confirmed or suspected) developed by the WHO, Centers for Disease Control and Prevention [19, 20]. A suspected case was defined as an influenza-like illness (temperature ≥ 37.5°C and at least one of the following symptoms: sore throat, cough, rhinorrhea or nasal congestion) and either a history of travel to a country where infection had been reported in the previous 7 days or an epidemiologic link to a person with confirmed or suspected infection in the previous 7 days. A confirmed case was defined by a positive result of a real-time polymerase-chain-reaction (RT-PCR) to identify the virus A (H1N1) from nasopharyngeal swabs of the patients.

Our research was conducted based on available medical records of 293 patients hospitalized between October 2009 and February 2010 in the Clinic for Infectious Disease in Novi Sad. Basic demographic data, underlying medical conditions, duration of the disease before the admission, clinical signs and symptoms, laboratory tests, radiographic findings, treatment, and the final outcome (survived, died) were all

noted. Factors associated with severe disease requiring ICU admission were determined by comparing ICU patients with control groups patients who were admitted to hospital but not to an ICU.

Indications for hospital admission included radiographic findings that were consistent with pneumonia, exacerbation of underlying medical condition, especially asthma or chronic obstructive pulmonary disease, hypoxemia, hemodynamic instability and dysfunction of other organs. Critically ill patients with low blood oxygen saturation ($SaO_2 \leq 92\%$) and with hemodynamic instability with the need for vasopressors were transferred to ICU.

Laboratory analyses were performed in the laboratory of the Clinical Centre of Vojvodina for all the patients including complete blood count, C-reactive protein (CRP), urea, creatinine, alanine aminotransferasae (ALT), gamma-glutamyl transpeptidase (GGT), creatine kinase (CK), lactate dehydrogenase (LDH) and gas analysis.

Chest radiography were performed at the Institute of Radiology, Clinical Center of Vojvodina. The diagnosis of influenza A (H1N1) was confirmed by RT-PCR from nasopharyngeal swabs of the hospitalized patients. The test was performed in a reference laboratory in the Institute of Immunology and Virology "Torlak" Belgrade.

Diagnosis of pneumonia was based on clinical data and radiographic infiltrates in the lung parenchyma. The diagnosis of acute respiratory distress syndrome (ARDS) was based on clinical findings consistent with acute respiratory infection, massive bilateral lung infiltrates on chest radiography, the absence of heart failure and low level of oxygen saturation (Sa02 < 92%).

The results were presented as numbers and percentages. The Fisher's exact test was used to compare proportions for categorical variables. For continuous variables, Student's *t*-test and χ^2 test to assess the significance in differences between the groups were used. A probability level of $p < 0.05$ was considered statistically significant.

Results

The age of the hospitalized patients in our study ranged from 2 to 84 years, the average age was 32.72 years. Totally 245 (83.6 %) patients were less than 50 years of age, and 61 (20.7%) were patients under the age of 19 years (Table 1). Both genders were equally represented, 152 (51.88%) of the patients were males and 141 (48.12%) were females.

Table 1
The age of 293 hospitalized patients with a novel influenza A (H1N1)

Age (years)	Patients n (%)
2–9	13 (4.3)
10–19	48 (16.4)
20–29	75 (25.6)
30–39	60 (20.5)
40–49	49 (16.8)
50–59	28 (9.6)
≥ 60	20 (6.8)

Most patients had clinical symptoms and signs of general infectious syndrome characteristic for influenza (Figure 1). Fever ≥ 38°C was registered in 282 (96.24%), myalgias in 119 (40.61%) and headache in 48 (16.38%) of the patients. Most patients manifested more or less pronounced symptoms and signs of the respiratory tract: cough was registered in 240 (81.91%), sore throat in 25 (8.53%), runny nose and sneezing in 17 (5.8%) of the patients, while dyspnea was registered in 110 (37.54%) of the patients. Symptoms of gastrointestinal tract were recorded in less than ¼ of the patients (24.2%).

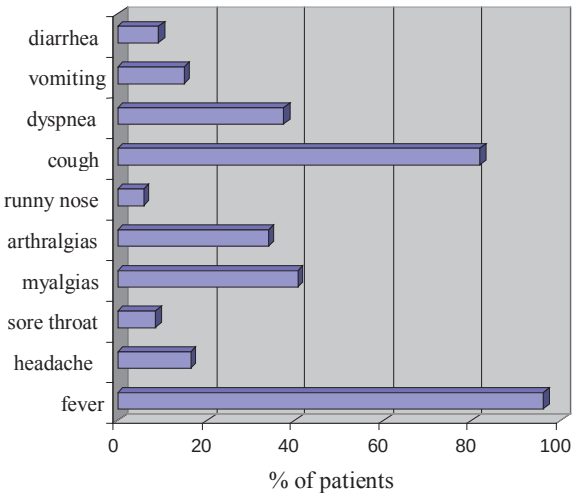

Fig. 1 – The signs and symptoms of a novel influenza A (H1N1) in hospitalized patients

The average time from the onset of illness to hospital admission in our series of patients was 3.7 days (range from 6 hours to 16 days). A total of 116 (39.59%) of them were admitted within 48 hours, 172 (58.7%) within 3 days, 92 (31.4%) were admitted 4–7 days upon occurrence of first symptoms, while 29 (9.9%) patients were hospitalized in the second week (Table 2).

Table 2
The time from the onset of influenza A (H1N1) to hospital admission in 293 patients

The time (days)	Patients n (%)
1–3	172 (58.7)
4–7	92 (31.4)
> 7	29 (9.9)

A total of 114 (38.9%) patients had an underlying medical condition on admission. Asthma and chronic obstructive pulmonary disease (COPD) were present in 44 (15.01%) of the patients, chronic cardiovascular diseases in 28 (9.56%), diabetes mellitus in 16 (5.46%), malignity in 15 (4.44%) of the patients and 11 (3.75%) of the patients were pregnant (Figure 2).

Laboratory data on admission in our series of patients are shown in Table 3. Totally 67 (22.87%) of the patients

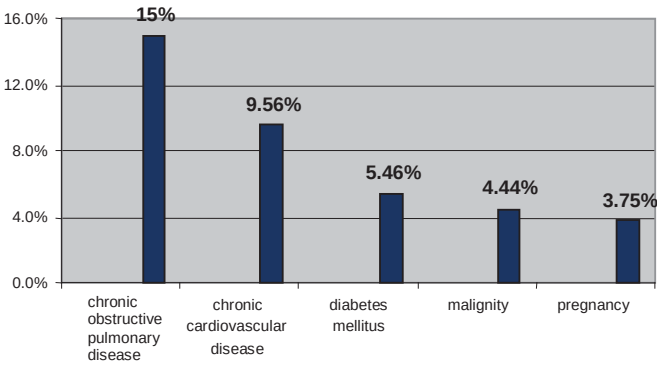

Fig. 2 – Underlying medical condition in hospitalized patients

Table 3

Laboratory data in 293 hospitalized patients with influenza A (H1N1)

Variables	Patients n (%)
WBC > 10 × 10^9/mL^3_3	39 (19.31)
WBC < 4.0 × 10 /mL	67 (22.87)
Lymphopenia	108 (36.86)
Lymphocytosis	42 (14.30)
Neutropenia	65 (22.18)
CRP > 5 mg/L	271 (92.49)
CRP > 200 mg/L	94 (32.08)
Fibrinogen > 4 g/L	62 (21.16)
Increased ALT	41 (14.0)
Increased GGT	10 (3.41)
Increased CPK	65 (22.18)
Increased LDH	24 (8.15)

WBC – white blood cells, CRP – C reactive protein; ALT – alanine aminotransferase; CPK – creatine phosphokinase; GGT – gamma-glutamyl transpeptidase; LDH – lactate dehydrogenase

had leukopenia while leukocytosis was noted in 39 (19.31%) of the patients. Thrombocytopenia was registered only in one case, as a part of pancytopenia after chemotherapy in a patient with leukemia. Elevated serum enzymes were found in less than ½ of the patients. ALT was elevated in 41 (14%) of the patients, GGT in 10 (3.41%), LDH in 24 (8.15%) and CK in 65 (22.18%) of the patients. Elevated CRP levels (> 5 mg/L) was noticed in 271 (92.49%) of the patients (median 133, range 7–312 mg/L) and CRP levels > 200 mg/L was noticed in 94 (32.08%) of the patients.

Novel influenza A (H1N1) infection was confirmed in 19 (4.06%) patients by the real-time PCR test for identification of influenza A (H1N1) virus.

A total of 292 patients underwent chest radiography on admission and 192 (65.53%) had findings that were consistent with pneumonia. Hypoxemia with the saturation of $SaO_2 \leq 92\%$ was noticed in 28 (9.5%) of the patients. ARDS developed in one (2.93%) patient.

Antiviral therapy was prescribed in 278 (94.88%) of the patients after admission to the Clinic. All the patients received oseltamivir. Totally 274 (93.5%) of the patients received antibiotics. Commonly used antibiotics included ceftriaxone, azithromycin and ciprofloxacin. The median time from the onset of illness to the initiation of antiviral therapy was 3.2 days (range, 1–12 days). A total of 154 (56.61%) patients received antiviral therapy within 48 h and 172 (76.1%) of the patients received antiviral therapy within 72 h after the onset of symptoms.

The outcome of illness among our patients was favorable in most cases. A total of 280 (96.24%) patients were discharged and 13 (4.44%) were transferred to ICU. Fatal outcome was noticed in 2/13 (15.3%) patients treated in the ICU and 11/13 (84.7%) patients survived.

The median age of patients admitted to ICU was 28 years (range 6–52 yars). A total of 6 (46.15%) ICU patients had an underlying medical condition on admission. There was no significant difference in the prevalence of background medical conditions among the patients required ICU hospitalization and those who did not. The median temperature and the duration of fever were similar for both hospitalized groups (Table 4).

Table 4

Comparison between variables of ICU and non-ICU patients

Patients parameters	Non-ICU (n = 280)	ICU (n = 13)	*p*
Age, median	32	28	> 0.05
Comorbidity, n (%)			
asthma / COPD	41 (14.64)	3 (23.07)	> 0.05
chronic cardiovascular diseases	26 (9.29)	2 (15.38)	> 0.05
malignity	14 (5.0)	1 (7.69)	> 0.05
Temperature (°C) , median	38.1	37.9	> 0.05
Duration of fever (days), median	3.3	5.1	> 0.05
Saturation $SaO_2 \leq 92\%$, n (%)	28 (10)	10 (76.92)	< 0.01
WBC ($\times 10^9/mL^3$), median	8.9	12.6	> 0.05
PMN (%), median	76	83	> 0.05
LYM (%), median	13	8	> 0.05
CRP > 200 mg/L, n (%)	85 (30.35)	9 (69.23%)	< 0.05
CRP (mg/L), median	93 (7–282)	198 (66–312)	< 0.01
Infiltrate on chest radiographs, n (%)	192 (68.57)	13 (100)	> 0.05
Time from the onset of illness to the initiation of antiviral therapy (days), median	3.2	7.1	< 0.05
Antiviral treatment recevied ≤ 48 h after symptom onset, n (%)	154 (55.0%)	1 (7.69%)	< 0.01

ICU – intensive care unit; COPD – chronic obstructive pulmonary disease; WBC – white blood cells; PMN – polymorphonuclears; LYM – lymphocytes; CRP – C reactive protein

Low blood oxygen saturation ($SaO_2 \leq 92\%$) was more common in the patients admitted to an ICU, 10/13 (76.92%), compared to those who were not, 28/280 (10%), and this difference was significant ($p < 0.01$).

Among the evaluated laboratory parameters, serum CRP levels on admission were the only significantly differentiated factor ($p < 0.01$) between ICU admitted patients and those who were not (median 93 mg/L *vs* 198 mg/L). Serum CRP levels > 200 mg/L was noticed in 9/13 (69.23%) patients admitted to an ICU and 85/280 (30.35%) patients who were not; the difference was also significant ($p < 0.05$).

Chest radiographic findings consistent with pneumonia were more prevalent among patients who required ICU care than among those who did not, but this difference was not statistically significant (100% *vs* 68.57%, $p > 0.05$).

A total of 12/13 (92.3%) patients admitted to an ICU received antiviral drugs, and all received antibiotics. The median time from the onset of illness to the initiation of antiviral treatment was 7.1 days (range 3–12) for ICU admitted patients and 3.2 days (range, 1–10) for those not admitted to ICU (the difference was statistically significant, $p < 0.05$).

Only one (7.69%) of the ICU patients received antiviral therapy within 48 h after the onset of symptoms in contrast to 154/280 (55%) of the non-ICU admitted patients who recevied oseltamivir within 48 h after the onset of symptoms (the difference was significant, $p < 0.01$).

Discussion

In the present study, we retrospectively analyzed clinical features, treatment and outcome of 293 patients with confirmed or suspected novel influenza A (H1N1) hospitalized between October 2009 and February 2010. The majority of the patients (79.3%) were between the age of 10 and 50 years. This is consistent with other studies on 2009 pandemic influenza which found greater affinity of the novel A (H1N1) virus for the younger population [21–27]. The median age of the patients in our study was 32.7 years in contrast to 27 years in the study of Louie et al. [28] and 22.6 years in the study of Xiao et al. [29]. Serologic studies suggest that many older people had preexisting antibodies that cross-reacted with the novel pandemic influenza A (H1N1) 2009 virus. This phenomenon may explain why older people were relatively protected against contracting the virus, while younger people, who lacked these antibodies, were more likely infected [30, 31].

Both genders were equally represented in our study. The structure of our patients by gender is consistent with the knowledge of equal sensitivity of both sexes to influenza A viruses, both seasonal and novel A (H1N1) [9], Mikić et al. [32] found twice as much male patients than female, which is consistent with the gender predominance of male patients at the Military Medical Academy in Belgrade.

Infection with pandemic influenza A (H1N1) 2009 virus causes broad spectrum of clinical syndromes, ranging from afebrile upper respiratory illness to fulminant viral pneumonia. Afebrile or atypical presentations of A (H1N1) infection occurred also in pregnant women, patients with immunosuppression and other chronic disorders [33]. Most patients have typical influenza-like illness with fever and cough that are sometimes accompanied by sore throat and rhinorrhea [2, 24, 33–36]. The commonest symptoms in our patients were: fever (96.24%), cough (81.91%), myalgias (40.61%) and dyspnea (37.54%). Our results concur with data from the United States [6, 13, 14], Mexico [2] and Shanghai [29] in which more than 80% of cases presented with fever. Mild illness without fever has been reported in 8% to 32% of infected persons [7, 34]. According to Burke et al. [37] occurrence of myalgia in addition to dyspnea, raises high suspicion of A (H1N1) infection. The incidence of diarrhea in our study (9.22%) was much lower than previously reported in the United States (25%) and in the United Kingdom (28%) [7, 38, 39]. In the study of Liang et al. [40] fever was reported in 90%, cough in 70%, myalgia in 30% of patients and none reported diarrhea.

The average time from the onset of illness to hospital admission in our series of patients was 3.7 days (range from 6 h to 16 days). It was not significantly different in comparison to some other studies. In the study of Loui et al. [28] the median time from the onset of symptoms to hospitalization was 2 days (range, 0–31 days), in the study of Kumar et al. [41] 4 days (0–18 days) and in the study of Jain et al. [7] it was 3 days (0–18 days).

History of an underlying medical condition was reported in 60%–83% of patients with pandemic influenza A(H1N1) [7]. The most common comorbid illness in our patients were asthma and COPD (15.01%) and chronic cardiovascular diseases (9.56%), In the study of Bewick et al. [42] asthma was also the most common comorbid illness (25.2%). Similarly to our results, chronic cardiovascular disease in the study of Jain et al. [7] was seen in 13% of patients and asthma in 27% of adults patients. Thus, some studies showed that exacerbation of underlying lung disease appears to be a more common indication for hospital admission in patients with seasonal influenza than with pandemic influenza [21].

A number of studies suggest that certain underlying chronic medical condition represent risk factors for complication and severe form of illness. However, in up to 50% of patients with severe disease, no conventional risk factor could be identified [21]. In our study, 38.9% of patients had an underlying medical condition on admission and we also found no significant difference in the prevalence of background medical conditions among the patients who required hospitalization in the ICU and those who did not.

Laboratory findings at presentation, in patients with influenza include normal or low-normal leukocyte counts with lymphocytopenia and elevations in the levels of serum aminotransferases, lactate dehydrogenase, creatine kinase and creatinine [2, 24, 25, 27]. Studies have shown that in the early and late stages of influenza infection, neutrophils play a vital role in inhibiting viral replication, and an inferior status in neutrophil activity may result in severe form of illness even if the viral strain has only medium virulence [43, 44]. In our study, 22.87% of the patients had leucopenia, 36.86% of them had lymphopenia and 19.31% had leucocytosis. The number of patients with leucopenia was similar to that of other authors. For example, in the study of Jain et al. [7] 20% of patients had leucopenia and only 18% of patients had leucocytosis. In the

study of Liang et al.[40] leucocytosis was noted in 30%, neutrophilia in 40%, lymphopenia in 50% patients while in the study of Mu et al. [45] almost 52% of patients had a neutrophil level higher than the upper normal limit, only 5.4% had elevated lymphocyte level and 32.6% had lymphopenia. The pandemic influenza A (H1N1) virus also affected hepatic functioning. This collateral damage to the liver may result from the viral activation of the Kupfer cells in the liver [45]. In our study 14% of the patients showed increased levels of serum ALT and 3.41% of the patients had increased GGT. This is consistent with the study of Mu et al. [45] who noticed 7.6% of the patients with increased levels of ALT and 4.9% of the patients with increased levels of GGT.

The diagnostic utility of biomarkers such as CRP and procalcitonin may be useful for differentiation between viral and bacterial mixed infection. Given the tendency to administer both antiviral and antibacterial therapy to patients infected with novel inflenza A (H1N1) virus, low procalcitonin and CRP levels when combined with clinical judgement, may allow earlier cessation of antibiotic therapy [46]. A retrospective observational study performed at an Australian hospital conducted by Ingram et al. [47] suggested a CRP cutoff of > 200 mg/L best identified patients with bacterial mixed infection (sensitivity 100%, specifity 87.5%). In this study the median value for CRP in the bacterial mixed infection was 363 mg/L and 103 mg/L in the A (H1N1) group. In our study 92.49% of the patients had increased CRP levels > 5 mg/L (median 133, range 7–312) while we noticed CRP levels > 200 mg/L in 32.08% of patients. Humoral immunity test in the study of Mu et al. [45] showed that 71.4% patients had a CRP level higher than the upper limit of the normal range (median 10.80 mg/L). In the study of Bewick et al. [42] median CRP level was 85 (range 34–199) and in the study of Liang et al. [40] it was 20 mg/L (range 7–57).

Song et al. [48] analysed clinical, laboratory and radiologic characteristics of pandemic influenza A (H1N1) pneumonia and concluded that both procalcitonin and CRP would be helpful to differentiate primary influenza pneumonia from concomitant secondary bacterial pneumonia (CRP cutoff value 86 mg/L, sensitivity 81% and specifity 59% was discriminative between patients with concomitant bacterial pneumonia and patients with primary influenza pneumonia). In the study of Ahn et al. [49] the sensitivity and specifity for detection of mixed bacterial infection pneumonia during the pandemic A (H1N1) influenza were 69% and 63% for CRP levels > 100 mg/L. The results of the study conducted by Zimmerman et al. [50] also confirmed that high serum CRP levels were associated with a severe course of influenza A (H1N1) and low initial serum CRP levels were an excellent predictor of good outcome. None of the patients with CRP levels lower than 33 mg/L in this study required ICU care.

Radiographic findings of patients with influenza A (H1N1) virus infection commonly include diffuse mixed interstitial and alveolar infiltrates, particularly in patients with bacterial coinfection [7]. A principal clinical syndrome leading to hospitalization and intensive care is diffuse viral pneumonitis associated with severe hypoxemia, ARDS, and sometimes shock and renal failure [38]. This syndrome accounted for approximately 49% to 72% of ICU admissions [41, 51]. In our study, 192/280 (68,57%) of nonICU patients and 13/13 (100%) of patients who admitted to ICU had radiological findings that were consistent with pneumonia. This number is much higher than in findings of Jain et al. [7] who reported 40% of patients with pneumonia. Similarly to our results, among hospitalized patients with influenza A (H1N1) presented by Louie et al. [28] as many as 66% had pneumonia.

The currently circulating influenza A (H1N1) virus is susceptible to the neuraminidase inhibitors oseltamivir and zanamivir. Therapy with a neuraminidase inhibitor is especially important for patients with risk factors, including pregnancy and those with severe or progressive clinical illness. According to the WHO guidelines on the pharmacologic management of influenza virus, patients who are at risk for pneumonia should be treated with oseltamivir or zanamivir as soon as symptoms develop [52–55]. Available findings suggested the importance of early use of antiviral drugs and antibiotics in the treatment of serious cases [33, 38].

Previous observational studies showed that oseltamivir could reduce the duration of symptoms and shorten the duration of fever in cases of influenza A (H1N1) infection [21, 28, 29]. Most of our patients (94.88%) were treated with oseltamivir and 56.61% of the patients received antiviral therapy within 48 h. The fever disappeared in our patients for the mean 2.38 days after the initiation of the therapy. This is consistent with the data of Hong et al. [39] who found that the fever disappeared for the mean 2.46 days. Totally 85.3% of patients in this study received oseltamivir within 48 h following the onset of symptoms.

Some studies showed that pulmonary complications are common in patients with influenza A (H1N1) infection admitted to ICU, which required early antibiotic therapy in combination with antiviral treatment [56]. In this regard, in most studies, antibiotic therapy was administered in almost all hospitalized patients [7, 32]. In a study of Jain et al. [7] 97% of patients with radiographic findings that were consistent with pneumonia were treated with antibiotics. Commonly used antibiotics included ceftriaxone, azithromycin, vancomycin and levofloxacin. In the study of Falagas et al. [57] 85% of patients received antibiotics and similarly to this, in our study antibiotics were given in 93.5% of the patients. Commonly used antibiotics included ceftriaxone, azithromycin and ciprofloxacin. We applied antibiotic treatment in all the patients with pneumonia and also in the patients with increased CRP levels because of suspect bacterial mixed infection. According to the literature data, *Staphylococcus aureus* is an important cause of secondary bacterial pneumonia with a high mortality rate and treatment of pneumonia should include empirical coverage for this pathogen [24]. Influenza A (H1N1) virus infection can induce rapidly progressive lower respiratory tract disease resulting in acute lung injury and acute respiratory distress syndrome wich calls for intensive care. Severe cases can occur at patients with extreme ages or with underlying chronic medical condition but they can also occur in young and previously healthy people [58–61].

In pandemic influenza A (HIN1), data suggested that timely antiviral treatment was associated with enhanced viral

clearance and improved survival in hospitalized patients. Unfortunately, many patients had a delay before starting antiviral therapy [21]. In the study of Jain et al. [7] 25% of the patients were admitted to ICU. These patients had longer time between the onset of illness and the initiations of antiviral therapy (median 6 days), as compared with patients who were not admitted to ICU (median 3 days). Similarly, in our study the median time from the onset of illness to the initiation of antiviral therapy was 7.1 days for patients admitted to ICU and 3.2 days for non-ICU patients. The small number of patients (4.44%) admitted to an ICU in our study might be attributed to the early administration antiviral therapy. Totally 55% of patients not admitted to ICU received oseltamivir within 48 h in contrast to only one of ICU patients who received antiviral therapy within 48 h after the onset of symptoms. This is consistent with the study of Sertogullarindan et al. [60] who found that none of the patients admitted to ICU had taken oseltamivir within 48 h.

Conclusion

The most novel influenza A (H1N1) 2009 infection presented the mild respiratory disease. Prompt antiviral therapy in patients with A (H1N1) virus infection seem to be the best approach to avoid serious form of disease requiring ICU admission. Special attention should be paid to patients with low level of peripheral oxygen saturation and raised CRP serum level. Early identification of risk patients for developing severe disease is an important aim in preparation for future waves of pandemic A (H1N1) influenza.

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Body weight and waist circumference as predictors of vitamin D deficiency in patients with type 2 diabetes and cardiovascular disease

Telesna masa i obim struka kao prediktori nedostatka vitamina D kod bolesnika sa dijabetesom tipa 2 i kardiovaskularnom bolešću

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Abstract

Background/Aim. Vitamin D deficiency is a well-established risk factor for bone disease, but emerging data suggest that altered vitamin D homeostasis may play a role in the development of type 2 diabetes mellitus (T2DM), dyslipidemia hypertension, and other cardiovascular diseases (CVD). The aim of this study was to investigate the prevalence of vitamin D deficiency in patients with T2DM with/without CVD, to correlate it with anthropometric and metabolic parameters and to determine the predictors of vitamin D deficiency. **Methods.** A total of 88 patients with T2DM (49 male/39 female, aged 61.0 ± 0.9 yrs, body mass index (BMI) 29.9 ± 0.4 kg/m^2) and 67 patients (44 male/23 female, aged 63.6 ± 1.0 yrs, BMI 29.2 ± 0.5 kg/m^2) with T2DM and CVD (myocardial infarction in 57 patients and angina pectoris in 10 patients) were included in this study. These patients were compared with 87 healthy subjects (35 male/52 female, aged 52.8 ± 1.4 yrs, BMI 27.2 ± 0.5 kg/m^2). Weight, height, waist circumference and BMI were recorded in all patients. Also, total cholesterol, triglycerides, hemoglobin A1c (HbA_{1c}) and 25-hydroxy-vitamin D [25(OH)D] levels were measured in all. According to 25(OH)D level, all subjects were divided into three categories: severe vitamin D deficiency (≤ 15 ng/mL), vitamin D insufficiency (15–20 ng/mL) and vitamin D sufficiency (20 ng/mL). We correlated vitamin D levels with anthropometric and metabolic status and determined the predictors of vitamin D deficiency. **Results.** Severe vitamin D deficiency was registered in 16.1% healthy subjects, in 21.6% patients with T2DM and in 26.9% patients with T2DM and CVD. Patients with T2DM who were vitamin D deficient had increased weight, waist circumference, cholesterol and triglyceride levels when compared with patients with T2DM who had sufficient vitamin D level. 25(OH)D levels correlated with BMI and waist circumference in all subjects, but did not correlate with metabolic parameters (lipids, HbA_{1c}). The best predictors of vitamin D level in all subjects were weight, waist circumference and BMI. **Conclusion.** The high prevalence of vitamin D deficiency in patients with T2DM and particularly in patients with T2DM and CVD suggests that supplementation with vitamin D may be beneficial although there is still not sufficient evidence for recommending prescribing vitamin D.

Key words:
diabetes mellitus, type 2; cardiovascular disease; vitamin d deficiency; body weight; body mass index; risk factors.

Apstrakt

Uvod/Cilj. Nedostatak vitamina D je poznat faktor rizika od oboljenja skeleta. Sve je više podataka o ulozi nedostatka vitamina D u razvoju dijabetesa melitusa tipa 2 (T2DM), dislipidemije, hipertenzije, i drugih kardiovaskularnih bolesti (KVB). Cilj studije bio je da se ustanovi učestalost nedostatka vitamina D kod bolesnika sa T2DM sa ili bez KVB, korelacija nedostatka vitamina D sa antropometrijskim i metaboličkim parametrima i odrede prediktori nedostatka vitamina D. **Metode.** U studiju je bilo uključeno 88 bolesnika sa T2DM [(49 muškaraca/39 žena, životno doba 61,0 ± 0,9 god, indeks telesne mase (ITM) 29,9 ± 0,4 kg/m^2)] i 67 bolesnika (44 muškaraca/23 žena, životno doba 63,6 ± 1,0 god, ITM 29,2 ± 0,5 kg/m^2) sa T2DM i KVB (infarkt miokarda kod 57 bolesnika i angina pektoris kod 10 bolesnika). Ovi bolesnici su upoređeni sa 87 zdrava ispitanika (35 muškarca/52 žena, životno doba 52,8 ± 1,4 god, ITM 27,2 ±

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0,5 kg/m²). Mereni su telesna masa, visina, obim struka i ITM, kao i nivo holesterola, triglicerida, hemoglobina A1c (HbA_{1c}) i 25-hidroksi vitamin D[25(OH)D]. Prema nivou 25(OH)D svi ispitanici bili su podeljeni u tri grupe: težak nedostatak vitamina D (≤ 15 ng/mL), nedostatak vitamina D (15–20 ng/mL) i zadovoljavajući nivo vitamina D (≥ 20 ng/mL). Ispitana je korelacija nivoa vitamina D sa antropometrijskim i metaboličkim parametrima i određeni su prediktori nedostatka vitamina D. **Rezultati.** Težak nedostatak vitamina D registrovan je kod 16,1% zdravih ispitanika, 21,6% bolesnika sa T2DM i 26,9% bolesnika sa T2DM i KVB. Bolesnici sa T2DM koji su imali težak nedostatak vitamina D imali su veću telesnu masu, obim struka, nivoe holesterola i triglicerida u poređenju sa bolesnicima sa T2DM koji su imali zadovoljavajući nivo vitamina D. Nivo 25(OH)D korelisao je sa ITM i obimom struka kod svih ispitanika, ali nije korelisao sa metaboličkim parametrima (lipidi, HbA_{1c}). Najbolji prediktori nivoa vitamina D kod svih ispitanika bili su telesna masa, obim struka i ITM. **Zaključak.** Visoka učestalost nedostatka vitamina D kod bolesnika sa T2DM, a posebno kod bolesnika sa T2DM i KVB ukazuje na potrebu supstitucije vitaminom D iako još uvek nema dovoljno podataka o tome.

Ključne reči:
dijabetes melitus, insulin-nezavisni; kardiovaskularne bolesti; vitamin d, nedostatak; telesna težina; telesna masa, indeks; faktori rizika.

Introduction

Vitamin D is crucial not only to maintain bone strength, but research also suggests it plays a role in immune system functioning, cancer prevention, glucose and lipid metabolism and cardiovascular health [1]. Just recently Task Force of the Endocrine Society released guidelines to clinicians for the evaluation, treatment and prevention of vitamin D deficiency with an emphasis on the care of patients who are at risk for deficiency [2]. Considering that vitamin D deficiency is very common in all age groups and that few foods contain vitamin D, Task Force recommended supplementation depending on age and clinical circumstances.

Vitamin D is formed mainly in the skin by photolysis of steroid precursors by ultraviolet B radiation and is also found in fish, eggs, fortified milk, cod liver oil, and supplements. Newly formed vitamin D is bound to vitamin D binding protein (DBP) and transported to the liver where is hydroxylated to 25-hydroxyvitamin D [(25(OH)D)]. 25(OH)D is further hydroxylated in the kidney to 1,25-dihydroxyvitamin D [$(25(OH)_2D)$], the most active metabolite of vitamin D [3]. However, serum 25(OH)D is regarded as the best indicator of vitamin D status in individuals without kidney disease, because it is the substrate for the renal and nonrenal production of $1,25(OH)_2D$, has a longer biological half-life than $1,25(OH)_2D$ and circulates in much higher concentrations. Serum 25(OH)D reflects the total production of vitamin D from both endogenous and exogenous sources, including exposure to ultraviolet-B radiation and intake of various dietary forms. People who are at high risk for vitamin D deficiency are the elderly, dark skinned, obese, those who cover all exposed skin or use sunscreen, patients with fat malabsorption syndromes or inflammatory bowel disease [4–7].

At present, there is no consensus on the optimal level of vitamin D; however, it has been suggested that serum 25(OH)D levels greater than 25 ng/mL (Endocrine Society Guidelines 30 ng/mL) are required for optimal health and that values less than 15 ng/mL (Endocrine Society Guidelines 20 ng/mL) have been associated with decreases in bone density and other negative effects of vitamin D deficiency [2, 8].

Recently, many studies have reported an inverse associations between serum 25(OH)D levels and the risk of a variety of diseases, including diabetes mellitus (DM), cardiovascular disease (CVD), cancer, autoimmune diseases, infections, or cognitive decline [9]. Clinically, vitamin D has been shown to be linked with glucose and insulin homeostasis. Observational studies show a relatively consistent association between low vitamin D status and DM, both type 1 DM and type 2 (T2DM) or metabolic syndrome [10–12]. It's already well-known that children living in areas of the world without much sunlight, such as Finland, have higher rates of type 1 diabetes than those in sunnier parts of the world. In fact, infants in Finland are 400 times more likely to develop DM than infants in Venezuela [13]. Some research indicates that infants and children given vitamin D supplements are less likely to develop type 1 DM. Many studies have shown that supplementing with vitamin D and calcium slows the progression to T2DM. Vitamin D deficiency is commonly found in people with poor DM control. As the deficiency worsened, so did DM control. A minority of DM patients took vitamin D supplements. The molecular mechanisms of association of vitamin D deficiency with DM, hypertension, obesity and CVD remain incompletely understood.

As low vitamin D status has been suggested to be a risk factor for T2DM and for CVD, the aim of this study was to determine vitamin D levels in patients with T2DM and in patients with T2DM and CVD and correlate the levels of vitamin D to their anthropometric and metabolic status.

Methods

A total of 88 patients with T2DM [49 male and 39 female, aged 61.0 ± 0.9 yrs, body mass index (BMI) 29.9 ± 0.4 kg/m²] and 67 patients (44 male and 23 female, aged 63.6 ± 1.0 yrs, BMI 29.2 ± 0.5 kg/m²) with history of both T2DM and CVD (myocardial infarction in 57 patients and angina pectoris in 10 patients were included in this study). These patients were compared with 87 healthy subjects (35 male and 52 female, aged 52.8 ± 1.4 yrs, BMI 27.2 ± 0.5 kg/m²) with no history of DM or CVD.

Informed consent for the study was obtained from all patients and from healthy subjects. The protocol was ap-

proved by the Ethical Committee of University Clinical Center Podgorica.

The patients' age, weight, height, waist circumference and calculated BMI were recorded. Serum samples were obtained in the morning after an overnight fast. In serum samples we measured total cholesterol, triglycerides, hemoglobin A_{1c} (HbA_{1c}) and 25(OH)D levels. HbA_{1c} was measured with immunoturbimetric analysis (COBAS INTEGRA), cholesterol was measured with enzymatic methodology, while triglycerides were measured with glycerol phosphate oxidase methodology. The HbA_{1c} provides an average measurement of blood sugar control over about a 12-week span. For people with DM, the goal is 7%, for healthy people, the normal range is 4%–6%. Serum levels of 25(OH)D were measured by commercial enzyme immunotest (EIA, Immunodiagnostic system, UK). The limit of detection for 25(OH)D was 5 ng/mL.

Parameters of descriptive statistics are presented as mean ± standard error (SE). For statistical analysis we used parametric *t*-test, nonparametric Mann-Whitney test and ANOVA. We analyzed the influence of sex, age, weight, waist circumference and BMI on vitamin D level. We analyzed the correlation between anthropometric and metabolic parameters and vitamin D level using Spearman's correlation. We used the multiple regression analysis for determination of predictors of vitamin D level. Statistical analysis was performed with SPSS for Windows (version 15.0). *P* value < 0.05 was considered as statistically significant.

Results

Clinical characteristics of three groups of participants in the study (patients with T2DM, patients with T2DM and CVD, and healthy subjects, are presented in Table 1.

HbA_{1c} and lipid levels in patients with T2DM and patients with T2DM and CVD are presented in Table 2. Patients with T2DM had higher levels of HbA_{1c} and cholesterol compared with patients with T2DM and CVD ($p < 0.01$).

Vitamin D levels in patients and healthy subjects are shown in Table 3.

There were no differences in 25(OH)D levels between male and female subjects in all investigated groups (data not shown). There was no difference in vitamin D levels between patients with hypertension (51.0 ± 1.8 ng/mL) and with normal blood pressure (54.1 ± 1.6 ng/mL; $p > 0.05$).

Table 2
Hemoglobin A_{1c} (HbA_{1c}), cholesterol and triglyceride levels in the patients with type 2 diabetes mellitus (T2DM) and the patients with T2DM and cardiovascular disease (CVD)

Parameters	T2DM	T2DM and CVD
HbA_{1c} (%)	7.6*	7.2
Cholesterol (mmol/L)	6.1*	5.5
Triglycerides (mmol/L)	2.8	2.3

*$p < 0.01$ *vs* T2DM and CVD

Table 3
25-hydroxyvitamin D [25(OH)D] level in the patients with type 2 diabetes (T2DM), the patients with T2DM and cardiovascular disease (CVD) and in the healthy subjects

Groups of patients	25(OH)D (ng/mL)
T2DM	21.7 ± 0.8 (6.8–44.8)
T2DM and CVD	19.4 ± 0.8* (7.6–33.2)
Healthy subjects	22.6 ± 0.8 (8.0–55.2)

*SE – standard error; $p < 0.01$ *vs* healthy subjects

In all subjects, 25(OH)D levels inversely correlated with BMI (r = -0.127, $p = 0.048$) and waist circumference (r = -0.165, $p = 0.010$), but did not correlate with metabolic parameters (lipids, HbA_{1c}). According to 25(OH)D level, we divided all subjects into three categories: severe vitamin D deficiency (less than 15 ng/mL), vitamin D insufficiency (15 to 20 ng/mL) and vitamin D sufficiency (above 20 ng/mL). Table 4 shows the vitamin D status in all studied patients. The percentages of patients with severe vitamin D deficiency and vitamin D insufficiency were the highest (26.9% both) in patients with T2DM and CVD.

Nineteen patients with T2DM (21.6%) were severe vitamin D deficient. Clinical characteristics of these patients are presented in Table 5. Patients with T2DM who were vitamin D deficient had increased body weight and waist circumference ($p < 0.05$) compared with patients with T2DM and vitamin D > 20 ng/mL. Cholesterol and triglyceride lev-

Table 1
Clinical characteristics of the patients with type 2 diabetes mellitus (T2DM), the patients with T2DM and cardiovascular disease (CVD) and in the healthy subjects

Characteristics	T2DM patients (n = 88)	T2DM and CVD patients (n = 67)	Healthy subjects (n = 87)
Sex (male/female), n	49/39	44/23	35/52
Age (years), $\bar{x} \pm SE$	61.0 ± 0.9	63.6 ± 1.0	52.8 ± 1.4
Duration of diabetes mellitus (years), $\bar{x} \pm SE$	7.6 ± 0.6	10.2 ± 0.8	–
Hypertension (yes/no), n	40/48	49/18	–
Weight (kg), $\bar{x} \pm SE$	88.2 ± 1.6*	85.5 ± 2.0	81.8 ± 1.5
Height (cm), $\bar{x} \pm SE$	172.6 ± 1.0	171.8 ± 1.2	173.6 ± 1.1
BMI (kg/m^2), $\bar{x} \pm SE$	29.9 ± 0.4*	29.2 ± 0.5*	27.2 ± 0.5
Waist (cm), $\bar{x} \pm SE$	99.5 ± 1.2*	99.7 ± 1.4*	91.5 ± 1.5
Therapy			
insulin	45	35	–
OAD	43	32	–

*$p < 0.01$ *vs* healthy subjects; SE – standard error; BMI – body mass index; OAD – oral antidiabetics

Table 4

Percentage of subjects with severe vitamin D deficiency, vitamin D insufficiency and vitamin D sufficiency in the patients with type 2 diabetes mellitus (T2DM), patients with T2DM and cardiovascular disease (CVD) and in healthy subjects

25(OH)D (ng/mL)	Vitamin D status	T2DM patients (%)	T2DM and CVD patients (%)	Healthy subjects (%)
< 15	severe deficiency	21.6	26.9	16.1
15–20	insufficiency	18.2	26.9	14.9
> 20	sufficiency	60.2	46.2	69

25(OH)D – 25-hydroxyvitamin D

Table 5

Clinical characteristics of 88 patients with type 2 diabetes mellitus (T2DM) divided in 3 categories according to vitamin D status (severe vitamin D deficiency, vitamin D insufficiency and vitamin D sufficiency)

Variables	25(OH)D in patients with T2DM		
	≤ 15	15–20	> 20
Number (%)	19 (21.6)	16 (18.2)	53 (60.2)
Age (years), ± SE	59.7 ± 1.9	62.4 ± 2.1	61.0 ± 1.3
Weight (kg), ± SE	88.8 ± 4.0*	85.9 ± 4.0	88.7 ± 1.9
Height (cm), ± SE	169.4 ± 2.5	169.3 ± 2.3	174.8 ± 1.3
BMI (kg/m^2), ± SE	31.0 ± 1.0	30.4 ± 1.1	29.4 ± 0.5
Waist (cm), ± SE	103.8 ± 2.6*	99.4 ± 3.2	97.9 ± 1.3

***p < 0.05 *vs* 20 ng/mL; BMI – body mass index; [25(OH)D] – 25-hydroxyvitamin D**

els were higher in patients with severe vitamin D deficiency compared to those with vitamin D 15–20 ng/mL and > 20 ng/mL ($p < 0.01$; Table 6).

Eighteen patients with T2DM and CVD (26.9%) had severe vitamin D deficiency and the same number of patients had vitamin D insufficiency. Clinical characteristics of these patients are presented in Table 7. There were no differences in various parameters (anthropometric, lipids, or HbA_{1c}) between three subgroups of patients divided according to vitamin D status (severe vitamin D deficiency, vitamin D insufficiency and vitamin D sufficiency, Table 8).

Table 6

Hemoglobin A_{1c} (HbA_{1c}), cholesterol and triglyceride levels in the patients with type 2 diabetes (T2DM) divided in 3 categories according to vitamin D status (severe vitamin D deficiency, vitamin D insufficiency and vitamin D sufficiency)

25(OH)D (ng/mL)	Vitamin D status	HbA1c (%)	Cholesterol (mmol/L)	Triglycerides (mmol/L)
< 15*	severe deficiency	7.7	6.8*	4.7*
15–20*	insufficiency	7.4	5.7	2.3
> 20*	sufficiency	7.6	5.9	2.3

***p < 0.05 *vs* group with 15–20 ng/mL and 20 ng/mL 25(OH)D**

Table 7

Clinical characteristics of 67 patients with type 2 diabetes (T2DM) and cardiovascular disease (CVD) divided in 3 categories according to vitamin D status (severe vitamin D deficiency, vitamin D insufficiency and vitamin D sufficiency)

Variables	Vitamin D status measured by 25-hydroxyvitamin D level in patients with T2DM and CVD		
	≤ 15 ng/mL	15–20 ng/mL	> 20 ng/mL
Number of patients (%)	18 (26.9)	18 (26.9)	31 (46.2)
Age (years), ± SE	63.7 ± 1.7	64.1 ± 2.4	63.2 ± 1.5
Weight (kg), ± SE	87.1 ± 5.1	85.0 ± 4.6	84.9 ± 1.9
Height (cm), ± SE	169.5 ± 2.1	173.7 ± 3.0	172.1 ± 1.6
BMI (kg/m^2), ± SE	30.2 ± 1.2	28.3 ± 0.9	29.3 ± 0.6
Waist (cm), ± SE	102.7 ± 3.2	98.6 ± 2.6	98.5 ± 1.7

Table 8

Hemoglobin A_{1c} (HbA_{1c}), cholesterol and triglyceride levels in the patients with type 2 diabetes (T2DM) and cardiovascular disease (CVD) divided in 3 categories according to vitamin D status (severe vitamin D deficiency, vitamin D insufficiency and vitamin D sufficiency)

25(OH)D (ng/mL)	Vitamin D status	HbA1c (%)	Cholesterol (mmol/L)	Triglycerides (mmol/L)
< 15*	severe deficiency	7.3	5.3	2.4
15–20*	insufficiency	7	5.4	2.3
> 20*	sufficiency	7.2	5.6	2.2

The best predictors of 25(OH)D levels in all subjects were weight (p = 0.0001), waist circumference (p = 0.006) and BMI (p = 0.001). In patients with T2DM predictors of 25(OH)D levels were weight (p = 0.0001) and waist circumference (p = 0.004). In patients with T2DM and CVD the best predictor of 25(OH)D level was BMI (p = 0.006).

Discussion

According to our results, a substantial percentage of patients with T2DM, patients with T2DM and CVD, as well as healthy subjects were vitamin D deficient. The worst situation was in the group of patients with T2DM and CVD in which 27% were vitamin D deficient. We found a negative correlation between serum vitamin D levels and BMI and waist circumference in our patients, which is in accordance with other studies [4, 7]. We did not find any gender differences in serum vitamin D levels, in contrast to other studies in which the authors reported higher levels in men than in women [5, 6]. We did not find any correlation between serum vitamin D levels and age or metabolic parameters ($HbA1_c$, lipids).

The strongest relationship (inverse) was found between 25(OH)D levels and BMI and waist circumference. The negative relationship between 25(OH)D and fat mass has been attributed to increased sequestration of fat-soluble vitamin D in adipocytes. It is hypothesized that vitamin D generated in the skin or orally ingested is sequestered into adipocytes before it is transported to the liver and converted to 25(OH)D [14]. It is not known whether the adipocytes simply store vitamin D or actively catabolize it. Other study showed that changes in 25(OH)D levels with age, gender, or fat mass are not due to genetic variability of vitamin D binding protein [15]. Consistently observed negative relationships of 25(OH)D with body composition have a biological origin other than adaptation to plasma transport. Thus obese people are at risk for vitamin D deficiency and may need 2–3 times more vitamin D for their age group to satisfy their bodies vitamin D requirement [2].

Vitamin D deficiency is highly prevalent worldwide and essentially everyone is at risk [2]. Low levels of 25(OH)D are present in as many as one third to one half of otherwise healthy middle-aged to elderly adults [1, 16, 17]. Limited cutaneous synthesis due to inadequate sun exposure or pigmented skin and inadequate dietary intake are the principal causes of low 25(OH)D levels. Although the best characterized consequences of vitamin D deficiency involve the musculoskeletal system, a growing body of evidence suggests that low levels of vitamin D may negatively affect the glucose and insulin homeostasis and the cardiovascular system as well, leading to hyperglycemia, insulin resistance, hypertension, heart disease and cognitive decline in the elderly population [18, 19]. Observational studies showed the association between vitamin D levels deficiency and impaired glucose tolerance or T2DM [10, 11, 20–22]. Glycemic control in T2DM depends on the season, with the lowest HbA_{1c} levels during summer [23]. Data from the Nurses' Health Study found that women who took a combination of 1,200 mg of calcium and more than 800 IU of vitamin D daily had a 33% lower chance of getting T2DM than women taking smaller amounts of these nutrients [10]. It seems that subjects with vitamin D deficiency are at higher risk of insulin resistance and the metabolic syndrome. The unredlying mechanism could be an effect of vitamin D on insulin sensitivity, on β-cell function, or on both [11]. The pancreatic β-cells express vitamin D receptor [24]. Vitamin D deficiency inhibits insulin secretion and modulates lipolysis [25]. Vitamin D supplementation improves insulin secretion, insulin sensitivity and glucose tolerance in vitamin D-deficient animals and in humans [26–28]. Treatment with vitamin D_3 in patients with T2DM increased plasma 25(OH)D and the first phase of the insulin secretion evaluated by an intravenous glucose tolerance test [28].

Cardiovascular system is also affected by vitamin D deficiency. Vitamin D deficiency has been observed in patients with acute myocardial infarction, stroke and heart failure and CVD [29–32]. Data from the Framingham Heart Study indicate that patients with the lowest levels of vitamin D were 62% more likely to have either a heart attack or a stroke than those with higher vitamin D levels. The results were so impressive that authors believe that people should take between 1,000 and 2,000 IU of vitamin D every day. Patients with heart failure have lower plasma levels of 25(OH)D and $1,25(OH)_2D$ than controls [31, 33]. Another large prospective study of Wang et al. [34] in more than 1,700 subjects showed that vitamin D deficiency is associated with increased cardiovascular risk [34]. The authors showed that the higher risk (2-fold risk) was evident among subjects with hypertension, in whom 25(OH)D levels were bellow 15 ng/mL. With this cut point, the prevalence of vitamin D deficiency in the study of Wang et al. [34] was 28%, similar to our results and results reported in other large epidemiological studies [35].

In the UK an increased cardiovascular morbidity was associated with low plasma 25(OH)D concentrations in winter. Similarly, blood pressure was higher in winter than in summer, varies with skin pigmentation and is higher in subjects with vitamin D deficiency [36, 37]. Vitamin D receptors are identified in many tissues, including vascular smooth muscle, endothelium and cardiomyocytes [1, 38]. *In vitro*, $1,25(OH)_2D$ suppresses renin gene expression, regulates the growth and proliferation of vascular smooth muscle cells and cardiomyocytes [39, 40]. In knockout mice model, the absence of vitamin D receptor activation results with the upregulation of the renin-angiotensin system, hypertension and left ventricular hypertrophy [27, 41]. Vascular smooth muscle cells and endothelial cells have the ability to convert circulating 25(OH)D to $1,25(OH)_2D$ [42]. Furthermore, atherosclerosis may be viewed as a chronic inflammatory disease that involves several cytokines (TNF-α, IL-6). Active vitamin D can suppress these cytokines *in vivo* and TNF-α is inversely related to plasma 25(OH)D *in vivo* [43].

Management of vitamin D deficiency may be a simple and cost-effective method to improve blood sugar control, arterial hypertension, dyslipidemia, atherosclerosis and prevent the serious complications associated with these pathological conditions. Diet alone may not be sufficient to manage vitamin D levels. Based on our and other studies people at risk for DM, dyslipidemia and CVD should be screened

for low vitamin D levels which allow health care professionals to identify a nutrient deficiency early on. Getting 25(OH)D consistently above 30 ng/mL may requier 1000–2,000 IU/day of vitamin D as suggested by the recent Endocrine Society Guideline [2].

Conclusion

The results of the present study suggest that vitamin D deficiency is highly prevalent in patients with T2DM and particularly in patients with T2DM and CVD. The best predictors of vitamin D status seem to be weight, BMI and waist circumference all related to the metabolic status. There is no sufficient evidence yet to recommend prescribing vitamin D to attain the benefit for cardiovascular protection, however interventional studies are awaited.

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Correlation of inflammation parameters and biochemical markers of cholestasis with the intensity of lipid peroxidation in patients with choledocholithiasis

Povezanost inflamatornih parametara i biohemijskih markera holestaze sa intenzitetom lipidne peroksidacije kod bolesnika sa holedoholitijazom

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Abstract

Background/Aim. During choledocholitiasis inflammatory oxidant stress involves the promotion of mitochondrial dysfunction through an intracellular oxidant stress in hepatocytes leading mainly to necrosis and less to apoptosis. The product of oxidative stress, malondialdehyde (MDA), is extremely cytotoxic and damages cell membranes and intracellular macromolecules. The toxicity of MDA is based on its ability to act as a mutagenic agent in a cell. Therefore, the aim of this prospective study was to establish correlation of the parameters of inflammation and biochemical markers of cholestasis with the intensity of oxidative stress in pathogenesis of liver function disorders. **Methods.** Seventy adult subjects of either sex included in the study were devided into two groups: I – 40 patients with obstructive icterus caused by choledocholithiasis, and II – 30 healthy individuals. All the participants were subjected to a clinical, laboratory and ultrasonic check-up at the Internal Department of the Military Hospital in Niš. The parameters of oxidative stress: MDA, a measure of lipid peroxidation, and inflammation parameters: C-reactive protein (CRP), fibrinogen, albumins, number of leukocytes (Leu), granulocytes (Gr), lymphocytes (Ly) and monocytes (Mo) and biochemical markers of cholestasis: activity of γ-glutamyltransferase (γ-GT) and alkaline phosphatase (AP) enzymes, the level of total, direct and indirect bilirubin were determined by standard biochemical methods. **Results.** Lower values of albumin ($p < 0.001$), and significantly higher values of fibrinogen ($p < 0.05$) and CRP ($p < 0.001$) were found in the blood of the patients with cholestasis due to choledocholithiasis in relation to the controls. Significantly higher values of Leu ($p < 0.01$) and Gr ($p < 0.001$) with decreasing number of Ly ($p < 0.001$) and Mo ($p < 0.001$) were found in blood of the patients with cholestasis due to choledocholithiasis in relation to the control. Similarly, higher values of γ-GT, and AP ($p < 0.001$), as well as the level of total, direct and indirect bilirubin ($p < 0.001$) were found in blood of the patients with cholestasis due to choledocholithiasis in relation to the controls. The concentration of MDA ($p < 0.001$) was increased in the patients with choledocholithiasis in relation to the controls. There was a significant positive linear correlation of the number of leukocytes ($r = 0.51$, $p < 0.05$) and the concentration of total ($r = 0.87$, $p < 0.01$), direct ($r = 0.85$, $p < 0.01$) and indirect ($r = 0.88$, $p < 0.01$) bilirubin with the concentration of MDA in the group of patients with choledocholithiasis. **Conclusion**. Neutrophils and the levels of total, direct and indirect bilirubin have a significant positive linear correlation with the level of lipid peroxidation in patients with choledocholithiasis. Neutrophilia and hiperbilirubinemia observed in this way represent important parameters in estimating the level of liver tissue damage in choledocholithiasis.

Key words:
choledocholithiasis; oxidative stress; cholestasis; inflammation.

Apstrakt

Uvod/Cilj. U toku holedoholitijaze inflamatorni oksidativni stres uzrokuje mitohondrijalnu disfunkciju kroz intracelularni oksidativni stres u hepatocitima dovodeći uglavnom do nekroze, a ređe i do apoptoze. Produkt oksidativnog stresa, malondialdehid (MDA), izuzetno je citotoksičan i oštećuje ćelijske membrane i intraćelijske makromolekule.

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Cilj ovog istraživanja bio je utvrđivanje povezanosti parametara inflamacije i biohemijskih markera holestaze sa intenzitetom oksidativnog stresa u patogenezi poremećaja funkcije jetre. **Metode.** Ukupno 70 odraslih osoba oba pola uključenih u ispitavanje bilo je podeljeno u dve grupe: grupa I – 40 bolesnika sa opstruktivnom žuticom izazvanom holedoholitijazom i grupa II – 30 zdravih ispitanika. Svi učesnici ispitani su klinički, laboratorijski i ultrazvučno na Internom odeljenju Vojne bolnice u Nišu. Parametri oksidativnog stresa: MDA, mera lipidne peroksidacije, inflamatorni parametri: C-reaktivni protein (CRP), fibrinogen, albumini, broj leukocita (Leu), granulocita (Gr), limfocita (Ly) i monocita (Mo); i biohemijski markeri holestaze: aktivnosti enzima γ-glutamiltransferaze (γ-GT) i alkalne fosfataze (AP), nivo ukupnog, direktnog i indirektnog bilirubina, određivani su standardnim biohemijskim metodama. **Rezultati.** Niže vrednosti albumina ($p < 0,001$) i značajno veće vrednosti fibrinogena ($p < 0,05$) i CRP ($p < 0,001$) nađene su kod bolesnika sa holestazom izazvanom holedoholitijazom u odnosu na kontrolu. Značajno veća vrednost Leu ($p < 0,01$) i Gr ($p < 0,001$), uz opadanje broja Ly ($p < 0,001$) i Mo ($p < 0,001$), uočena je u grupi bolesnika sa holestazom izazvanom holedoholitijazom u odnosu na kontrolnu grupu. Povećane vrednosti enzima γ-glutamiltransferaze (γ-GT) i alkalne fosfataze (AP) ($p < 0,001$), kao i nivo ukupnog, direktnog i indirektnog bilirubina ($p < 0,001$) nađene su u krvi bolesnika sa holedoholitijazom u odnosu na kontrolu. Koncentracija MDA bila je povišena kod bolesnika sa holedoholitijazom u odnosu na kontrolnu grupu. Ustanovljena je značajna pozitivna linearna povezanost između broja leukocita ($r = 0,51$, $p < 0,05$) i koncentracije ukupnog ($r = 0,87$, $p < 0,01$), direktnog ($r = 0,85$, $p < 0,01$) i indirektnog ($r = 0,88$, $p < 0,01$) bilirubina sa koncentracijom MDA u grupi bolesnika sa holedoholitijazom. **Zaključak.** Neutrofili i nivo ukupnog, direktnog i indirektnog bilirubina pokazuju značajnu pozitivnu linearnu povezanost sa stepenom lipidne peroksidacije kod bolesnika sa holedoholitijazom. U skladu sa tim, neutrofilija i hiperbilirubinemija predstavljaju značajne parametre u proceni stepena oštećenja jetrinog tkiva kod holedoholitijaze.

Ključne reči:
holedoholitijaza; stres, oksidativni; holestaza; zapaljenje.

Introduction

The biochemical syndrome occurring in patients with choledocholithiasis is called cholestasis [1]. Hyperbilirubinaemia, which is the integral part of cholestasis syndrome, leads to liver function damage, dysfunction of gastrointestinal barrier, immunodeficiency, coagulation disorders and disorders in detoxification, accompanied by impeded wound healing [2].

Choledocholitiasis (CHDL) also initiates an inflammatory response [3]. The mechanism by which cholestasis initiates an inflammatory response in the liver, however, is not known. In the study of Allen et al. [4] two mechanisms of inflammation were examined. Firstly, activation of Toll-like receptor 4 (TLR4), either by bacterial lipopolysaccharide or by damage-associated molecular pattern molecules released from dead hepatocytes, triggers an inflammatory response. Secondly, bile acids act as inflammagens, and directly activate signaling pathways in hepatocytes that stimulate production of proinflammatory mediators. Koutelidakis et al. [5] have reported that the development of inflammatory response in the liver is a very complex process, involving a coaction of numerous pro- and anti-inflammatory mediators and immune cells.

Numerous experimental studies proved more intense oxidative stress and increased intensity of lipid peroxidation in the plasma and liver tissue in animals with experimentally induced cholestasis [6–7].

Studies have demonstrated that oxidative stress occurs in human livers with choledocholitiasis [3, 5, 8]. Jaeschke [8] has reported that bile duct obstruction is associated with hepatocellular injury, cholangiocyte proliferation, stellate cell activation, Kupffer cell activation, oxidative stress, inflammation and fibrosis.

Oxidative stress is a process of tissue injury due to the free radicals effect. They can damage almost all important biomolecules and cells in an organism [9]. Inflammatory oxidant stress insufficient to directly cause cell damage can induce transcription of stress defense genes including antioxidant genes [8].

The mechanism of reactive oxigen species (ROS)-induced cell killing during inflammation involves the promotion of mitochondrial dysfunction through an intracellular oxidant stress in hepatocytes leading mainly to necrosis and less to apoptosis [10]. The products of oxidative stress, such as malondialdehyde (MDA), have been found in in the blood of patients with cholestasis. These products are extremely cytotoxic and damage cell membranes and intracellular macromolecules [9]. MDA is an end product of lipid peroxidation and is a good indicator of oxidative stress. Consistent with previously reported findings from studies of obstructive jaundice in rodents [11,12], the toxicity of MDA is based on its ability to act as a mutagenic agent in a cell [13].

There is a lack of information about the correlation between the inflammatory parameters and markers of cholestasis with intensity of lipid peroxidation in the patients with choledocholitiasis.

Therefore, the aim of this study was to establish the correlation of parameters of inflammatory disorders and characteristic biochemical markers of cholestasis with the intensity of lipid peroxidation in pathogenesis of liver function disorders. Such knowledge in patients with choledocholithiasis may contribute to better understanding of the disease and possibly to its more rational treatment.

Methods

The study included 70 subjects divided into two groups: the group I – 40 patients with obstructive jaundice caused by choledocholithiasis and the group II (control) – 30 healthy individuals.

The patients with extrahepatic cholestasis due to mechanical obstruction caused by choledocholithiasis were included in the study. The obstruction of biliary ducts caused by other factors was not considered.

The diagnosis of obstructive icterus was made according to anamnestic data, clinical features, and biochemical and ultrasound examination of biliary ducts. For the ultrasound examination of biliary ducts in the supine position a Sono et Medison Co. Ltd ultrasound was used.

All the patients were anamnaestically and clinically observed at the Internal Department of Military Hospital in Niš. Basic biochemical indicators and parameters of oxidative stress were determined in Biochemical Laboratory of Military Hospital in Niš and the Laboratory of the Biochemistry Institute at the Faculty of Medicine in Niš.

All the patients with choledocholitiasis were tested in the first three days since the occurrence of cholestasis syndrome and before surgery or endoscopic retrograde cholangiopancreatography (ERCP) with papillotomy.

Biochemical analysis

Inflammatory and cholestasis parameters: C-reactive protein (CRP), fibrinogen, albumins, sedimentation (SE) rate, number of leukocytes (Leu), granulocytes (Gr), lymphocytes (Ly), monocytes (Mo), activity of γ-glutamyltransferase (γ-GT) and alkaline phosphatase (AP) and the level of bilirubin were determined.

The previously mentioned biochemical parameters were determined by the ready tests produced by Ellitech Company, on the biochemical analyzer BTS–370 (Bio–system).

The intensity of lipid peroxidation in plasma was measured spectrophotometrically, and based on the thiobarbituric response products as described by Ohkawa et al. [14]. Malondialdehyde (MDA – lipid peroxidation end-product) concentration was expressed as μmol/L, using the MDA molecular absorbance coefficient (1.56×10^{-5} mol cm^{-1}).

Statistical analysis

The data were analyzed by means of the commercially available statistic software package (SPSS® for Windows, v. 9.0, Chicago, USA) using the Student's *t*-test and χ^2 test. The results were presented as means ±/SD. Statistical significance was set to $p < 0.05$. To determine the correlation of the parameters of inflammatory disorders and characteristic biochemical markers of cholestasis with the intensity of lipid peroxidation the Pearson's correlation coefficient (*r*) was used.

Results

The demographic characteristics in the control group and the patients with choledocholitiasis are shown in Table 1.

Table 1

Demographic characteristics in the control group and the patients with choledocholitiasis (CHDL)

Parameter	Control	CHDL	Total
Age (years), $\bar{x} \pm SD$	55.5 ± 18.0	61.4 ± 14.1	58.8 ± 15.9
Gender, n (%)			
male	18 (60)	19 (47.5)	37 (53)
female	12 (40)	21 (52.5)	33 (47)

χ^2 test and Student's *t*-test did not reveal any significant differences in gender distribution and in the average age between the examined groups.

Participants of both groups did not differ in gender and age structure. Out of the total number of studied subjects, there were 40 patients with extrahepatic cholestasis caused by choledocholitiasis and 30 control (healthy) individuals; 37 (53%) were men and 33 (47%) women. The average age of the patients was 58.8 ± 15.9 years.

The clinical characteristics of the patients with choledocholitiasis are shown in Table 2.

Table 2

Clinical characteristics of the patients with choledocholitiasis

Clinical characteristics	Number of patients (%)
Icterus	21 (52.5)
Subicterus	19 (47.5)
Abdominal pain	40 (100)
Nausea and vomiting	23 (57)
Aholic stool	32 (80)

Abdominal pain was presented in all the patients with choledocholitiasis and 89% of the patients had aholic stool. All the patients with choledocholitiasis had icterus or subicterus while nausea and vomiting were registered in about half of the patients.

The level of inflammation and cholestasis, measured *via* biochemical indicators, and the intensity of lipid peroxidation measured in the form of MDA, are shown in Table 3.

In the patients with choledocholithiasis, statistically significantly lower values of albumins ($p < 0.001$), as well as significantly higher values of fibrinogen ($p < 0.05$) and CRP ($p < 0.001$) compared to the control group were found. Statistically much higher values of SE ($p < 0.05$), Leu ($p < 0.01$), and Gr ($p < 0.001$), with a decreasing number of Ly ($p < 0.001$) and Mo ($p < 0.001$) were found in the group of patients with choledocholithiasis compared to the control group.

The activity of alkaline phosphatase (AP) and γ-GT and the levels of total, direct and indirect bilirubin in the blood plasma of the patients with extrahepatic cholestasis caused by choledocholithiasis showed a significant increase ($p < 0.001$) compared to the control group.

Also, the values of MDA were significantly increased ($p < 0.001$) in the patients with extrahepatic cholestasis caused by choledocholithiasis, compared to the control group.

Table 3

The results of laboratory parameters in the control group and the patients with choledocholitiasis (CHDL)

Parameter	Control	CHDL
Albumin (g/L)	46.1 ± 4.3	36.7 ± 6.6***
Fibrinogen (g/L)	3.5 ± 1.1	5.1 ± 1.2*
CRP (mg/dL)	4.7 ± 1.3	11.2 ± 7.1***
Leu (G/L)	6.1 ± 1.4	9.9 ± 6.3**
Ly (%)	28.8 ± 9.4	15.3 ± 8.2***
Mo (%)	8.5 ± 3.0	5.2 ± 3.9***
Gr (%)	62.2 ± 8.9	79.4 ± 10.6***
AP (U/L)	81.4 ± 37.7	385.0 ± 459.0***
γ-GT (U/L)	24.1 ± 6	364.0 ± 382.0***
Bil – total (mmol/L)[1]	9.5 ± 2.8	123.2 ± 101.1***
Bil – direct (mmol/L)[1]	3.01 ± 1.09	55.1 ± 39.4***
Bil – indirect (mmol/L)[1]	6.6 ± 2.4	73.6 ± 61.8***
MDA (μmol/L)	21.6 ± 2.	51.2 ± 8.6***

CRP – C-reactive protein; Leu – leukocytes; Ly – lymphocytes; Mo – monocytes; Gr – granulocytes; AP – alkaline phosphatase; γ-GT – γ-glutamyl transferase; Bil-bilirubin; MDA – malondialdehyde.
***p* < 0.05; ***p* < 0.01; ****p* < 0.001 compared to the control.**

The correlation of biochemical parameters of inflammation and cholestasis with the intensity of lipid peroxidation in the patients with choledocholithiasis is shown in Table 4.

Table 4

Correlation between biochemical parameters of inflammation and cholestasis with the intensity of lipid peroxidation in the control group and the patients with choledocholitiasis (CHDL)

Parameter	Correlation with MDA (*r*) Control CHDL	CHDL (*r*)
Albumin (g/L)	-0.05	-0.11
Fibrinogen (g/L)	0.53*	-0.23
CRP	0.55*	-0.24
Leu (G/L)[3]	0.15	0.51*
Ly (%)[4]	0.34	0.02
Mo (%)[5]	-0.10	0.32
Gr (%)[6]	-0.33	-0.13
AP (U/l)	0.12	0.01
γ-GT (U/L)	0.15	-0.03
Bil – total (μmol/L)	-0.05	0.87**
Bil – direct (μmol/L)	0.05	0.85**
Bil – idirect (μmol/L)	0.15	0.88**

CRP – C-reactive protein; Leu – leukocytes; Ly – limphocytes; Mo – monocytes; Gr – granulocytes; AP – alkaline phosphatase; γ-GT – γ-glutamyl transferase; Bil-bilirubin; MDA – malonidialdehyde.
***r* – Pearson's correlation coefficient**
***p* < 0.05; ***p* < 0.01 compared to the control**

The concentration of fibrinogen ($r = 0.53$, $p < 0.05$) and CRP values ($r = 0.55$, $p < 0.05$) were in a direct positive linear correlation with the intensity of lipid peroxidation in healthy individuals, but the correlation was not present in the patients with choledocholithiasis There was a significant positive linear correlation between the number of leukocytes ($r = 0.51$, $p < 0.05$) and the concentration of MDA in the group of patients with choledocholithiasis, while the other inflammation parameters in this group did not show such correlation.

The concentration of total ($r = 0.87$, $p < 0.01$), direct ($r = 0.85$, $p < 0.01$) and indirect ($r = 0.88$, $p < 0.01$) bilirubin was in statistically significant positive linear correlation with the level of lipid peroxidation, while the other biochemical markers of cholestasis do not show such correlation.

Discussion

In this study the patients with choledocholitiasis were examined in the first three days since the occurrence of cholestasis syndrome. Abdominal pain, icterus or subicterus were presented in all the patients with choledocholitiasis. Nausea and vomiting were registered in about half of the patients while 89% of the patients had aholic stool.

Cholestasis syndrome includes liver function disorder due to the obstruction of bile drainage into the intestine, with the consequent retention of bile constituents in liver and their regurgitation in the blood [15, 16].

The intensity of oxidative stress, measured as MDA values, was significantly increased in patients with cholestasis caused by choledocholithiasis ($p < 0.001$). This is in accordance with numerous experimental and clinical studies [3, 5–8]. Obstructive jaundice points to intestinal oxidative stress, which can be the key factor in the loss of intestinal barrier and development of septic complications in these patients [13].

Correlation of the inflammation parameters and the intensity of lipid peroxidation

Albumins as negative inflammatory reactants and markers of impaired synthetic liver function were significantly lower in patients with extrahepatic cholestasis. A decrease in the concentration of albumin indicates liver disorder lasting longer than three weeks, but in rapidly progressive diseases, the decrease can occur even sooner [17].

In this study, the values of both fibrinogen (positive inflammatory reactant) and CRP were significantly higher in the patients with cholestasis. A significant increase in fibrinogen concentrations, as an inflammatory parameter in patients with obstructive icterus, is in accordance with the results of other authors [18]. Inflammatory cytokines inducing fibrinogen transcription in the liver, tissue factor in monocytes, endothelial and muscle cells, VIII factor in the liver, PAI-I in the liver and adipocytes, lead to procoagulation states. There was also a positive correlation between fibrinogen and C-reactive protein in healthy common population [19].

The results of this study show that choledocholithiasis leads to increased number of leukocytes, with the decrease in lymphocyte and monocyte percentage.

The increase in the number of granulocytes is in accordance with the results of other authors stating that the clinical problem in patients with obstructive icterus is mainly the consequence of disorders in neutrophil function [20]. The experimental study by Gujarol et al. [21] shows that activated neutrophils take part in the liver parenchymal damage during a short period of obstructive icterus manifestation (during 5 days from the obstruction of mutual biliary duct). Increased number of leukocytes, neutrophils, hemotaxic neutrophils, increased production of superoxide radicals and increased expression of adhesive molecules, damage endothelial cells of liver sinusoids and apoptosis, playing thus the key role in organic dysfunction [22, 23].

Monocytes start to accumulate in three days from the beginning of obstruction and their number increases synchronously with the increase of concentration of intercellular adhesion molecule (ICAM) – 1 during obstructive jaundice development. Simultaneously, their number returns to normal on the 14th day form the obstruction of ductus choledochus, which indicates the time trend of change in cholestatic organism [24]. These results are not in accordance with the changes in the number of monocytes registered in our study. One of the possible explanations is that all the patients were tested in the first three days since the occurrence of cholestasis syndrome during which there was no significant increase in the number of monocytes.

There is a positive linear correlation of fibrinogen concentration ($r = 0.53$, $p < 0.05$) and CRP values ($r = 0.55$, $p < 0.05$) with the intensity of lipid peroxidation in the control group of the patients. The correlation of inflammatory markers synthesized in the liver, such as albumins, fibrinogen and CRP, with the level of oxidative stress, has not been proven in the patients with extrahepatic cholestasis. This is in accordance with the results of other authors stating that dysfunction of hepatocytes and damaged synthetic liver function in the states of cholestasis make the significance of these markers quite uncertain. Therefore, extrahepatic cholestasis should involve analysis of other markers of inflammation, such as tumor necrosis factor alpha (TNF-a), interleukin (IL)-1, IL-6, number of leukocytes and neutrophils [5].

There was a significant positive linear correlation of the number of leukocytes ($r = 0.51$, $p < 0.05$) with the intensity of lipid peroxidation. Namely, this knowledge in patients with choledocholithiasis might not only contribute to better understanding of pathophysiology of the disease, but also to a possible improvement of its treatment. Accumulated neutrophils are adhered to vascular endothelium due to increased expression of adhesive molecule ICAM-1 and they penetrate into mucosa of involved organs and hepatic tissue, causing oxidative stress and tissue damage [25, 26]. These neutrophiles represent the source of large quantity of free radicals that damage the mentioned structures and increase the content of carbonyl groups [27, 28].

The correlation of the number of leukocytes with the intensity of lipid peroxidation is in accordance with the results of other authors indicating the correlation between the oxidative stress and inflammation *via* cytokine TNF-a, transforming growth factor beta (TGF-β), IL-1b and IL-6 [29–31] and transcription factor, NFkB [32]. NF-kB plays a central role in the transcription of cytokines, adhesion molecules, and other mediators involved in the inflammatory reaction and oxidative damage [33, 34].

The level of inflammation is directly proportional to the level of oxidative stress and capability of antioxidative mechanisms, primarily in hepatocytes, to counteract the increased production of free radicals *via* immune cells, such as neutrophils and monocytal macrophageal cells [23].

Correlation of the biochemical markers of cholestasis and the intensity of lipid peroxidation

The values of alkaline phosphatase (AP) in the patients with extrahepatic cholestasis caused by choledocholithiasis were almost 7 times higher than in the control group ($p < 0.001$). According to the results of other authors, the increase of AP is a reliable indicator of biliary obstruction, especially in incomplete and segment obstructions (obstructive icterus) where the values of bilirubin remain normal, while the values of AP are increased [35].

The increased activity of γ-GT in the plasma of patients with extrahepatic cholestasis found in this study, is in accordance with the results of other authors [36]. This is due to the necrosis of epithelial cells of biliary ducts (rich in γ -GT) and their proliferation leading thus to the significant increase of γ-GT activity in the serum [37].

In the patients with extrahepatic cholestasis, there was a significant increase in the values of total, conjugated and unconjugated bilirubin in the plasma, compared to the control patients ($p < 0.001$). The increased values of direct bilirubin in the plasma in cholestasis are the consequence of the increased concentration gradient between the cells and plasma or of wasting bilirubin due to the damage of the cells caused by the obstructive bile drainage. Unlike the conjugated one which is not toxic, the unconjugated bilirubin in free state is

very toxic to cells. Its effect is explained by the strong detergent effects of biliary salts and their ability to solubilize cell membranes [38, 39].

The concentration of total ($r = 0.87$, $p < 0.01$), direct ($r = 0.85$, $p < 0.01$) and indirect ($r = 0.88$, $p < 0.01$) bilirubin shows a significant positive linear correlation with the level of lipid peroxidation. Based on the above results, we support the idea that bilirubin can act *in vivo* as efficient scavenger of ROS and that bilirubin plays a key physiological role in cytoprotection against an oxidant-mediated damage [40].

The correlation between oxidative stress and disorders of production and secretion of bilirubin is in accordance with the results of other authors. Namely, they have shown that a reduced amount of glutathione and decreased activity of glutathione peroxidase in patients with cholestasis, decrease hepatobiliary transport of toxic organic components [41] leading to the development of complications accompaning cholestasis.

The correlation between hyperbilirubinaemia and oxidative stress is an expected result due to insolubilization of cytoplasmic membrane, dysfunction of mitochondrial membrane and freeing of reactive oxygen radicals [13]. Considering the results of other authors, indicating that the products of hems have a significant anti-inflammatory role and decrease mortality in experimental models, the correlation between hyperbilirubinaemia and oxidative stress can be considered as a form of protective effect. It has been found that the products of hems and bilirubin decrease adhesion of leukocytes for the vascular endothelium as a response to oxidative stress [42]. This is achieved by the inhibition of expression of adhesive molecule (VCAM-1) and the reduction of inflammation, increasing the risk of infection set-on [43]. Hyperbilirubinaemia viewed this way could represent a significant factor that leads to immune system disorder and the development of infection complications.

Conclusion

According to the results of this prospective study, it can be conclude that neutrophils and the level of total, direct and indirect bilirubin show a significant positive correlation with the level of lipid peroxidation, measured by MDA as its final product, while the other determined inflammatory parameters and biochemical markers of cholestasis do not show such correlation. Thus, neutrophilia and hiperbilirubinemia observed in this way, easily established with inexpensive and routine laboratory tests, represent an important parameter in estimating the level of liver tissue damage in choledocholithiasis.

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Age-related structural changes in the myenteric nervous plexus ganglion along the anterior wall of the proximal human duodenum – a morphometric analysis

Morfometrijska analiza ganglijskih struktura mijenteričkog nervnog spleta prednjeg zida proksimalnog dela duodenuma čoveka u toku procesa starenja

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Abstract

Background/Aim. Aging is one of the most complex biological processes which probably affect structure and function of the enteric nerve system. However, there is not much available information on this topic, particularly in humans. The aim of this study was to investigate the influence of aging on the structure of the myenteric ganglia in the anterior wall of the human proximal duodenum. **Methods.** We examined the myenteric ganglia in the proximal duodenal anterior wall specimens obtained from 30 cadaver persons aged from 20 to 84 years. Tissue samples were classified into three age groups: 20–44, 45–64 and 65–84 years. After standard histological preparation, specimens were stained with HE, Cresyl Violet and $AgNO_3$. Morphometric analysis of all the specimens, using a multipurpose test system M42, was performed. The data were subjected to the *t*-test. **Results.** The myenteric ganglia of very old humans contains an empty space, i.e. the respective parts of ganglia show a decreased number of neuron as compared to younger population. The average number of neuron per cm^2 of the duodenum in the youngest people (20–44 years) was 69,370 ± 1,750.00, in the people aged 45–64 years 69,211 ± 1,573.33, and in the oldest persons (65–84 years) 57,951 ± 1,291.52. The loss of neurons in the oldest persons was 16.46%. The applied statistic test demonstrated a significant difference between the observed groups ($p < 0.0001$). **Conclusion.** Aging does not induce changes in size and surface of neurons in the ganglia, but it decreases the number of neurons. The nerve structures in the elderly are partly emptied of bodies of nerve cells ("empty ganglions"), which indicates the existence of changed myenteric ganglia in the duodenum. These changes could be related to the duodenum motility disorder associated with aging.

Key words:
myenteric plexus; duodenum; aging.

Apstrakt

Uvod/Cilj. Starenje je jedan od bioloških procesa koji verovatno utiče na strukturu i funkciju enteričkog nervnog sistema. Međutim, veoma je malo informacija o ovoj temi, posebno kada je u pitanju ljudska vrsta. Cilj ove studije bio je istraživanje uticaja starenja na strukturu mijenteričkih ganglija proksimalnog dela humanog duodenuma. **Metode.** Ispitivan je mijenterički nervni splet prednjeg zida proksimalnog dela duodenuma uzoraka uzetih od 30 kadavera osoba starih od 20 do 84 godine. Uzorci tkiva proksimalnog dela duodenuma prema starosti bili su razvrstani u tri grupe: od 20 do 44 godine, od 45 do 64 i od 65 do 84 godine. Nakon standardne histološke obrade preparati su bojeni hematoksilin-eozin (HE), Cresyl violet i $AgNO_3$ metodom bojenja. Gotovi preparati bili su podvrgnuti morfometrijskoj analizi korišćenjem višenamenskog sistema za testiranje M42. Dobijeni rezultati obrađivani su *t*-test. **Rezultati.** Mijenteričke ganglije veoma starih osoba sadrže prazne prostore, odnosno pojedini delovi ganglija pokazuju deficit broja neurona u odnosu na mlađu populaciju. Prosečan broj neurona/cm^2 duodenuma kod najmlađih (20–44 godine) iznosio je 69 370 ± 1 750, kod osoba starosti 45–64 godine 69 211 ± 1 573,33, a kod najstarijih (65–84 godine) 57 951 ± 1 291,52. Procentualni gubitak neurona kod najstarijih iznosio je 16,46%. Primenjeni statistički test ukazuje na postojanje značajnih razlika između starosnih grupa ($p < 0,0001$). **Zaključak.** Tokom starenja ne dolazi do promena veličine gangliona, ni površine neurona, već do gubitka broja neurona. Ganglijske strukture kod starijih osoba delimično su ispražnjene od tela nervnih ćelija („prazni ganglioni"), što ukazuje na postojanje izmenjenih mijenteričkih ganglija u duodenumu. To najverovatnije doprinosi poremećaju motiliteta duodenuma kod starije populacije.

Ključne reči:
plexus myentericus; duodenum; starenje.

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Introduction

Aging is an universal biological phenomenon that everyone has to undergo at a certain point of life. All parts of a living organism during aging go trough some changes, leading to a large number of functional disorders, generally characteristic for older people. Changes that occur during aging are deleterious; they decrease person's ability to cope with its environment. A large number of functional gastrointestinal disorders are also present in older people [1, 2]. It is generally known that the dysfunction of the gastrointestinal system, including dysphagia, constipation, diarrhea, irritable colon, is pronounced in the older population [3, 4]. A research on animal species has also shown the existence of disorders of intestinal function in elderly animals [5, 6]. Very rapid progress and development of neurobiology have led to a better understanding of the central nervous system, as well as aging caused changes in the enteric nervous system. It is accepted that many of age-dependent motility disorders are caused by abnormalities in nerves and muscles of the gastrointestinal tract, but a direct evidence for this is scarce. A number of studies in experimental animals showed that the number of myenteric nerve plexus neurons of the small and large intestine in older is significantly lower when compared to younger animals [7, 8].

Some researchers report that the number of neurons of myenteric nerve plexus ganglia submitted to the histochemical technique to stain the nerve cells through the activity of the NADH-diaphorase (NADH-d) enzyme was significantly reduced to approximately 15% in older rats [8]. In contrast, the number of neurons stained immunohistochemically with PGP9.5 (protein gene product 9.5) was not reduced but rather increased in older animals [9]. These findings support the hypothesis that age-related loss of myenteric plexus nerve cells of the small and large intestines affect only cholinergic neurons, while nitrergic are partially spared [10, 11]. In addition, a reduced number of neurons in the small and large intestine during aging follows a significant reduction in supporting or glial cells. The loss of glia was proportional to the loss of nerve cells [12]. In fact, most of the studies in animal models described a reduction in the number of myenteric neurons with age. In terms of changes in the ganglions of myenteric nerve plexus, previous studies on the human colon have shown an increased number of ganglia with aging, however, containing a smaller number of neurons compared to the ganglia of younger people [13].

The aim of our study was detailed examination of the myenteric nerve plexus in the human proximal duodenum by applying adequate histological, morphometric and stereological methods. Using methods that provide opportunities for quantification, we sought to determine the presence of individual differences and verify eventual changes in the structure of the myenteric nerve plexus of the anterior wall of the proximal part of the duodenum during aging. According to this, the number of ganglion cells per unit area in ganglionic structures of the myenteric nerve plexus of the anterior proximal duodenum was determined. At the same time the surface of ganglion structure was determined and the range of the ganglion surface that goes to the surface of nerve cells.

Methods

Tissue samples of the human duodenum were obtained from autopsy material at the Institute of Forensic Medicine from 30 cadavers of both sexes, aged between 20 and 84 years. Ethical approval for the study protocol was obtained from the Ethics Committee. Samples were taken from the anterior wall of the proximal duodenum. After taking, tissue samples were divided into three predefined age groups: 20–44 years, 45–64 years and 65–84 years. Tissue slice preparations the size of 1 × 1 cm were fixed in 10% buffered paraformaldehyde for 48 hours. After the routine processing of conducting, series of alcohol samples were embedded in paraffin blocks, which were then cut in two ways; sections perpendicular to the longitudinal axis of the front wall of the duodenum (classical) and sections parallel to the surface of the proximal duodenum serosa until plexus myentericus and through it. Histological preparations were stained with the routine hematoxylin-eosin method, and to ensure reliable identification of ganglion, cells and structures were stained with silver nitrate by the method of Mason Fontana and cresyl-violet color.

Silver nitrate staining by the method of Mason Fontana was performed as follows: the hydrated preparations were put in a previously prepared solution of silver nitrate for 2 h at 56°C, rinsed in distilled water and tones with 0.2% gold chloride solution for 2–3 min, rinsed again with distilled water and 1 min down to 5% sodium thiosulfate. Preparations were again rinsed with distilled water and 5 min immersed in nuclear-fast red and then mounted on glass slides and covered the outer husks. The result of staining was following: argentaffin granules in neural cells were black, nuclei were pink-reddish, and cytoplasm pale-pink.

Cresyl-violet staining (Cresyl violet) for nerve cells was performed as follows: hydrated preparations were left in the previously prepared solution of cresyl-violet stain during 30 min. They were then discolored in 96% alcohol which was added 1 drop of HCl and bleaching was controlled under the microscope. When they get the desired color, preparations were dehydrated and mounted on glass slides. The result of staining was: dark blue nucleus, cytoplasm, slightly lighter, nerve fibers were not colored.

Microscopic techniques

Tissue samples were cut with microtome on section thickness of 6 μm. Analysis was made by the M42 test system that calibrated to the proper magnification of the light microscope (Carl Zeis Jena). For measuring the average diameter of cells and their nuclei, ocular micrometer calibrated at the appropriate magnification was used. On each preparation of the anterior wall of the duodenum the number of point test-system on the area occupying ganglion structure and the number of point on nerve cell body profile of the myenteric ganglia were counted. In addition, it was numer-

ous the total numbers of neurons located in the complex nerve structure. On each preparation 10 visual fields were analyzed. The data were entered into spread sheets. On the basis of them the number of neurons per square centimeter surface of nerve plexus, ganglion size range structure, the total area of all neurons in the ganglion structure and surface profiles of individual nerve cell bodies were mathematically calculated.

The results presented in the text and tables are expressed as mean ± standard deviation. Estimation of statistical significance between mean values was performed by the independent Student's *t*-test. A significance was expressed as $p < 0.05$ or $p < 0.001$.

Results

The myenteric nerve was woven between the circular and longitudinal layer of the duodenal wall smooth muscle. It was composed of ganglion cells by linking the bundles of nerve fibers to form a polygonal network between the muscle layers. On the cross sections ganglion structures of the myenteric plexus were relatively small in size and within each of them was noticed the large number of neurons (Figure 1). Neurons were interconnected with each other, and with the muscle cells through nerve fibers.

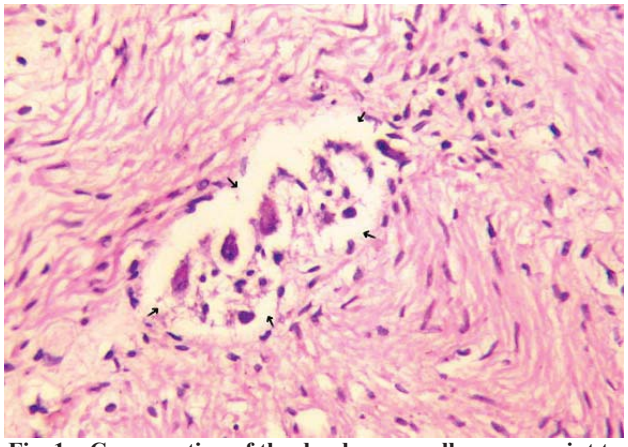

Fig. 1 – Cross-section of the duodenum wall-arrows point to the myenteric ganglion (HE, ×200)

On longitudinal sections made of serosa to mucosa, i.e. through the longitudinal axis of the plexus, could be seen ganglion structures of various shapes and sizes. The shape and size of ganglion structures depend on the extent to which in particular it is affected by the cut. Around the nerve structures were irregularly scattered bundles of smooth muscle fibers (Figure 2). In the very ganglionic structures are ganglion cells that display a wide diversity of shapes and sizes and were grouped into smaller or larger groups.

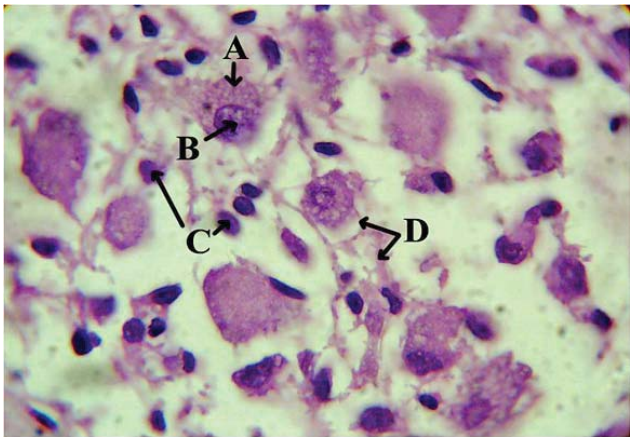

Fig. 2 – Longitudinal section of the duodenum wall (HE, ×1000)
A – ganglion cell; B – nucleus with nucleolus; C – nuclei of glial cells; D – nerve fibres

Neurons can be oval, round, spindle or polygonal with vesicular nuclei containing little chromatin. Around the ganglion cells could be seen scattered, irregularly oval nuclei and supporting glial cells whose cytoplasm was not stained by this method.

Using the method of quantification we determined the number of ganglion cells of the myenteric nervous plexus of the anterior proximal duodenum per unit area (cm^2) in all age groups. The average number of neurons in the duodenal myenteric nerve plexus is shown in Table 1.

A reduction in the number of neurons per unit area of the myenterc plexus in people over 65 showed a very high statistical significance ($p < 0.0001$) compared with other age groups.

Table 1

Influence of age on parameters of the myenteric nerve plexus of the human duodenum

Parameters	Age (years)		
	20–44	45–64	65–84
Number of ganglion (cells/cm^2), $\bar{x} \pm SD$	69.370 ± 1.750,00	69.211 ± 1.573,33	57.951 ± 1.291,52*
Surface area of ganglion structure (mm^2), $\bar{x} \pm SD$	0.01169 ± 0.00174	0.01186 ± 0.00182	0.01203 ± 0.00137
Surface phase of the ganglion structure belonging to the profiles of nerve cells (mm^2), $\bar{x} \pm SD$	0.00280 ± 0.00028	0.00267 ± 0.00030	0.00203 ± 0.00027**
(%)	24.17	22.66	17.17
Surface of neurons (μm^2), $\bar{x} \pm SD$	323.11 ± 8.71	338.78 ± 13.85	297.82 ± 48.09

$\bar{x}$ – Mean value; SD – standard deviation;
*** $p < 0.0001$ compared to other groups (*t*-test)**
**** $p < 0.001$ compared to other groups (*t*-test)**

According to this results it can be calculated that the loss of neurons of the duodenal myenteric nerve plexus in the oldest (65–84 years) compared to the youngest (20–44 years), expressed as a percentage is 16.46% and to the middle age group (45–64 years) 16.26%.

In further analysis, apart from the number of neurons per cm^2 surface, the area of the ganglion structure of the myenteric nerve plexus in the duodenum was also determined. The surface area of ganglion structure was expressed in mm^2. The results presented in Table 1 were subjected to statistical testing (*t*-test).

The values obtained show that the surface area of ganglion structure is equivalent regardless of age. Statistical analysis of values range of ganglion surface structure does not show a statistically significant difference with age.

In the morphometric analysis it was interesting to determine the surface occupied with the profiles of neurons inside the ganglion structure, i.e. surface phase of the ganglion structure of myenteric nerve plexus belonging to the profiles of nerve cells. The obtained values expressed in mm ² are shown in Table 1.

The data statistically analyzed by the Student's *t*-test, showed that the total area of ganglion neurons structure of myenteric plexus of the duodenum in the oldest (65–84 years) were significantly different as compared to other age groups. The level of statistical significance was $p < 0.001$.

We came up with the idea that the difference of the total area of ganglion cells within the ganglion structure between different age groups was more apparent if the computation of the total area was expressed in percentage. The results are presented in Table 1.

It can be seen that the smallest percentage of surface in the ganglion structures occupy ganglion cells in the old people (65–84 years), i.e. 17.17%, and highest in the young adults (20–44 years), 24.17%.

In the morphometric analysis surface of individual ganglion cells of the myenteric nerve plexus of the duodenum of men at different ages was determined. The obtained data were tested by the Student's *t*-test which showed no significant differences between the examined groups (Table 1).

Discussion

Studies on the myenterc plexus of human material are very rare. In this study we used tissue samples of 30 people, age range from 20 to 84 years. Our study shows that on cross section of the duodenum the myenteric plexus is visible as a thin, folded, discontinued lamellar structure, inserted between the layers of the smooth muscle, practically, on the form of plate transverse cut off. It was noticeable that thickness of these plane was spotty; at the places of thickness groups of nerve cells were visible.

It was observed that the thickness of the plate was uneven and in thickened places were visible groups of nerve cells.

Longitudinal sections of duodenum tissue proved to be useful for the analysis of the myenteric nervous plexus. This manner of cutting tissue request strain and request more continuous native control of section under light microscope. Therefore, it is necessary to made a great number of serial sections from the serosal towards the mucosal side, make enough sections for analysis and separate only those that pass through the myenteric nerve plexus.

On longitudinal sections, myenteric plexus appears as a small or a large cluster of ganglion cells around which are irregularly scattered over bundles of smooth muscles. A fundamental objective of this study was to establish neuronal density per cm^2 of the surface of the myenteric plexus of the human duodenum in different age groups.

In our researches the myenteric plexus of the duodenum showed a very high neuronal density. The average number of neuron/cm^2 duodenum among the youngest (20–44 years) amounted to 69,370 ± 1,750, in the people aged 45–64 it was 69,211 ± 1,573.33, and in the elderly (65–84 years) 57,951 ± 1,291.52. In the oldest, it was seen a drop in the number of neurons of the 16.46% compared with the children and 16.26% compared with those in the middle-aged group. The number of neurons in the oldest age group compared to the first and second group, tested by the Student's *t*-test, showed a level of statistical significance $p < 0{,}0001$. In the available literature we found no data to compare our results obtained on human material. However, some studies show high neuronal density in the myenteric plexus of the mouse duodenum (20,212 ± 3,038 neuron/cm^2) [14] and in the rat colon (30,968 neuron/cm^2) [15]. Other types of studies suggest a significantly greater percentage loss of neurons of the small intestine (over 30%) [16] and esophagus (22–62%) of man in old age [17]. A rational answer to the question why this is so, can be found in the fact that in all these works different techniques of tissue staining have been used.

In addition to the number of neurons/cm² interesting question is whether aging is connected to the emergence of differences in the surface area of ganglion structures of the duodenal myenteric plexus. The obtained results indicate that aging does not decrease the surface area of ganglion structure of the myenteric plexus. The obtained values are almost equal in all age groups. However, it is noticeable that ganglions of older people within their borders have often completely blank spaces in which there are no nerve cells bodies and also no nerve fibers. Ganglion spaces like this, some authors call "cavity". They also conclude that in ganglia of myenteric plexus the frequent "cavities" occur with age [18].

From that reasons our research included determination of total surface inside the area of ganglion structure. The obtained results show a decreased range of nerve plexus structures of the myenteric plexus of the stomach with age, which belong to the body surface of neurons present in the ganglion. Expressed in percent 24.17% surface area of ganglion structure belongs to the body surface of neurons in young (20–44 years), and only 17.17% in the elderly (65–84 years). It can be concluded that aging process can arise ganglions that are partially emptied from the body of neurons ("empty ganglions"). Similar results have been presented in studies of human colon [18].

We can conclude that the process of aging lead to a decreased total surface area of ganglion cells within the range of ganglion structure of myenteric plexus. In fact, a reduction in the surface phases of ganglion structure belongs to the bodies

of nerve cells. This, most likely, indicates that increased surface phase belongs to connective, glial and vascular elements. In our research we did not work on the surface phase range ganglion structures belonging to the nerve fibers, connective and vascular elements for several reasons. One reason is that the presentation of glial elements and connective structures apply different coloring methods which can not clearly identify nerve cells. The second reason is contained in the fact that the structure of this phase is irregularly distributed around neurons and across the nerve cell bodies. For this reason, stained myenteric plexus nerve cell bodies would not be visible, and thus would be excluded an important parameter for comparing phase of surface structures within the ganglion. We can say that what does not belong to the phase of body surface of myenteric neurons belongs to glial, connective and, of course, to vascular elements of tissue. We conclude that the decrease in neuronal density depends on the age and is associated with the appearance of increased fibrous components of the myenteric ganglia [13]. Investigations included the determination of the value of the average body surface of individual ganglion cells in the anterior wall of the proximal part of the human duodenum of the myenteric nerve plexus in all age groups. The surface of neurons ranged from 297.82 ± 48.09 in the oldest to 323.11 ± 8.71 in the youngest. Similar values were obtained for the duodenum in studies of other authors [19]. The obtained values show that there are no significant differences in body surface of ganglion cells in relation to age. It may be noted that the standard deviation of the oldest is considerably higher than in the plexus of the duodenum in younger age groups. The explanation may be in the fact that in the course of the study in the elderly we found the existence of neurons of significantly larger and much smaller surfaces.

Conclusion

The myenteric nerve plexus of the anterior wall of the proximal human duodenum is characterized by the presence of a large number of neurons. As all other structures of human organism, the myenteric plexus is also a subject to change during aging. During aging, a loss in the number of neurons occurs. Ganglion structures are in the elderly partly emptied of bodies of nerve cells ("empty ganglions"). The number of neurons in older people (65–84 years) decreases in relation to younger from 16.26% up to 16.46%. This finding corroborates with the fact that within the range of ganglion structure in the elderly there is some reduction in the surface phase, which belongs to the body surface of neurons located in a given structure. However, the surface area of the ganglion was not changed. We conclude that the reduction in neuronal density compensates increased fibrous components, so that the size of the myenteric ganglia is practically unchanged. With aging, there is no significant change in the size of neurons.

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Comparative analysis of autodermal graft and polypropylene mesh use in large incisional hernia defects reconstruction

Uporedna analiza upotrebe autodermalnog grafta i polipropilenske mreže u rekonstrukciji velikih incizionih hernija

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Abstract

Background. Large defects of the abdominal wall caused by incisional hernia still represent a challenging problem in plastic, reconstructive, and abdominal surgery. For their successful tension-free repair a proper selection of reconstructive material is essential. In the last decades, the use of synthetic meshes was dominant while biological autodermal grafts were rarely used. The aim of the study was to comparatively analyse efficacy and safety of autodermal graft and polypropylene mesh in surgical treatment of large abdominal wall defects. **Methods.** This prospective comparative clinical study enroled 40 patients surgically treated for large incisional hernia repair in a 10-year period. The patients were divided into two equal groups consisting of 20 subjects and treated either by biological autodermal graft or by synthetic polypropylene mesh. The surgical techniques of reconstruction, duration of surgery, the occurrence of early, minor, and major (severe) and delyed complications and hospital stay were analysed. The average follow-up took 2 years. **Results.** Statistically significant differences in demographic characteristics of patients and in size of defects were not found. The surgical technique of reconstruction with an autodermal graft was more complicated. The duration of surgery in patients treated with autodermal grafts was significantly longer. There was no statistically significant difference regarding occurrence of early, minor postoperative complications and hospital stay in our study. Two severe complications were registered in the synthetic mesh group: intestinal obstruction and enterocutaneous fistula. The recurrence rate was 10% in the autodermal graft group and 15% in the group with a synthetic mesh. **Conclusion.** Tension-free repair of large incisional hernia with autodermal grafts was unjustly neglected despite the fact that it is safe and effective. It can be applied in all cases where synthetic mesh are not indicated (presence of infection, immunodeficient patients, after radiotherapy). They are especially important in war surgery and in lack of funds when commercial grafts cannot be purchased.

Key words:
hernia, abdominal; reconstructive surgical procedures; transplantants; polypropylenes; treatment outcome.

Apstrakt

Uvod. Veliki defekti trbušnog zida kod incizionih kila još uvek su veliki izazov u plastično rekonstruktivnoj i abdominalnoj hirurgiji. Za njihove uspešne bestenzione rekonstrukcije, kojim se postižu najbolji rezultati, pored adekvatnih indikacija i hirurške tehnike presudan je i pravilan izbor rekonstruktivnog materijala. U poslednjim decenijama dominirala je primena sintetskih graftova dok su biološki autodermalni graftovi retko korišćeni. Cilj rada bio je da se uporedi efikasnost i bezbednost sintetičkih i autodermalnih graftova u rekonstrukcijama velikih defekata trbušnog zida. **Metode.** Ova prospektivna, randomizirana, komparativna klinička studija obuhvatila je 40 bolesnika hospitalizovanih i operativno lečenih u periodu od 10 godina primenom bioloških autodermalnih i sintetskih polipropilenskih graftova. Bolesnici su bili podeljeni u dve brojčano jednake grupe. Formirane grupe bile su slične u odnosu na osobine defekata i opšte stanje bolesnika. Analizirana je hirurška tehnika izvođenja rekonstrukcija, trajanje operacija, nastanak lakih ranih i teških, kao i kasnih postoperativnih komplikacija i dužina hospitalizacije. Bolesnici su prospektivno praćeni

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prosečno dve godine. **Rezultati.** Nije bilo statistički značajne razlike u demografskim karakteristikama ispitivanih bolesnika niti veličine defekata. Hirurška tehnika rekonstrukcije primenom autodermalnih graftova bila je složenija. Dužina operacija u grupi bolesnika operisanih primenom autodermalnih graftova bila je statistički značajno veća. Nije bilo statistički značajne razlike u učestalosti lakih, ranih postoperativnih komplikacija u ispitivanim grupama. U grupi sa sintetskim graftovima registrovane su dve teže komplikacije u obliku enterokutane fistule i adhezionog ileusa. Učestalost kasnih komplikacija u obliku recidiva iznosila je 10% u grupi sa autodermalnim graftom, a 15% u grupi sa sintetskim graftom. **Zaključak.** Bestenziona rekonstrukcija autodermalnim graftovima iako bezbedna i efikasna, neopravdano je zapostavljena metoda. Ona se može primeniti u svim slučajevima gde su sintetski graftovi kontraindikovani (prisustvo infekcije, imunodeficijentni bolesnici, stanje posle radioterapije), u vanrednim situacijama, kao što su ratovi ili elementarne katastrofe, i u nedostatku finansijskih sredstava, kada se industrijski proizvedeni graftovi ne mogu nabaviti.

Ključne reči:
hernija, ventralna; hirurgija, rekonstruktivne procedure; graftovi; plastične materije; lečenje, ishod.

Introduction

Abdominal wall defects of different origin, size and location are defined as partial or complete loss of its anatomical structures. They lead to functional disabilities and compromise health, quality of life, work ability and aesthetic appearance of the patient. In addition, complications occured in clinical course of large incisional hernia could be life threating. For these reasons, their repair takes an important place in contemporary reconstructive and abdominal surgery.

The most challenging problem in surgical treatment are defects larger than 10 cm in diameter. They are most frequently found in incisional, postoperative hernia in 90% of cases and less common in posttraumatic and defects of infective ethiology or after neoplasma resection [1, 2].

Incisional hernia can occur in the region of any previous laparotomy. Theoretically, any prior abdominal surgery can subsequently be followed by an incisional hernia formation at the laparotomy line, despite of laparotomy extention. Incisional hernia is the most frequent delayed complication in abdominal surgery. The ethiology and pathogenesis of incisional hernia has not yet been clearly undersood. From anatomical aspect of view, incisional hernia is incomplete abdominal wall defect where the musculoaponeurotic layer is lost, but peritoneal and skin layers are intact. The estimated incidence of incisional hernias ranges from 2%–11%, despite a significant progress in surgical technique and surgical suture material, but still represent significant and frequent problem in everyday surgical practice [3].

The goals of incisional hernia repair are to restore anatomical structure and function of abdominal wall, avoid complication and perform reconstruction safely and easily.

The last two decades have produced a dramatic change in surgical treatment of incisional hernias. Based on the results of numerous experimental and clinical studies around the world, conventional direct closure technique has been abandoned and tension-free repair accepted as a prefered method for achieving optimal results [4]. This surgery technique enables substitution of missing or damaged structures of the abdominal wall with free grafts or flaps without tension and traction on the suture line thus avoiding increase of intraabdominal pressure and abdominal compartment syndrome occurrance. Due to the much simpler surgical technique, open, tension-free reconstruction with mesh is the most frequent method for hernia reapir in everyday surgical practice.

A successful outcome depends primarly on proper selection of a suitable implant for each defect individually, but good knowledge of properties of defects and available implants are crucial. In the absence of an "ideal implant", which would be able to restore any defects, a wide range of implants with different nature and properties is now available. Implants can be classified into two major, basic, different groups – biological (auto-, allo- or xeno-) [5] and non-biological (synthetic mesh) implants [6, 7]. Among biological autografts the most freqently used is autodermal graft because of its excellent properties. Its good and safe alternative is full-thickness skin graft.

The choice of appropriate implant for individual patient is the crucial point in surgery planning, but there are a lot of controversial opinions and dilemmas. One of the current dilemma is whether biological auto-grafts are still unjustly neglected in comparison to commonly applied synthetic mesh.

The aim of this study was to compare the results achieved with tension free reconstruction of large abdominal wall defects either by autodermal graft (most commonly used biological autograft) or polypropylene mesh (the most frequently applied synthetic mesh), as well as to analyse their advantages and disadvantages, thus to contribute to solving this dilemma.

Methods

This prospective, comparative clinical study enrolled 40 patients hospitalized and surgically treated for large abdominal wall defects at site of incisional hernia with tension-free reconstruction in the Clinic of Plastic and Reconstructive Surgery and Department of Abdominal Surgery of Surgical Clinic, Clinical Center of Niš during a 10-year period (2000–2009). All the treated patients gave their written consent and were prospectively observed in the study period. The patients were divided into two equal study groups. The group D included 20 patients with autodermal graft. The group M included 20 patients with synthetic polypropylene mesh used for reconstruction. Both groups of patients had similar characteristics of abdominal wall defect and general condition for an objective assessment and comparision of results.

Before hospital admission all the patients underwent routine laboratory testing, electrocardiography and radiography and were also clinicaly examined by internist and anesthesiologist. Routinely, all the patients were suggested to stop smoking, reduce body weight, and use medications for chronic heart and lung diseases as well as to regulate hypertension and diabetes mellitus and other comorbidites in order to reduce postoperative complication rate. Prophylactic doses of cephalosporins and low-molecular weight heparin were administred appropriately. All the elective open surgeries were performed under general anesthesia.

The key points of the sugical technique in the group D were as follows: autodermal graft was harvested from the distended skin of incisional hernia formation by sharp dissection. The deepitelisation was made by a surgical blade. Following trimming grafts were perforated with small incision 2–3 mm in lenght and 1 cm for one from another. The prepared grafts were placed in saline with an antibiotic (gentamicin). Then, the hernia sac and musculoaponeurotic layer were prepared 2 to 3 cm from the edge of hernia defect. A tailored graft, with deepitelized surface directed towards the peritoneum, was laid over the defect, by definition 2 cm overlapping the defect (in the lay position). A crucial point is to fix the graft under maximal tension like "skin on the drum" to the abdominal wall by 4 non-absorbable polypropylene 0 or 1 sutures at its four corners, followed by a continuous polypropylene suture between the corners (Figure 1). Two aspiration drains were used and subcutaneous tissue was closed directly with continuous absorbable sutures and skin with interrupted non-absorbable sutures. All the patients used to wear abdominal belts for three months.

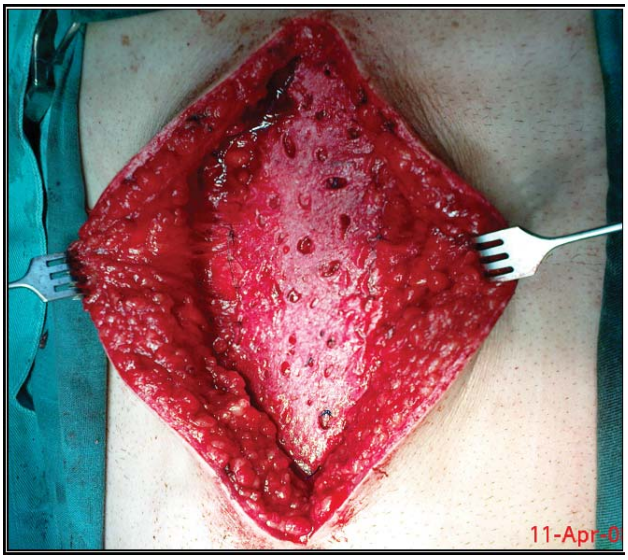

Fig. 1 – Abdominal wall defect reconstructed with autodermal graft

In the group M, after hernia sac preparation, the musculoaponeurotic layer was prepared 2–3 cm from the defect edge. A polypropylene mash was tailored and fixed peripherally to the musculoaponeurotical layer in the in-lay position, too, but without tension in the same way as in the group D (Figure 2). Drainage and closure of the skin and subcutaneous tissue were performed in the same way as in the group D with a set of abdominal belts.

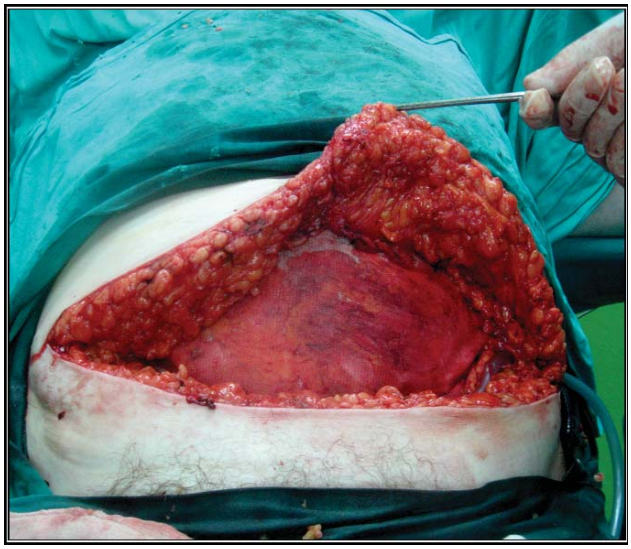

Fig. 2 – Abdominal wall defect repair with polypropylene mesh

The postoperative early minor complications (seroma, hematoma, wound infection) and major complications (graft infection, intestinal obstruction, enterocutaneous fistula), the duration of surgery, the duration of hospital stay and quality of life following surgery were noted. All the patients were provided with printed instructions upon discharge to avoid risk factors for recurrence. Follow-up was carried out in an outpatient clinic or telephone contact, 4 weeks after surgery, every 6 months for the first year, yearly thereafter. A thorough history and physical examination, with particular attention to the operative site, were undertaken on every visit.

The results were analyzed and presented in tables and figures (Excel 2000, Word 2000), and analyzed using descriptive statistics and quantitative analysis (SPSS v15 for Windows v5 Statcalc Epi Info).

Results

All the patients enrolled in study had been admitted for elective large incisional hernia repair. All hernias were uncomplicated (without incarceration or skin necrosis). Defects size ranged from 10.5 cm to 18.6 cm. The mean size of hernia defects was lower in the group D (14.54 $\pm$ 2.34 cm) than in the group M (14.78 $\pm$ 1.63 cm), but there were no statistically significant differences between the groups (Table 1).

Demographic characteristics of patients are shown in Table 2. There were more male patients in both groups and the average age was 57.6 ± 10.91 years and slightly higher in the group M. There were no statistically significant differences in gender and age among the patients.

The mean operating time for the group D was 1 h and 20 min (range 50 min to 3 h and 45 min) and that for the

Table 1

Defect size in the musculoaponeurotic layer

Defect size of a hernial defect (cm)	Groups of patients		*p*
	D (n)	M (n)	
10–14.9	12	11	0.99
≥ 15	8	9	0.99
The mean size of a hernial defect ($\bar{x}$ ± SD)	14.54 ± 2.34	14.78 ± 1.63	0.716

D – autodermal graft; M – polypropylene mesh

Table 2

The demographic characteristics of the study groups

Parameter	Groups of patients		*p*
	D (n)	M (n)	
Sex (n)			
females	7	8	0.747
males	13	12	
Age (years)			
≤ 40	1	1	0.99
41–55	7	9	0.747
56–70	10	6	0.333
≥ 71	2	4	0.661
mean ± SD	57.5 ± 10	57.7 ± 11.43	0.954

D – autodermal graft; M – polypropylene mesh; SD – standard deviation

froup M 1 h and 5 min (range 35 min to 2 h and 22 min). The surgery in 2 patients in the group D and in 10 patients in the group M took less than 120 min, while in the other patients the operation was longer as shown in Figure 3. There was a statistically significant difference in surgery time between the two groups, and surgery was significantly longer in patients with biological auto-grafts (χ^2 test, $p < 0.05$).

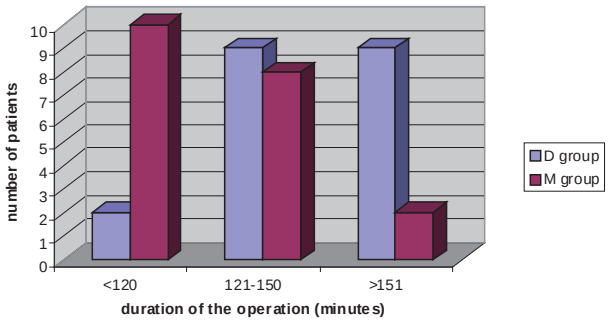

Fig. 3 – Comparation of operative time in the study groups

D – autodermal graft; M – polypropylene mesh

The type and the number of early minor complications are shown in Table 3. Seroma occurred in 20% and 18% of the patients with autodermal graft, and with polypropylene mesh, respectively. Hematoma formation was not found in any of the patients. Only one patient with a synthetic mesh graft had a light wound infection. No statistically significant difference in the number of complications was found between the two groups (χ^2 test, $p > 0.05$). These complications occurred in the first 6 months after the surgery.

Serious complications were registered only in the group M. In this group, one patient was reoperated because of adhesional intestinal obstruction 6 months after the reconstruction and the formation of an enterocutaneous fistula with graft infection was registered in another after 18 months. In both patients the reconstruction of the abdominal wall in the repeated surgery was done using autodermal graft. There was no hospital mortality in both groups.

The length of hospital stay in the groups is shown in Table 4. The average hospital stay was 6.8 ± 2.8 days in the group D and 6.4 ± 2.5 days in the group M. There was no statistically significant difference in the length of hospital stay between the two groups (χ^2 test, $p > 0.05$).

Table 3

Early minor complications in the study groups

Early minor complications	Groups of patients		*p*
	D, n (%)	M, n (%)	
Seroma	4 (20)	3 (15)	
Wound infection	0 (0)	1 (5)	0.99
Total	4 (20)	4 (20)	

D – autodermal graft; M – polypropylene mesh

Table 4

Hospital stay in the study groups

Hospital stay (days)	Group of patients		*p*
	D (n)	M (n)	
< 7	11	14	
7–10	5	4	
11–14	4	2	0.566
Total	20	20	

D – autodermal graft; M – polypropylene mesh

Prospective follow-up of 40 patients regarding the recurrence revealed recidivant hernias in 10% of the patients in the group D and 15% in the group M. There was no significant difference in the recurrence rate between the two groups (log rank test, $p > 0.05$). All recurrences occurred in the first two years of monitoring.

In the group M 3 patients referred constantly foreign body sensation and rigidity of the abdominal wall, and two patients were dissatisfied with the aesthetic appearance of the abdominal wall.

Discussion

Large abdominal wall defects in incisional hernia present significant and actual problem in plastic and reconstructive and abdominal surgery, due to its frequency and complex surgical strategy that require multidisciplinary approach.

The best results are achieved by tension-free group of techniques using free (biological or synthetic) implans or flaps. Due to a simple surgical technique, implant reconstruction is widely used while flaps are reserved for the most complex defects. There is no "ideal implant" and the types of implants available for use in complex ventral hernias repair are numerous.

Successful outcome of abdominal wall reconstruction primarily depends on choosing an adequate implant for each individual patient. However, adequate choice is only possible with extensive knowledge of the properties (advantages and disadvantages) of the available implants. The most commonly used biological autograft in clinical practice is autodermal graft, while nonbiological synthetic one is unresorbable polypropylene mesh (Marlex Mesh). Therefore, these implants are selected and used for our research.

Data from the literature refer to the use of autodermal grafts before synthetic mesh. First description of autodermal graft in clinical practis was given by Otto Loewe [8] and Edvard Rehn [9] 1913–14 year, and in America in 1939 by Uihlein [10]. However, after the explosive development of industrial polymers and production of synthetic implants, skin grafts went into the margins for almost 40 years. Their reaffirmation began in the 80's primarly due to experimental and clinical work of German surgeons [11].

Numerous investigations have defined the exellent properties of those grafts. From immunology and biology viewpoint, they are referred as skin autotransplants which do not cause rejection. Because of the outstanding characteristics of human skin, these grafts have good physical properties - sufficient strength, elasticity, flexibility and proper resistance to traction and tension.

Abdominal wall repair using these grafts goes throught specific biological processes and reactions through synchronized degradation and transformation of grafts. After the implantation, autodermal graft was remodeled into formation of dense fibrous sheet which provided proper integrity of the abdominal wall [12]. Skin autografts can be used in two forms: like full-thickness skin graft without removing epidermal layer and as a dermal graft without epidermis.

The disadvantage of autodermal graft harvesting is longer operative time due to the removal of epidermal layer. In case of full-thickness skin graft use, experimental studies reveal epidermal cyst formations and a prolonged time of remodeling.

Considering good revascularization and smooth surface, autodermal grafts without epidermal layer are less prone to the development of infection and adhesions.

The main advantage of autodermal grafts compared to synthetic mesh is the fact that they are available at any time from the patient's body, an "always open bank". This is significant, especially in emergency situations such as wars or natural disasters when the production, market availability and use of all other implants are impossible.

Utilisation of nonbiological synthetic mesh made of polypropylene fibers began in 1962, in America by Usher [13, 14]. During the last four decades, these sinthetic meshes were implanted worldwide to millions of patients. They are biocompatibile and do not cause severe inflammation, anaphylactic and allergic reactions and host reaction. Furthermore, not carcinogenic, they are chemically inert and are not disassembled in the body. Also, they are resistant to traction and tension and are thermostable.

Synthetic mesh incorporation is caused by inflammatory response and fibrous tissue proliferation through the pores of meshes. Thus, incorporation of mesh in the abdominal wall structures and its complete isolation as a foreign body occur. A lifetime reconstruction of abdominal wall defects is achieved by the presence of synthetic mesh reinforced by surrounding fibrous tissue.

However, mesh structure has its disadventages. It decreases the resistance to bacterial infection because bacterial inudation in pores provide them with better survival and reproduction. Mesh infection can be solved only by implant removal. Use of synthetic mesh is related to higher incidence of intraabdominal adhesions.

Analysis of our clinical material showed that large defects in the abdominal wall incisional hernia are common and important problem in our society. According to the avaialble data, the overall incidence of incisional hernia following laparotomy remains reported to be up to 11% [15, 16]. Incisional hernias typically occur two to four years after laparotomy [15, 17]. The incidence of recurrent incisional hernia is 24%–58% and the rate remained unchanged in the last 50 years, which accentuates the importance of finding the optimal method of reconstruction of muscleoaponeurotic layer defects of the abdominal wall [16].

Our clinical study on applying autodermal grafts and polypropylene mesh was conducted only on patients with large abdominal wall defects (greater than 10 cm). The average size of the defect in the two groups of patients was not significantly different, so the size of the defect could not be considered as an independent factor for complications ($p = 0.716$).

The average age of the patients in our study was of 57.6 ± 10.91 years correlating with the reported data on the average patient's age of 45.5 to 62 years [16–19]. The patients were predominantly male. In several studies [18, 19] females are pre-

dominant, whereas in studies of Mc Greevy et al. [20] and La Mura et al. [21] dominate males.

In our study, the operative time was significantly longer in the group with autodermal graft ($p = 0.03$), which is consistent with the literature data with the mean operative time with synthetic grafts of 1.7 h [20]. In the study of Chan and Chan [22] conducted on 135 patients, the duration of surgery using mesh technique was up to 40 min in 15 cases, up to 60 min in 77 cases, up to 90 minutes in 41 cases and up to 120 min in 2 cases. The time required for graft harvesting was significantly longer if the graft was taken by a surgical blade and less when taken by dermatome. In our Clinic there is no dermatome, so it may be for the longer duration of surgery. Also, surgical techniques using dermatomes provide an ideally smooth surface of the graft, which reduces the possibility of tearing the graft, forming keratine cysts and other complications, particularly adhesion. Operation of large abdominal wall defects should be performed as a team work, with the participation of the abdominal and plastic and reconstructive surgeon in order to shorten operative time.

Application of autodermal graft reduces the frequency of pain and intensity of inflammatory response, but correlate with higher complications rate, even up to 25% [15]. The overall incidence of early complications in our study in both groups was 20%. The available literature data reported the incidence of infection and bleeding of 10% [23]. Some authors [24] stated that seroma after applying synthetic mesh may occur even one year after surgery, but such a complication was not observed in our study. Kingsnorth et al. [19] in their study reported the early complications rate of 34%. Our study results correlate well with other studies, where the percentage of early minor complications (seroma, hematoma, infection) ranged from 16% to 18%, and severe complications from 6% to 27% (intestinal obstruction, intraperitoneal infection and enterocutaneous fistula), accordingly [6, 20].

A literature review shows the most common use of synthetic mesh for incisional hernia repair. Artificial materials such as synthetic grafts, represent a strong stimulus for the development of intestinal adhesions, which can lead to serious complications, such as intestinal obstruction and enterocutaneous fistula [25, 26]. In our research we recorded one patient with intestinal obstruction and one with enterocutaneous fistula.

Enterocutaneous fistula is rarely formed with synthetic mesh placed extraperitoneally [27], although some authors reported increased incidence of complications with intraperitoneally placed mesh [6, 25, 28, 29]. In this study, enterocutaneous fistula was observed in one patient with synthetic mesh. During surgery, the most important point is to avoid contact of mesh and skin [20]. When infection occurs mesh must be removed and definitive abdominal wall reconstruction have to be done after 6 months. Although some literature data indicate a higher complication rate in patients treated with autografts [15], in our study there were no significant differences in the early minor complication rate between the groups.

Hospital stay analysis for the patients who required autodermal grafts showed 6.8 ± 2.8 days, and by the use of synthetic mesh 6.4 ± 2.5 days, but statistical significance was not found. These data correlate with the literature, where the average duration of hospital stay was 5–13 days [1, 30]. In the group of patients with synthetic mesh, two of all the patients had repeated hospitalization increasing the overall cost of treatment.

In contemporary clinical practice, there are numerous studies that analyse indications, complications, length of hospitalization and economical aspects of the treatment [31]. A total cost of frequent severe complications treatment after the use of synthetic mesh, was lower when biological materials (allo- or xenograft) were applied regardless the fact that they are more expensive [32]. Application of autografts in this study, despite the longer operative time led to lower overall costs of treatment because autodermal graft is free of charge.

In our research, the recurrence rate observed in autografts was 10% in the first 20 months, and with synthetic mesh the observed rate was 15% in the first 42 months following the surgery. According to the literature data, recurrence rate after the use of synthetic mesh varies from 15% to 36%, where 45% of recurrences occur within the first year, 19% in the second year, 14% in the third and the rest later [33, 34].

The reestablishment of the anatomical and functional integrity of the abdominal wall using synthetic mesh is safe and secure in general but is accompanied by a number of adverse effects, most notably reduced flexibility of the abdominal wall, due to the presence of the permanently rigid structure, which consists of the mesh and the fibrous capsule, unsatisfactory aesthetic appearance, particularly with lean patients, feeling the presence of foreign body, as well as granuloma formation. Therefore, the use of synthetic mesh should be avoided in young patients.

Applying autodermal graft could be an ideal choice for patients with infection or exposure to synthetic implant, and in patients with intraabdominal infections, immunocompromised patients and after radiotherapy, where a synthetic mesh is contraindicated.

Conclusion

We believe that tension-free reconstruction with an autodermal graft is a safe operation with minimal morbidity and mortality. Autodermal graft reconstruction is technically more difficult and prolongs the duration of operation but it is more cost effective. The incidence of early minor and major postoperative complications is lower with autodermal graft, and they can be efficiently treated. We believe that the dominance of synthetic mesh in reconstruction of large defects of the abdominal wall is unduly favored and biological autografts should be more often applied in everyday surgical practice. They can be applied when synthetic implants are contraindicated (the presence of infection, immunodeficient patients, or after radiotherapy) and in unusual conditions such as natural disastres or war surgery with no idustrial manufactur of grafts.

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Mortality rate of lip, oral cavity and pharynx malignant tumors in Serbia within a period 1991–2009

Stopa mortaliteta od malignih tumora usne, usne duplje i ždrela u Srbiji u periodu 1991–2009. godine

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Abstract

Background/Aim. Lip, oral cavity and pharynx malignant tumors account for 3.7% of all cancer deaths worldwide, with significant geographic variations in frequency and distribution. The aim of this descriptive epidemiologic study was to analyze the mortality rate of lip, oral cavity and pharynx malignant tumors in Serbia proper within a period 1991–2009. **Methods.** Mortality rates standardized directly using the world population as the standard were used in data analysis. Linear trend and regression analyses were used to analyze rate trends in mortality. **Results**. The Serbian population demonstrated an increase in the mortality of lip, oral cavity and pharynx malignant tumors (y = 3.32 + 0.03×; p = 0.002; average annual percent change = + 0.8). The male population showed a significant increase in mortality trend (y = 5.90 + 0.03×; p = 0.020; % change = + 0.9), while the female population did not show a significant increase in mortality. The male/female cancer mortality ratio was 5.5:1. Mortality rates for lip, oral cavity and pharynx cancer increased with age in both genders, with rates being the highest in the population aged 85 and older. Increasing trends of lip, oral cavity and pharynx cancer mortality were observed in males aged 50–54; the average annual percent change was + 7.4 % (95% CI, 6.2–9.0). The population of both genders aged 55–59 demonstrated an increase in lip, oral cavity and pharynx cancer mortality, the increase being + 1.8% (95% CI, 1.4–2.2) in men and + 34.3% (95% CI, 28.4–40.2) in women. **Conclusion.** The increasing trend in lip, oral cavity and pharynx cancer mortality points to the necessity to investigate etiology and improve primary and secondary prevention measures.

Key words:
mouth neoplasms; pharyngeal neoplasms; serbia; mortality; risk factors.

Apstrakt

Uvod/Cilj. Maligni tumori usne, usne duplje i ždrela uzrok su 3,7% svih smrtnih slučajeva od raka u svetu, sa značajnim geografskim varijacijama u učestalosti i distribuciji. Cilj ove deskriptivne epidemiološke studije bila je analiza mortaliteta od malignih tumora usne, usne duplje i ždrela u Srbiji u periodu od 1991. do 2009. godine. **Metode.** U analizi podataka korišćene su standardizovane stope mortaliteta, dobijene metodom direktne standardizacije sa populacijom sveta kao standardom. Linearni trend i regresiona analiza korišćeni su za analizu trenda mortaliteta. **Rezultati.** U populaciji Srbije zabeležen je porast mortaliteta od malignih tumora usne, usne duplje i ždrela (y = 3,32 + 0,03×; p = 0,002; prosečna godišnja procentualna promena = + 0,8). Kod muškaraca je zabeležen značajan trend porasta mortaliteta (y = 5,90 + 0,03×; p = 0,020; % promena = + 0,9), dok kod žena nije utvrđen značajan porast mortaliteta. Odnos mortaliteta među polovima (muškarci/žene) bio je 5,5 : 1. Stope mortaliteta od malignih tumora usne, usne duplje i ždrela povećavale su se sa starošću kod oba pola, pri čemu su stope bile najviše u populaciji starih od 85 i više godina. Trend porasta mortaliteta od malignih tumora usne, usne duplje i ždrela uočen je kod muškaraca starosti 50–54 godine: prosečna godišnja procentualna promena iznosila je + 7,4% (95% IP = 6,2–9,0). U populaciji oba pola u uzrasnoj grupi 55–59 godina zabeležen je trend porasta mortaliteta od malignih tumora usne, usne duplje i ždrela, pri čemu je porast iznosio + 1,8% (95% IP = 1,4–2,2) kod muškaraca i + 34,3% (95% IP = 28,4–40,2) kod žena. **Zaključak.** Trend porasta mortaliteta od malignih tumora usne, usne duplje i ždrela ukazuje na neophodnost etioloških istraživanja i unapređenja mera primarne i sekundarne prevencije.

Ključne reči:
usta, neoplazme; farinks, neoplazme; srbija; mortalitet; faktori rizika.

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Introduction

The International Association of Cancer Registries in the most recent estimate reports 128,000 deaths from oral cavity cancer (including lip cancer) and 147,000 pharyngeal cancers in 2008 worldwide [1–3]. Oral cavity and pharynx cancers account for 3.7% of all cancer deaths in the world. Geographic variations in mortality were observed [3–5].

Globally, the highest lip, oral cavity and pharynx cancer mortality rates for both males and females are found in Melanesia (11.3 per 100,000 people), South-Central Asia (9.1), and South-East Asia (8.8) [1, 3]. The lowest death rates are found in Central America (1.3), North America (1.5) and Australia/New Zealand region (1.9). Nearly 80% of lip, oral cavity and pharynx cancer cases (216,425 cases) occur in developing countries (150,820 men *versus* 65,605 women) [1, 3]. The greatest number of deaths (70.1%) was recorded in Asia, almost 50% of which occurred in India.

These cancers are more than twice as common in men as in women [5, 6]. Most people of both genders, diagnosed with oral cavity and pharynx cancer are older than 50 years [5]. In the United States, from 1975 through 2002, age-adjusted mortality rates were higher among males than females and highest for black males [7]. By the mid 1980s, mortality rates were declining for white and Afro-American males and females, but however disparities persist [7, 8]. In the United States the lowest mortality rates were recorded in Latinos [8]. The age-adjusted death rate for cancer of the oral cavity and pharynx was 2.4 per 100,000 men and 0.7 per 100,000 women per year in 2005–2009 in the US Latinos, with a significant declining trend (average annual percentage change was – 3.6% in males and – 1.5 % in females). Though oral and pharyngeal cancer mortality in Europe has declined in the last decade in men, there are still rises in a few Central and Eastern European countries, reaching exceedingly high rates in Hungary and Slovakia, which now have the highest rates on a European scale [9, 10].

The aim of this descriptive epidemiologic analysis was to estimate death rates for lip, oral cavity and pharynx cancer and their secular trends in the population of Serbia over a period 1991–2009.

Methods

This descriptive epidemiologic study used data on individuals who had died of lip, oral cavity and pharynx malignant tumors (codes 140–149 revision 9 and C00–C14 revision 10 of the International Classification of Diseases to classify death, injury and cause of death), all malignancies (revision 9 codes 140–209 and revision 10 codes C00–C97), symptoms and undefined states (revision 9 codes 780–799 and revision 10 codes R00–R99), and all causes of death (revision 9 codes 001–999 and revision 10 codes A00–Z99) collected by the Statistical Office of the Republic of Serbia, which receives death certificates and compiles mortality data by gender, age, year, and cause of death. The research included the entire population of the Republic of Serbia (all ages), excluding the Province of Kosovo, from 1991 to 2009. Data for internally displaced 2009. Data for internally displaced persons and refugees were included in the population of Serbia, but could not be set aside as a special contingent. Data on the number and composition of the population of the Republic of Serbia by gender and age were obtained from the population censuses (1991 and 2002 national censuses) for the years 1991 and 2002, and for inter-census years estimates published by the Statistical Office of the Republic of Serbia. The age-standardized rates (per 100,000 people per year) were calculated by direct standardization, using the World Standard Population as proposed by Jensen et al. [11].

An estimate of the linear trend of the age-adjusted lip, oral cavity and pharynx cancer mortality rates was obtained by fitting Poisson regression models to the data observed over the period 1991–2009. Two-sided p values were reported and considered to indicate statistical significance when they were less than 0.05. Age-specific mortality rates were computed for 5-year age groups. Percent changes of mortality rates were calculated as a percent difference between the adjusted rates of the two successive years and then as an average of these changes for the entire observation period. Confidence intervals (CI) for the average age specific rates were assessed with 95% level of probability.

All statistical analyses were conducted using the Statistical Package for the Social Sciences software (SPSS Inc, version 19.0, Chicago, IL, USA).

Results

Over the 19-year observation period, in the Republic of Serbia, excluding the Province of Kosovo, a significant decrease in total mortality was observed ($y = 799.31 – 7.78\times$; $p = 0.000$; % change = - 0.7), with a significant increase in deaths from all malignant tumors ($y = 119.69 + 1.18\times$; $p = 0.000$; % change = + 1.0) (Figure 1). Mortality rate of lip, oral cavity and pharynx malignant tumors increased ($y = 3.32 + 0.03\times$; $p = 0.002$; % change = + 0.8). The nonsignificant declining trend ($y = 63.33 - 0.83\times$; $p = 0.151$; % change = - 0.2) was observed for mortality in which the causes of death were symptoms, signs and abnormal clinical and laboratory findings.

In the same period, mortality of lip, oral cavity and pharynx malignant tumors significantly increased among males ($y = 5.90 + 0.03\times$; $p = 0.020$; % change = + 0.9), whereas the increase in mortality among women was not statistically significant (Figure 2). On average, men died of lip, oral cavity and pharynx malignant tumors 5.5 times more frequently than women.

Trends analysis of age adjusted mortality rates of oral cavity cancer (including lip cancer) showed a significant increase ($y = 1.79 + 0.02\times$; $p = 0.001$; % change = + 1.1) in the population of Serbia (Figure 3), with an increase in both genders ($y = 3.20 + 0.03\times$; $p = 0.010$; % change = + 0.9 in men, *versus* $y = 0.57 + 0.02\times$; $p = 0.038$; % change = + 4.6 in women). On the other hand, mortality rates of malignant pharyngeal tumors have not decreased significantly (Figure 4).

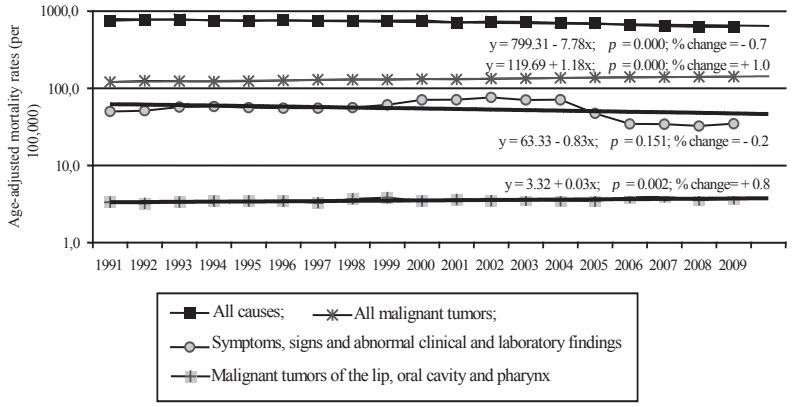

Fig. 1 – The mortality rates of the choosen causes in Serbia, excluding the Province of Kosovo, in 1991–2009

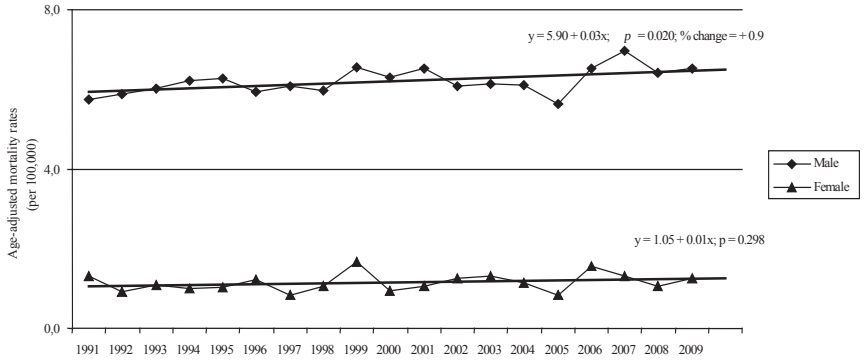

Fig. 2 – The mortality rates of malignant tumors of the lip, oral cavity and pharynx by gender in Serbia, excluding the Province of Kosovo, in 1991–2009

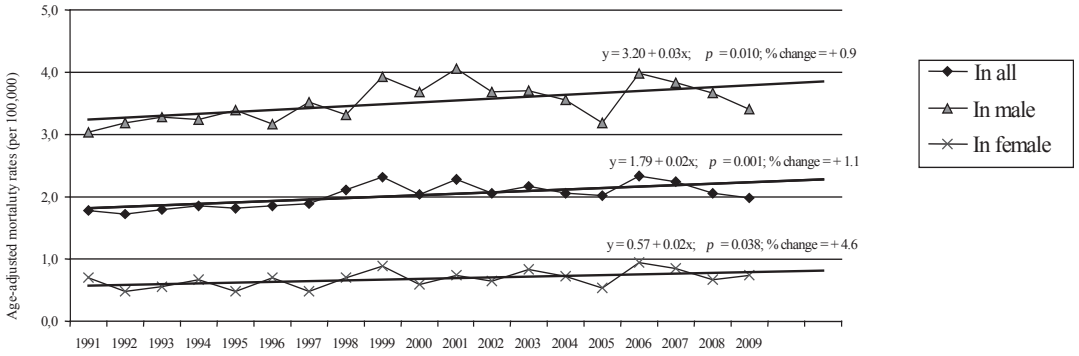

Fig. 3 – The mortality rates of malignant tumurs of the lip and oral cavity by gender in Serbia, excluding the Province of Kosovo, in 1991–2009

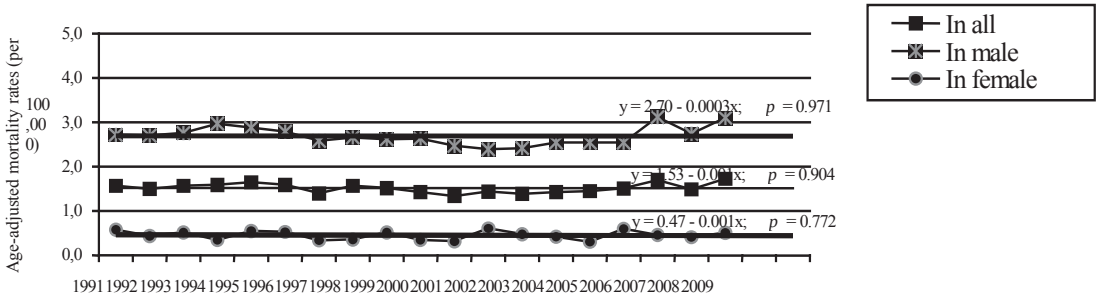

Fig. 4 – The mortality rates of malignant tumors of the pharynx by gender in Serbia, excluding the Province of Kosovo, in 1991–2009

Lip, oral cavity and pharynx cancer mortality rates increase with age and are highest in people aged 85 and older (Table 1). Low mortality rates were recorded in both men and women aged under 45. In terms of gender, age-specific rates were notably higher among males than among females, and the differences were highest for the age 45–64. In the observation period, a significant increase in the number of deaths caused by lip, oral cavity and pharynx cancer was recorded in people aged 55–59, the increase being + 1.8% (95% CI, 1.4–2.2) in men and + 34.3% (95% CI, 28.4–40.2) in women. The male population also demonstrated an increase of 7.4 % (95% CI, 6.2–9.0) in deaths caused by lip, oral cavity and pharynx cancer in a younger age group (50–54 years of age). None of the regression analysis models corresponded to mortality data for other age groups.

Table 1

The average age-specific mortality rates and linear trend in lip, oral cavity and pharynx malignant tumors in Serbia, excluding the Province of Kosovo, in 1991–2009

Age (years)	Age-specific rates* (per 100,000)	Linear trend	*p*	Average annual percentage change (95% CI)
Male				
≤ 19	0.05	†		
20–24	0.02	†		
25–29	0.21	†		
30–34	0.28	†		
35–39	1.24	†		
40–44	4.31	†		
45–49	11.88	†		
50–54	18.16	y = 15.04 + 0.31×	0.049	+7.4 (6.2–9.0)
55–59	25.82	y = 22.03 + 0.38×	0.003	+1.8 (1.4–2.2)
60–64	27.94	†		
65–69	29.51	†		
70–74	28.04	†		
75–79	26.77	†		
80–84	29.25	†		
85+	33.43	†		
Female				
≤ 19	0.05	†		
20–24	0.04	†		
25–29	0.17	†		
30–34	0.22	†		
35–39	0.34	†		
40–44	0.93	†		
45–49	1.45	†		
50–54	2.73	†		
55–59	3.26	y = 0.56 + 0.95×	0.027	+34.3 (28.4–40.2)
60–64	3.74	†		
65–69	4.44	†		
70–74	6.51	†		
75–79	9.09	†		
80–84	13.86	†		
85+	20.56	†		

† None of the regression analysis models corresponded to mortality data for this age group; CI − confidence interval

Discussion

Lip, oral cavity and pharynx cancer rates varied considerably among countries [2–4, 7]. The highest standardized male population death rates in 2008 were recorded in Hungary (19.4 per 100,000 individuals), Slovakia (15.6) and India (14.4), and the lowest mortality rates (approximately 1 per 100,000) are recorded in Israel, Kuwait and Cyprus [2, 3]. Countries with female population mortality rates higher than 5 per 100,000 individuals are Bangladesh, Pakistan and India, and countries with the rates less than 1 per 100,000 individuals are countries of North and South America, Australia, New Zealand and Northern and Western Europe.

In the period 1991–2009, the average standardized mortality rate of lip, oral cavity and pharynx cancers in Serbia was 6.2 per 100,000 in men and 1.2 per 100,000 in women. These mortality rates place Serbia among the countries with medium mortality values. Serbian male and female mortality rates are more similar to mortality rates of Central and Western European countries rather than to those of Eastern and South European countries [3, 12].

In last decades, the USA records a decline in deaths of oral cavity and pharynx cancers in all races (white, Afro-American, Asian/Pacific Islander, American Indian/Alaska Native, Latinos), for both sexes, although disparities persist [7, 8]. Oral cancer mortality has been rising appreciably in most European countries up to the late 1980s, essentially for men [6, 10]. From 1990 to 2004, the European Union records a decline in mortality of approximately 7% in men and an in-

crease of nearly 16% in women [9, 13]. However, in some countries, such as Hungary, Romania, Slovakia and Czech Republic, an increase in oral and pharynx cancer mortality in both men and women is recorded. Oral cancer mortality in men has been declining since the late 1980s in most western countries, although some persisting upward trends were recorded in Denmark, United Kingdom, England and Wales, and Scotland. These trends should be essentially interpreted in terms of patterns and changes in exposure to alcohol and tobacco in Central and Eastern Europe [4, 9].

For the youngest age categories in the Serbian population, mortality rates were generally less than 1 per 100,000 individuals per year; however, after about the third decade of life, rates began to increase notably, with the sharpest increases seen for males. Lip, oral cavity and pharynx cancer mortality rates in 11 countries (USA, Asian and European countries, and Australia) during the period 1990–2006, were from 3 to 10 times higher in males than in females [5]. The reason for this may be that men had been more likely to use tobacco and alcohol in the past [4, 7, 10]. Among females, few differences in mortality rates were observed for all the countries studied with the exception of China (Hong Kong) [5]. Although the age-standardized rates in China (Hong Kong) have evidently decreased over the period, they were still 5 times higher for both genders.

Oral cavity cancer mortality rates ranged from 12.1 per 100,000 among males and 5.9 among females in Melanesia, 6.3 among males and 3.6 among females in South-Central Asia, to less than 1 for both genders in North America, Northern and Western Europe, and Australia/New Zealand [1, 3]. The highest pharyngeal cancer mortality rates in 2008 were recorded in South-Central Asia and Southern Africa (7 per 100,000 among males and approximatively 2 per 100,000 among females). The lowest pharyngeal cancer mortality rates (1 per 100,000) for both genders were recorded in North America, Northern and Western Europe, and Australia/New Zealand. In India, among all malignant tumors, lip, oral cavity and pharynx cancer mortality rates have a leading position in the structure of mortality when considering the entire population (9.7 per 100,000) and the male population alone (14.4), while they rank number 3 in female population (5.4) [1, 3]. In India, pharyngeal cancer death was more frequent than oral cavity cancer death in men (7.6 versus 6.8), while in women oral cavity cancer mortality rates were twice as high as those of pharyngeal cancer.

Some potential explanations for this apparent differences among the countries may be discrepancies in the disease early detection and availability of the improved treatment methods. However, numerous epidemiological studies indicated that the increase was attributed primarily to changes in the patterns of smoking and alcohol use (especially among women) in recent decades; in addition, nutritional, lifestyle and other factors [3, 14]. The difference in rates between black and white population is attributable to racial differences in patterns of alcohol intake, especially among current smokers, as well as to higher risks associated with alcohol intake among blacks [7, 10, 15]. A Swedish population-based case-control study showed that risk factors for oral and oropharyngeal squamous cell carcinoma were poor oral hygiene, dental status (defective and missing teeth), oral mucosal lesions, alcohol and tobacco use, human papilloma virus (HPV) infection, and lifestyle-related factors [16]. The findings in England and Wales [17] about a positive correlation between liver cirrhosis and intraoral cancer suggested that rising alcohol consumption is more closely related to increasing intraoral cancer incidence and mortality than smoking, most notably among younger males since the early 1970s.

In India [14], tobacco chewing emerged as the strongest risk factor for oral cancer, while the strongest risk factor for pharyngeal cancer was tobacco smoking in current smokers. Oral tobacco products (snuff or chewing tobacco) are related to cancers of the cheek, gums, and inner surface of the lips. In Southeast Asia, South Asia, and some other areas of the world, many people chew betel and/or gutka [18, 19]. Several studies have found that a diet low in fruits and vegetables is related to an increased risk of oral cavity and oropharyngeal cancers [20, 21].

The rising rate of HPV related cancers is thought to be due to changes in sexual practices in recent decades, particularly to the increase in oral sex [22]. The International Agency for Research on Cancer conducted a multicenter case–control study of oral cavity and oropharyngeal cancer in nine countries, where HPV DNA was detected in biopsy specimens of 3.9% of oral cavity cancers with valid polymerase chain reaction (PCR) results and 18.3% of oropharyngeal cancers [23].

Mortality rates in the Republic of Serbia for which symptoms, signs and ill-defined states were indicated as causes of death suggest that caution must be present when interpreting statistical data on mortality in international comparisons. However, it is not likely that these had a significant impact on the increasing trend of lip, oral cavity and pharynx cancer mortality, for which the increasing trend ($y = 3.31 + 0.02$; $p = 0.033$) was also observed for the 1991–2003 period, when mortality rates of undefined death causes also demonstrated a considerable increasing trend ($y = 47.23 + 1.93$; $p = 0.000$).

A similar increasing trend of the mortality of lip, oral cavity and pharynx malignant tumors (average annual percent change = + 0.8) and the mortality of all malignant tumors (average annual percent change = + 1.0) in Serbia can be only partially explained by the lack of organized programs for primary and secondary prevention, especially during the recent decades which characterised the economic sanctions against Serbia, the war and the 1999 NATO bombing of Yugoslavia. Other than, it was not possible to give specific information about internally displaced persons and refugees, although they may have a different exposure, which could be of great importance for understanding the trends in mortality of malignant tumors. Despite changes in recent years, the most significant exposures to risk factors for malignant tumors in Serbia are still higher than in developed countries. The prevalence of smokers in the adult population has decreased from 40.5% in 2000 to 33.6% in 2006, while a third of young people in the 15–19 age group consumed alcoholic beverages [24]. In Serbia,

an increase in the number of deaths of lip, oral cavity and pharynx malignancies and of all malignant tumors was observed for the period from 1991 to 2009, is pointing to the need to conduct analytical epidemiologic studies to help identify risk factors for lip, oral cavity and pharynx malignant tumors in the Serbian population.

Conclusion

Lip, oral cavity and pharynx cancer mortality rates place Serbia among the countries with medium mortality values. The increasing trend in lip, oral cavity and pharynx cancer mortality points to the necessity to investigate etiology and improve primary and secondary prevention measures.

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Health-related quality of life assessment in Serbian schoolchildren hospitalized for malignant disease

Kvalitet života dece školskog uzrasta u Srbiji hospitalizovane radi lečenja maligne bolesti

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Abstract

Background/Aim. Although long-term survival of childhood cancer patients is significantly improved, prolonged treatment and hospitalization might have negative impacts on child development. The aim of this study was to verify profile of health-related quality of life parameters in population of schoolchildren during hospitalization and treatment for malignant disease. **Methods.** The Serbian version of Pediatric Quality of Life Inventory Version 4.0 (PedsQL™4.0) Generic Core Scales was applied. A total of 120 schoolchildren were analyzed: 60 patients hospitalized for prolonged malignant disease treatment and 60 healthy schoolchildren from public schools. The study was done at the Institute for Oncology and Radiology of Serbia, as well as in four schools. **Results.** Generally, schoolchildren hospitalized for cancer treatment demonstrated lower scores on physical, emotional, social and school functioning when compared to healthy schoolchildren from regular public schools. Significant differences were observed for all the 8 items of the Physical Health Scale, in 2 out of 5 items of the Emotional Functioning Scale, in 4 out of 5 items of the Social Functioning Scale, and 3 out of 5 items of the School Functioning Scale. **Conclusions.** The Serbian version of PedsQL™ 4.0 Generic Core Scales could be successfully used to evaluate physical, emotional, social and school functioning of hospitalized children and adolescent. Schoolchildren hospitalized for prolonged tumor treatment have poorer HRQOL scores compared to general healthy population, however the level of remaining physical, emotional and social parameters should provide solid foundation for their potential rehabilitation, education and inclusion.

Key words:
quality of life; neoplasms; questionnaires; child; adolescent.

Apstrakt

Uvod/Cilj. Iako je dužina života dece obolele od maligne bolesti značajno povećana, produženo lečenje i hospitalizacija mogu imati negativni uticaj na njihov razvoj. Cilj našeg istraživanja bio je da se utvrdi profil zdravstvenih parametara kvaliteta života populacije školske dece obolele od maligne bolesti tokom njihove hospitalizacije i lečenja. **Metode.** Primenjena je srpska verzija testa „Pediatric Quality of Life Inventory Version 4.0 (PedsQL™4.0) Generic Core Scales“. Testirano je ukupno 120 školske dece, od toga 60 hospitalizovane radi produženog lečenja maligne bolesti i 60 zdrave školske dece iz osnovnih i srednjih škola. Istraživanje je sprovedeno u Institutu za onkologiju i radiologiju Srbije i u četiri škole. **Rezultati.** Generalno, školska deca hospitalizovana radi lečenja kancera pokazala su slabije rezultate u fizičkim, emocionalnim, socijalnim i školskim parametrima u poređenju sa zdravom školskom decom iz redovnih škola. Značajne razlike uočene su u svih osam parametara na fizičkoj skali, u dva od pet parametara na emocionalnoj skali, u četiri od pet parametara na socijalnoj skali i u tri od pet parametara na školskoj skali. **Zaključak.** Srpska verzija “PedsQL™ 4.0 Generic Core Scales“ testa može biti uspešno upotrebljena za utvrđivanje fizičkih, emocionalnih, socijalnih i školskih parametara funkcionisanja hospitalizovane dece i adolescenata. Školska deca hospitalizovana radi produženog lečenje maligne bolesti pokazala su lošije rezultate pri analizi kvaliteta života od zdrave školske dece. Međutim, preostali stepen funkcionisanja na fizičkom, emocionalnom i socijalnom nivou obezbeđuje solidnu osnovu za njihovu rehabilitaciju, obrazovanje i inkluziju.

Ključne reči:
kvalitet života; neoplazme; upitnici; deca; adolescenti.

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Introduction

During the past decades, long-term survival of children and adolescents diagnosed with malignant diseases significantly improved. Yet, some evidence suggests that medical treatment results in improved health status of patients and their prolonged and frequent hospitalization during this sensitive period of life could negatively impact child development. Therefore, there is an increasing interest in the development and inclusion of measures for health-related quality of life (HRQOL) outcome in modern clinical practice [1].

The HRQOL is a multidimensional construct compiled of the patient's perception of the impact of the disease and treatment on his/her functioning in different aspects of life including physical, mental, and social domains [2]. Pediatric HRQOL measurement instruments must in addition be sensitive to cognitive development and to include both child self-report and parent proxy-report [3]. Furthermore, a relatively short self-report questionnaire is essential for administration to children given their short attention spans when compared to adults. Results obtained using HRQOL measurement instrument could be used in health status tracking, evaluation of treatment outcomes, research, school health settings and community population [3].

The Pediatric Quality of Life Inventory (PedsQL) is standardized assessment instrument developed by Varni et al. [4]. This instrument systematically evaluates patients' and/or parents' perception of HRQOL in pediatric patients with chronic health conditions using pediatric cancer as an exemplary model [4]. The PedsQL 4.0 Generic Core Scales were constructed to measure core physical, mental, and social health dimensions as delineated by the World Health Organization, but also to include school functioning [5]. The measurement properties, reliability and validity of the PedsQL 4.0 in pediatric cancer were demonstrated in several studies [2, 5–12]. On the other hand, only a few studies were performed with pediatric malignant disease patients during ongoing oncological treatment and hospitalization.

HRQOL instruments are expected to be simultaneously developed across different cultures and languages in order to ensure measurement equivalence between original and new local versions. PedsQL Generic Core Scales are translated into 73 languages or dialects among which is Serbian language [13, 14]. Recently, the Serbian version of PedsQL 4.0 Generic Core Scales self-report version for children and adolescents has been evaluated [14]. The study confirmed appropriate internal consistency reliability of the scales that is sufficient for group evaluations, and good convergent validity against psychological constructs. However, the Serbian PedsQL was validated and tested only on general healthy population of children.

The aim of our study was to determine the profile of HRQOL parameters in population of schoolchildren diagnosed with malignant diseases during the first year of the treatment and hospitalization. Based on the previous reports cited above, we hypothesized that children and adolescents hospitalized for malignant disease treatment would demonstrate lower HRQOL scores compared to general population. Since all the tested patients were enrolled to special hospital school service during hospitalization, and due to the fact that their parents were present for most of the hospitalization time, we expected to identify a significant level of preserved psychosocial functions.

Methods

The study comprised a total of 120 schoolchildren, aged 8–18 years and equally boys and girls from Serbia. Out of 120 children and adolescents 60 were patients recruited from the Institute for Oncology and Radiology of Serbia (IORS). The patients were considered eligible if they were: 8–18 years old, diagnosed with malignant disease confirmed with histopathological examination, without comorbid disease or major developmental disorders, receiving treatment and hospitalized in the Department of Pediatrics at the IORS. The patients were excluded if they were not clinically stable or not cognitively able to complete the questionnaire. All the patients from the IORS were enrolled to hospital school service according to their age and previous grade they attended in regular school. Another group of 60 healthy schoolchildren of the same age and sex were chosen as control from two primary and one secondary public schools.

All the children and adolescents, as well as their parents, were informed about the purpose of the examination and they gave consent. Child-report questionnaires were self- or interviewer-administered to participants.

The study was approved by the Ethical Committee of the IORS (study approval No 1005/1-01) and from Institutional Review Board of IORS.

To assess health-related quality of life factors in both, patients and healthy schoolchildren, the PedsQL™ 4.0 Generic Core Scales [13] were applied. The 23-items of this scale encompass: Physical Functioning Scale (8 items), Emotional Functioning Scale (5 items), Social Functioning Scale (5 items), and School Functioning Scale (5 items).

The scales ask about the frequency of a problem of each item within the past month. All items are in a 5-point response scale (0 = never a problem; 1 = almost never a problem; 2 = sometimes a problem; 3 = often a problem; 4 = almost always a problem). Items are reversely scored and linearly transformed to a 0–100 scale (0 = 100, 1 = 75, 2 = 50, 3 = 25, 4 = 0), so that higher scores indicate better health-related quality of life parameters. Scale scores are computed as the sum of the items divided by the number of items answered. If more than 50% of the items in the scale were missing, the Scale Score was not calculated.

The Serbian version of PedsQL™ 4.0 was provided by the MAPI Research Trust and permission was obtained from the author, Prof James W. Varni [13]. Serbian PedsQL™ 4.0 is feasible, short and easily scored questionnaire for health-related quality of life assessments in children and adolescents. The Serbian version of PedsQL™ 4.0 has appropriate internal consistency reliability as confirmed in the previous report [14].

To describe parameters from PedsQL™ 4.0 scales we calculated mean values, standard deviations (SD), frequen-

cies and range values. As statistical instruments, we used Kolmogorov-Mirnov test (K-S test), Histogram, Scatterplot and QQ Plot. To test the difference between parameters we used Welch Two Sample *t*-test, Fisher Exact Test and Pearson χ^2 test.

A statistical significance in correlations was only accepted at $p < 0.05$.

All the tests were performed using free statistical package "R Project for Statistical Computing" (version 2.6.0).

Results

This research enrolled a total of 120 schoolchildren during the years 2006, 2007 and 2008. A descriptive information about the sample is presented in Table 1. For both healthy and patient group, equal number of 60 participants was selected with equal proportion of boys and girls (50 : 50). Children were selected to represent a complete range of years from 8 to 18 (Table 1 and data not presented) with equal age distribution. The median age of the children from the healthy group was 13.22 years, and median age for the patient group was 12.98 years, showing statistically non-significant difference. Children from both groups were enrolled in a primary school (68.33%) or secondary one (31.67%). Healthy schoolchildren were chosen from regular public schools, and the patients were receiving special education services synchronized with regular school programs they were enrolled prior to hospitalization.

The sample of patients was heterogeneous with respect to tumor pathology (solid tumors and malignant hemopathies), disease stadium (localized and disseminated tumors) and duration of the disease (median time from diagnosis was 2.6 months). All the patients were subjected to the malignant disease treatment. Thirty seven patients (61.67%) went under chemotherapy treatment, two patients (3.33%) radiation treatment, and twenty one patients (35%) a combined therapy. In 8 cases malignant disease was previously diagnosed in one or more members of the family (13.33%) (Table 1).

Table 1

Demographic and clinical characteristics of the sampled children

Characteristic	Healthy n (%)	Patients n (%)
Gender		
total	60 (50.0)	60 (50.0)
female	30 (50.0)	30 (50.0)
male	30 (50.0)	30 (50.0)
Age, years		
average (mean ± SD)	13.22 ± 3.15	12.98 ± 3.05
8–12	30 (50.0)	30 (50.0)
13–18	30 (50.0)	30 (50.0)
School enrollment		
primary school	41 (68.33)	41 (68.33)
secondary school	19 (31.67)	19 (31.67)
Medical diagnosis		
solid tumor		43 (71.67)
malignant hemopathy		17 (28.33)
Disease stadium		
localized		21 (35.0)
disseminated		39 (65.0)
Duration of the disease, years		
average (mean ± SD)		2.6 ±1.08
up to 1 month		12 (20.0)
1–2 months		15 (25.0)
3–6 months		18 (30.0)
more than 6 months		15 (25.0)
Treatment received		
chemotherapy		37 (61.67)
radiation		2 (3.33)
combined therapy		21 (35.0)
Family history of cancer		
no		52 (86.67)
yes		8 (13.33)

SD – standard deviation

A total of 120 questionnaires on child self-report were analyzed for HRQOL scores. Items on which less than 50% of respondents gave clear and definitive answer were not calculated for the statistics. Mean values for items from PedsQL Generic Core Scales are presented with SD for both groups in Table 2. In order to determine differences in HRQOL scores between the healthy schoolchildren group and the group of schoolchildren hospitalized for cancer treatment the *t*-test was conducted.

Comparisons with the reference healthy group show that a entire patient sample has a lower health-related quality of life values in several domains. Significant differences

Table 2

Scale descriptives for PedsQL Generic Core Scales Child Self-Report and comparisons with Healthy Children Scores

Scale	Healthy mean ± SD	Patients mean ± SD
Physical health		
It is hard for me to walk more than one block	81.25* ± 22.84	52.5* ± 35.86
It is hard for me to run	79.17* ± 25.7	39.58* ± 32.96
It is hard for me to do sports activity or exercise	80.83* ± 23.64	42.08* ± 34.29
It is hard for me to lift something heavy	68.75* ± 28.97	44.17* ± 31.68
It is hard for me to take a bath or shower by myself	85.42* ± 26.56	69.58* ± 32.58
It is hard for me to do chores around the house	70.83* ± 28.06	53.75* ± 32.16
I hurt or ache	81.67* ± 23.41	72.08* ± 25.25
I have low energy	78.75* ± 25.97	67.5* ± 24.05
Emotional functioning		
I feel afraid or scared	68.33 ± 19.46	63.75 ± 25.39
I feel sad or blue	65 ± 22.64	58.75 ± 24.28
I feel angry	70.83* ± 22.15	59.17* ± 24.77
I have troubled sleeping	76.25 ± 23.66	72.92 ± 23.6
I worry about what will happen to me	66.25* ± 30.82	55* ± 28.3
Social functioning		
I have trouble getting along with other kids	90.83* ± 17.81	79.58* ± 25.83
Other kids do not want to be my friends	82.5 ± 21.74	80 ± 27.15
Other kids tease me	85* ± 23.11	52.5* ± 30.08
I cannot do things that other kids my age can do	89.58* ± 24.48	67.08* ± 33.03
It is hard to keep up when I play with other kids	78.75* ± 25.14	63.33* ± 29.28
School functioning		
It is hard to pay attention in class	76.25* ± 32.03	55.83* ± 31.68
I forget things	67.08* ± 30.07	43.33* ± 25.98
I have trouble keeping up with my schoolwork	70* ± 31.82	35.42* ± 36.04

***Result shows statistical significance – $p < 0.05$**

between healthy schoolchildren and patients hospitalized for treatment were observed for all 8 items of the Physical Health Scale. The children from the healthy group showed the expected mean values, while the patients demonstrated significantly lower mean values ($p < 0.05$). Schoolchildren on treatment for cancer demonstrated lower scores on all the items of the Emotional Functioning Scale, although a statistically significant difference was detected only for 2 items. Healthy schoolchildren revealed expected median values in all the items of the Social Functioning Scale, while the patients showed lower scores in all the items and a statistically significant difference in 4 out of 5 items. Lower mean values for the patients were also observed in the School Functioning Scale for 3 items, showing a significant difference.

Discussion

The PedsQL™ 4.0 Generic Core Scales designed for application in both healthy and patient populations [3, 15, 16] is one of the most used instruments of HRQOL measurement. The reliability and validity of the PedsQL™ 4.0 Scale was previously demonstrated, confirming that the Scale may be utilized as an outcome measure in clinical trials, research and clinical practice [5]. Recently, some psychometrics properties for the Serbian child self-report version of PedsQL were reported. Shortly, authors [14] demonstrated that the Serbian version of PedSQL 4.0 has sufficient basic measurement characteristics, adequate overall internal consistency reliability sufficient for the group evaluations and good convergent validity against psychological construct. Although, authors reported that alpha value did not exceed 0.70 for the School (0.65) and Emotional Functioning (0.69) this level is appropriate for comparing the groups [14].

We assessed HRQOL scores of schoolchildren diagnosed with malignant disease and hospitalized for treatment, and compared them to healthy schoolchildren population. In general, we detected a negative impact of malignancies on HRQOL parameters tested with PedsQL scales. We found a statistically significant difference in all the parameters of Physical Health Functioning were found: walking, running, exercising, lifting, bathing, cleaning, pain and low energy. The mean values for physical parameters are comparable to the results published in the similar previous studies [5, 11, 12, 14]. As expected, the obtained results demonstrate that malignancy and prolonged hospitalization of patients were accompanied by difficulties in activities of daily living and impoverished quality of life.

The mean values for five parameters of the Emotional Scales for healthy population were lower when compared to those obtained in some other reports [5, 17], but similar to those reported in a recent study on Serbian general population [14]. This might be due to the fact that alpha value for the Serbian version of PedsQL did not exceed 0.70 for the Emotional Functioning Scale (0.69). Still, this level should be appropriate for comparing the groups, but not for individual patient scale scores. Only two out of five parameters showed a significant difference in hospitalized schoolchildren when compared to healthy schoolchildren. A statistically significant difference was not detected in all items probably due to hospitalization of patients in the institution with the special child care unit, spe-

cial education service, supportive programs and constant presence of their parents. We believe that comparable Emotional Functioning Scale scores for patients and healthy schoolchildren might be the consequence of this practice.

Social functioning was strongly affected in schoolchildren hospitalized for malignant disease treatment. The mean values for all five parameters of the Social Functioning Scale are comparable to those obtained in similar studies [5, 7, 11, 12]. The mean values were decreased in patients compared to healthy schoolchildren population, and four out of five parameters showed statistically significant differences. Obviously, children with malignancies felt and reported difficulties in making friends and keeping pace with other children.

As expected, malignant disease and prolonged hospitalization due to the treatment strongly affected school functioning parameters like attention, memorizing the facts and schoolwork. These parameters also demonstrated a statistically significant difference when compared to the schoolchildren from the healthy group.

Conclusion

Our results revealed that the Serbian version of PedsQL™ 4.0 Generic Core Scales could be successfully used to evaluate physical, emotional, social and school functioning of children and adolescents. Schoolchildren hospitalized for prolonged tumor treatment have poorer HRQOL scores compared to general healthy population, as expected. On the other hand, the level of remaining physical, emotional and social parameters in schoolchildren hospitalized for prolonged treatment should provide solid foundation for their potential rehabilitation, education and inclusion. Further testing should be done on this population after they finish hospital treatment to determine achievement of process of rehabilitation. We believe that child self-reports of HRQOL parameters using PedsQL Scales regardless some imperfections might be important tool in future determination of programs for rehabilitation and education of hospitalized schoolchildren.

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Frequency of antimicrobial resistance in thermophilic *Campylobacter* strains from humans, poultry and pigs

Učestalost antimikrobne rezistencije termofilnih *Campylobacter* sojeva poreklom od ljudi, živine i svinja

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Key words:
campylobacter; anti-bacterial agents; drug resistance; humans; animals; poultry.

Ključne reči:
campylobacter; antibiotici; lekovi, rezistencija; ljudi; životinje; živina.

Introduction

Campylobacteriosis is classified as zooanthroponosis. It is an infection caused mainly by thermophilic campylobacters: *Campylobacter jejuni, Campylobacter coli, Campylobacter lari, Campylobacter upsaliensis. Campylobacter jejuni* and *Campylobacter coli* are causing the most important bacterial intestinal infections in modern era, with 400 000 000 patients in the world every year. A very important factor in intestinal campylobacteriosis development is a very low infective dosis of only 500 bacteria [1].

Humans get infected by this bacteria consumming insufficiently thermally processed meat, mostly poultry meat, pork and beef [2, 3], consumming unpasteurised milk and contaminated water [4], and beeing in contact with domestic pets [5]. Important role of poultry in human infections is demonstrated in Belgium during the Dioxin crisis in 1999, when, due to high levels of poison dioxin detected, domestic poultry and eggs were withdrawn from the market, resulting in lowering number of campylobacteriosis cases by 40% [2].

Thermophilic *Campylobacter* spp. mostly produce intestinal disorders, but could also produce extraintestinal disorders. Gut lesions in intestinal campylobacteriosis, similar to infections due to *Salmonella* and *Shigella* genera, are manifested as inflammatory infiltrates in lamina propria and abscesses in crypts [6]. The most frequent extraintestinal forms of disease are: meningitis, endocarditis, septic arthritis, osteomyelitis and neonatal sepsis. Several cases of myocarditis as a complication of *Campylobacter jejuni* infection were reported.

Secondary diseases reported by various authors [5, 7] as a consequence of thermophilic *Campylobacter* spp. primary infection, are Guillain-Barré syndrom (GBS) and Reiter's syndrom. Arthritis, GBS and Miller-Fisher's syndrom (a form of GBS) are possible complications in campylobacteriosis. Campylobacteriosis is generally a mild and self-limiting disorder. In patients with more severe and prolonged forms, an antibiotic treatment is recommended [8].

Although a significant percentage of animals is colonized, they rarely develop a disease, but they are reservoirs of infection for humans. Poultry aged 2–3 weeks are in 50%–90% of cases colonized by thermophilic *Campylobacter* spp. [3]. Swines are less than poultry colonized by the same bacteria. Tambur et al. [9] demonstrated that 80.88% of poultry and 77.27% of swines are contaminated by thermophilic *Campylobacter* spp.

After inoculation to newborn calves thermophilic *Campylobacter* spp. produce a mild and self-limiting enteritis and bacteriemia. *Campylobacter* spp. can produce dysentery in cattle and swine [7]. *Campylobacter jejuni* produces abortions in sheeps, acute enteritis in calves, dogs and cats, and hepatitis in poultry. Clinical symptoms of hepatitis in poultry are somnolence, weakness, diarrhoea and eggs-laying disorders [10].

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Treatment of campylobacteriosis and investigation of susceptibility to antibiotics

Drugs, generally used in human campylobacteriosis treatment are: erythromycin, quinolones, tetracyclin, ampicillin, chloramphenicol and gentamycin. Disk-diffusion test, E-test with strips and agar dilution test are used in investigations of susceptibility to antibiotics.

Different results were obtained by the three methods applied in investigation of susceptibility [2].

Antimicrobial susceptibility testing in *Campylobacter* spp. and methodology standardization

At present, several methods have been employed for *Campylobacter* susceptibility testing. Agar dilution is recommended by many authors [8, 11], but it is time consuming and not suitable for routine laboratory work. The E-test, a diffusion method with MIC determination, gives results faster than agar dilution, but its cost and need for standardization can be limitating [12]. Also, some authors recommended broth dilution method [13] (microbroth dilution by Trek, VetMic etc.) as suitable for routine use. Although disc diffusion is the simplest method, absence of available standards limits its application in clinical laboratories.

Disc diffusion and agar dilution are often compared in order to obtain diameter zone for application in routine work. With respect to these methods, Gaudreau and Gilbert [14] reported complete agreement for tetracycline and ciprofloxacin, with only minor differences for erythromycin but poor correlation coefficient for ampicillin. Similarly, Luangtongum et al. [15], revealed an excellent correlation between the agar dilution and the disk diffusion for aminoglycosidesand, quinolone/fluoroquinolones; a high-level correlation for erythromycin, clindamycin, and tetracycline, and a weak correlation for ampicillin. They suggested setting the MIC breakpoint for erythromycin-susceptible *Campylobacter* strains at ≤ 2 μg/mL and ≥ 8 μg/mL for resistant isolates and the zone diameter breakpoints of the disk diffusion method at ≥ 23 mm for susceptible isolates and ≤ 18 mm for resistant isolates. Also, they recommended the MIC breakpoints for clindamycin to be ≤ 2 μg/ml for susceptible isolates and ≥ 8 μg/mL for resistant strains and the zone diameter breakpoints ≥ 17 mm for susceptible isolates and ≤ 12 mm for resistant ones. Proposed values for the zone diameter breakpoints for tetracycline are ≥ 28 mm for susceptible strains and ≤ 8 mm for resistant strains. Authors also suggested that the disk diffusion method can be used as a reliable alternative method for susceptibility testing of thermophilic *Campylobacter* to several classes of antimicrobial agents, particularly to quinolone/fluoroquinolones and aminoglycosides.

Gaudreau et al. [16] recommended zone diameters of 6 mm and ≥ 20 mm around the erythromycin disk as resistant and susceptible breakpoints of *C. jejuni* isolates. Also, for ciprofloxacin susceptibility testing of *C. jejuni* isolates, zone diameters of ≤ 17 mm and ≥ 21 mm around the ciprofloxacin disk and the absence or the presence of an inhibition zone around the nalidixic acid disk are suggested as breakpoints for resistance and susceptibility, respectively.

With disk diffusion, the following zone diameters were proposed to be resistant and susceptible breakpoints, respectively, for susceptibility testing of *Campylobacter* coli: no inhibition zone and ≥ 15 mm for erythromycin, and ≤ 20 mm and ≥ 25 mm for ciprofloxacin, in the absence or presence of an inhibition zone around the nalidixic acid disk. For susceptibility testing of *C. coli* and *C. jejuni*, diameter zones ≤ 20 mm and ≥ 26 mm for tetracycline were recommended [17].

A recommendation, followed by these findings, is given that disk diffusion could be used to detect *C. jejuni* and *C. coli* isolates with reduced susceptibilities to ciprofloxacin and erythromycin in clinical laboratories [18].

Up to date, The Clinical and Laboratory Standards Institute (CLSI), has established minimal inhibitory concentration (MIC) breakpoints for agar dilution for erythromycin, ciprofloxacin, tetracycline and doxycycline. In addition, for disc diffusion, zone diameter is given only for erythromycin and ciprofloxacin [19]. EUCAST (the European Committee on Antimicrobial Susceptibility Testing) is still working on standards and epidemiological cut off is proposed for *C. jejuni* and *C. coli* for erythromycin, ciprofloxacin, tetracycline, streptomycin, gentamicin, chloramphenicol, and nalidixic acid [20].

Molecular techniques, also, can be applied for resistance determination as the Mismatch Amplification Mutation Assay (MAMA-PCR) [21], and the Lightcycler mutation assay [22] for the detection of ciprofloxacin-resistant *C. jejuni* and *C. coli* isolates. However, these and similar techniques can be applied only if prior knowledge about genetic basis for resistance exist. Usually, they cannot be refered to a routine resistance detection, and may not detect resistance if a new resistance mechanism emerge [22]. Some authors consider that combination of phenotypic and genotypic methods in resistance detection should be more convenient [23].

Mechanisms of erythromycin resistance in campylobacters

Erythromycin and other macrolide antibiotics bind to the subunit 50S of bacterial ribosome and restrict elongation of polypeptide chain [24]. Sites for macrolide action are parts of subunits 23S rRNA, and ribosomal proteins L4 and L22. Proteins L4 and L22 form parts of exit channel for polypeptide in bacterial ribosome 70S and they are described in several bacterial species [25]. Eyithromycin resistance can be mediated by enzymatic inactivation, can evolve through target modification by mutation or methylation, and by active efluxe [26]. In *Campylobacter*, resistance to macrolides confer to gene mutation with change of target site for drug binding to bacterial ribosome [27]. Other mechanism that confer resistance is active effluxe [28]. Resistance occurs as synergy between gene modification and efflux pumpe CmeABC activity [29]. Two types of resistance to macrolides are described: resistance to high levels of drug concentration (high level resistance - HLR) [25] and resistance to lower drug concentration (low level resistance - LLR) [28]. In HLR, MICs for erythro-

mycin are higher than128 mg/L, and in LLR, MICs are in range from 8–16 mg/L [25, 30]. In *C. jejuni* and *C. coli* strains, HLR is a consequence of mutation in 23S rRNA V domen in target gene at the positions 2074 and 2075. LLR can be a result of effluxe pumpe activity [31]. Also, it is recognized that modifications of L4 and L22 contribute to low level Ery resistance in *C. jejuni* [32].

Mechanisms of fluoroquinolones resistance in campylobacters

Fluoroquinolones inhibit the activity of DNA gyrase due to mutations in the DNA gyrase and DNA topoisomerase IV genes in most bacterial species [8]. Enzyme DNA gyrase is composed of two pairs of subunits, GyrA and GyrB, while topoizomerase IV consists of ParC and ParE [33]. Resistance to fluoroquinolones is a result of aminoacid changes in topoisomerase as well in gyrase. In *Campylobacter* strains, resistance to fluoroquinolones is a consequence of mutation in gene gyrA which encodes GyrA subunit of DNA gyrase [8]. Up to date, no mutations in DNA gyrase B have been associated with FQ resistance in *Campylobacter* [34]. The most frequently observed mutation in fluoroquinolones resistant isolates of *Campylobacter* is the point mutation Thr-86-Ile in gyrA gene [35] which leads to the T86I substitution in the gyrase and confers HLR to fluoroquinolones [33]. Other reported mutations of gyrA in C. *jejuni* include Thr-86-Ala (HLR to nalidixic acid and LLR to ciprofloxacin), Ala-70-Thr, Thr-86-Lys, Asp-90-Asn, and Pro-104-Ser [35, 36]. Double point mutations of gyrA have also been reported [35].

In *C. jejuni* and *C. coli*, a unique modification in the GyrA subunit is sufficient to confer a fluoroquinolone-resistant phenotype. Also, decrease in permeability of outer membrane and activity of effluxe system confer the fluoroquinolones resistance [37]. In *Campylobacter jejuni/coli* strains, apart of the mutations in GyrA, the multidrug efflux pump, CmeABC, also contributes to fluoroquinolones resistance by reducing the accumulation of the agents in *Campylobacter* cells [38]. Thus, CmeABC functions synergistically with the gyrA mutations in mediating fluoroquinolones resistance [39].

To understand the roles of multidrug efflux transporters in the pathobiology of *C. jejuni*, Jean et al. [40] characterized the function of an MFS transporter (Cj1375) designated CmeG. The results indicated that CmeG functions as a multidrug efflux transporter contributing to antibiotic resistance especially to fluoroquinolones and oxidative defense in *Campylobacter*.

Mechanisms of tetracyclines resistance in campylobacters

Tetracyclines, (e.g. tetracycline, chlortetracycline, and minocycline) bind to the ribosome and inhibit accommodation of the aminoacyl-tRNA (aa-tRNA) into the ribosomal A site and, therefore, prevent the elongation phase of protein synthesis [41]. Tetracycline resistance can be mediated by different mechanisms: efflux, the enzymatic degradation of drug, protection of the ribosomal binding site and mutations in 16S rDNA [42]. In *C. coli* and *C. jejuni*, genes for tetracycline resistance are located on self-transmissible plasmids. They have been identified as a ribosomal protection gene and designated *tet*(O) [43]. These genes are widely present in *Campylobacter* isolates recovered from various animal species [23]. They encode ribosomal protection proteins (RPPs) [41]. *Tet(*O) confers resistance by binding to the ribosome inducing a conformational change with subsequent release of the bound tetracycline molecule and its displacing from its primary binding site, such that the aa-tRNA can bind to the ribosomal A site and protein synthesis can continue [44].

The presence of *tet*(O) in different Gram-positive bacteria [45] suggest the origin of the resistance genes and their sharing between species. In *C. jejuni, tet*(O) was first cloned from a transferable plasmid pUA466 [46]. Sequencing of two tetracycline-resistance plasmids, one from *C. jejuni* strain 81–176 [47], and other from *C. coli* strain CC31, revealed a high level of sequence identity and genomic organization despite their temporal and spatial distance [48].

Although, in most strains, the *tet*(O) gene is plasmid-encoded, it can be located on the chromosome, which is reported for 33% of tetracycline-resistant *C. jejuni* isolates from Alberta, Canada [49] and 76% of tetracycline-resistant isolates from Australia Pratt, Korolik [50]. On *tet*(O)-carrying plasmids it is described the presence of an insertion element IS607 and therefore it is possible that mobile genetic elements other than transmissible plasmids may be involved in the acquisition and dissemination of tet(O) [51].

Tetracycline resistance in *C. jejuni* is also associated with the CmeABC multidrug efflux pump [52].

Resistance of thermophilic *Campylobacter* strains isolated from humans, poultry and swines to erythromycin

Alarming is the rise of resistance to erythromycin, the first choice drug for treatment of campylobacteriosis. Detection of the resistant strains started with the use of macrolides, generally thylosine in veterinary practice, mostly in swine farming [8, 13, 53].

An investigation [53] detected 12.5% *Campylobacter* strains isolated from humans resistant to erythromycin. These results are in accordance with the results of other authors [54–56]. Lower levels of resistance to erythromycin, ranging from 3.4% to 9.1% are reported by the authors in Brasil, Australia, USA and India [5, 57–59].

A tendency of rising frequency of resistant *Campylobacter* to erythromycin is evident. For example, in Canada there were 3% *Campylobacter jejuni/coli* resistant strains in 1998, but the percentage increased to 12% in 2001 [60].

A high percentage of *Campylobacter jejuni/coli* strains isolated from broilers was found [61] contrary to the fact that erythromycin has not been used in poultry farming. A low level of resistance to erythromycin in thermophilic *Campylobacter* strains was recorded in Great Britain (0–8%) [62], USA (3.1%) [63] and Czech Republic (6%) [64]. A high percentage of *Campylobacter coli* strains resistant to erythromycin isolated from broilers and eggs-laying hens (25% and 40%)

was found in Japan [65]. Authors in Italy reported a high level of resistance to erythromycin, up to 45%, in *Campylobacter coli* strains isolated from poultry faeces [55]. In Africa, high erythromycin resistance levels were observed in human clinical isolates, but low resistance rate to this antibiotic were noticed in *C. jejuni* and *C. coli* isolated from husbandry animals [66]. Reports from Asia describe low resistance of C. *jejuni* to macrolides, but higher resistance of *C. coli* strains [67]. Also, increased resistance to macrolides was observed among *C. coli* isolates from pigs in Australia [68].

Macrolides are widely used in swine farming and, as a consequence of intensive pressure of drugs included in this thylosine group, an increase of *Campylobacter* strains resistant to erythromycin originating from swines occured.

The investigation detected that even 40% of thermophilic *Campylobacter* spp. strains isolated from swines were resistant to erythromycin [61]. According to data from Spain, percentage of resistant *Campylobacter coli* was 81%, in Denmark percentage of resistant *Campylobacter jejuni* was 33% and of Campylobacter coli 74% [69].

Resistance of thermophilic *Campylobacter* strains isolated from humans, poultry and swines to quinolones

A rising frequency of thermophilic *Campylobacter* spp. originating from humans resistant to quinolones, drugs most frequently used in campylobacteriosis treatment [61, 70] is alarming. Emergence of the resistant strains coincided with the beginning of quinolones use in veterinary practice [8, 71].

Thermophilic *Campylobacter* spp. strains resistant to quinolones were produced diarrhea of mean duration 13.2 days, contrary to susceptible strains with mean duration of diarrhea of 10.3 days [72].

Investigation of resistance to ciprofloxacin of *Campylobacter* strains isolated from humans in Serbia, detected 50% resistance [73]. This results are in accordance to the results of others [5, 55, 58, 74–76]. In Chile the resistance of *Campylobacter jejuni/coli* to ciprofloxacin has not been recorded [77]. Fifty percent of thermophilic *Campylobacter* spp. originating from humans were characterized as resistant to ciprofloxacin in a controlled investigation of susceptibility to antibiotics [73]. A high level of resistance to ciprofloxacin (71.4%) was demonstrated in *Campylobacter jejuni/coli* isolated in India from humans, generally children in rural areas [58]. A high level of resistance to ciprofloxacin was registered in Spain, too. Resistance to this antibiotic was found in 75% *Campylobacter jejuni* and 70.7% *Campylobacter coli* strains [56].

A permanent trend of resistance increase to fluoroquinolones is spread worldwide. Enrofloxacin is licenced in Netherlands for use in veterinary medicine in 1987. Resistant *Campylobacter jejuni/coli* strains isolated from humans represented 8% in 1998, 11% in 1989 and 29% in 1997. A similar trend is registered in Austria, Denmark, Finland, France, Italy, Spain, Thailand, Great Britain and USA [8]. In Canada there were no resistance to ciprofloxacin in 1985/86. In the following period, 1995/97, 12.7% resistant *Campylobacter jejuni/coli* strains were isolated from humans [60]. A high level of thermophilic *Campylobacter* spp. resistant to ciprofloxacin has been registered (50% to 60%) [55, 62, 78]. Cardinale et al. (2002) [70], citing several other authors, reportspercentages of *Campylobacter jejuni/coli* resistance to ciprofloxacin in several countries: Germany 46%, Japan 46%, USA 23-100%, Kenya 7.7%, Belgium and Spain up to 100%, Taiwan and Thailand 56–84% and Senegal 34%. In Switzerland a very low level of thermophilic *Campylobacter* spp. isolated from poultry meat resistant to fluoroquinolones is registered: only 0.5%. Resistance to ciprofloxacin in thermophilic *Campylobacter* spp. isolated from poultry in Norway was also low (2.7%). The reason for this results could be found in the fact that fluoroquinolones were not approved for use in broilers in Norway [78].

Fluoroquinolones have not been applied in such extent in swine farming as in poultry farming, this being the reason that the percentage of *Campylobacter jejuni/coli* strains resistant to fluoroquinolones is lower in swines than in poultry. Results of an investigation [79] demonstrated 26.7% resistant *Campylobacter* strains isolated from swines. Similar results were reported in Italy and Switzerland [55, 80]. A low level of resistance to fluoroquinolones, only 0.5%, was registered in *Campylobacter jejuni/coli* strains isolated from swines in USA [81]. Hart et al. [82] did not register a resistance to ciprofloxacin in *Campylobacter jejuni/coli* isolated from swines in Australia, due to the fact that quinolones are not approved for use in veterinary medicine.

Resistance of thermophilic *Campylobacter* strains isolated from humans, poultry and swines to tetracyclines

It was noted that tetracyclines were used in human medicine without appropriate control [83]. According to numerous authors in the world 30%–40% thermophilic *Campylobacter* strains isolated from humans are resistant to tetracycline [73, 74]. High percentage of resistant thermophilic *Campylobacter* strains isolated from humans, ranging from 43% to 85%, are reported in Spain, USA and Finland [54, 58, 75, 84]. A lower level of resistance to tetracycline, ranging from 12% to 16%, was reported in Australia, India and Turkey [57, 59, 76]. Very low level of thermophilic *Campylobacter* spp. isolated from human, resistant to tetracyclines, only 1.8%, was registered in Chile [77]. The trend of resistance increase to tetracycline in many countries is annoying [2, 53]. Many authors report higher percentages of resistance to tetracycline of thermophilic *Campylobacter* spp. strains isolated from poultry [4, 56, 61, 65] but some reported lower percentages of resistance [83, 85–89]. It was noted that as far as 80% strains of thermophilic *Campylobacter* spp. originating from swines were resistant to tetracycline [69, 82, 90], but some authors registered lower percentages of resistance [80, 81]. Aarestrup and Wegener [85] in Denmark, found a low resistance level to tetracyclin in *Campylobacter jejuni/coli* strains isolated from swines (1%).

Investigation of sensitivity to antibiotics of thermophilic *Campylobacter* spp. collected from humans, applying disc-diffusion test, detected 47.1% strains resistant to two antibiotics, and 11.8% strains resistant to three antibiotics [91].

Hakanen et al. [92] detected 22% *Campylobacter jejuni* strains resistant to three or more antibiotics. Multiresistance to antibiotics of thermophilic *Campylobacter* spp. strains in India was 30.6%, most fequently to erythromycin, tetracycline and ciprofloxacin [59].

It is necessary to emphasize recorded multiresistance of thermophilic *Campylobacter* isolated from poultry and swines [54, 70, 81].

Conclusion

Consuming of food contaminated with thermophilic *Campylobacter* spp. results in transmission of strains resistance to antibiotics and resistency genes from animals to humans. Humans infected with strains resistant to antibiotics, get illness with more severe symptomatology and with prolonged course. High level of resistance to antibiotics of thermophilic *Campylobacter* spp. collected from humans and animals, even in high industrialized countries, is a consequence of irregular use and misuse of antibiotics, predominantly in veterinary medicine and husbandry, the fact demonstrated in many investigations. It should be emphasized that the level of resistance of 12.5% to erythromycin of *Campylobacter* strains collected from humans and poultry was detected, contrary to the fact that erythromycin was not being used in poultry farming. Resistance to ciprofloxacin of *Campylobacter* strains collected from humans and broilers was 50% or more. It was demonstrated that 30% strains originating from humans and 80% strains originating from swines are resistant to tetracycline. A trend of resistance increase to antibiotics of campylobacters collected from humans and animals is extensively evident.

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Lekarska greška kao razlog pravne odgovornosti lekara i zdravstvenih ustanova

Medical error as a basis for legal responsibility of physicians and health facilities

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Uvod

Osnovni zadatak savremene medicine je zaštita života i zdravlja, odnosno omogućavanje što kvalitetnijeg lečenja i produženja samog života, uvažavajući sva dostignuća u savremenoj medicini. Imajući u vidu broj stanovnika na svetu i njihove dnevne potrebe u delu zaštite zdravlja, jasno je da je rizik od nastanka lekarske greške tokom lečenja značajno povećan. „Samo društvo pretvorilo se u kliniku i svi građani postali su pacijenti“ [1]. Ne postoje tačni podaci o broju lekarskih grešaka, odnosno broju žrtava koje trpe značajnu štetu usled nestručnog lečenja ili pogrešaka nastalih u toku samog lečenja i rehabilitacije. Primera radi, iznosimo da su u osamdesetim godinama nemački stručnjaci iz Instituta „Robert Koh“ iz Nemačke objavili da se u Nemačkoj godišnje podnese 40 000 prigovora zbog lekarske greške, od čega više od 12 000 ostane nedokazano, kao i da su izračunali da je broj žrtava lekarskih grešaka tokom jedne godine daleko veći od broja saobraćajnih nezgoda [2]. Isto tako, prema istraživanju koje je sproveo Institut za medicinu u Sjedinjenim Američkim Državama (SAD) u 2000. godini, smatra se da za osnovu lekarske greške u SAD godišnje umire između 44 000 i 98 000 pacijenata [3]. Britanski izvori, takođe, upozoravaju da je u proteklih 30 godina dramatično (za 1 200%) povećan broj odštetnih zahteva pacijenata na osnovu lekarske greške [4].

Podaci ukazuju na mnogobrojnost prigovora koje pacijenti upućuju, ali to ne mora da znači da su ti prigovori pretežnim delom opravdani i da je zaista postojala lekarska greška. Pošto medicinsku pomoć po pravilu traže bolesni ljudi, postoje znatne teškoće da se ustanovi jasna uzročna veza između nastalog pogoršanja zdravlja pacijenta i preduzetog načina lečenja, pa se ponekad nekritički svaki nepovoljan ishod lečenja bez razloga pripisuje lekarskoj grešci. Bez obzira na napredak savremene medicine, koji je omogućio produženje ljudskog života i lakše izlečenje od mnogih povreda i bolesti, nerealna su i preterana očekivanja pacijenata da će preduzeto lečenje u svakom slučaju imati povoljan ishod po njihovo zdravlje, pa se zbog toga, razočarani ishodom lečenja, često pritužuju na „lekarsku grešku“. I treće, što će ovde biti samo pomenuto, a trebalo bi da bude tema posebnog rada, česti su slučajevi doprinosa pacijenta pogoršanju vlastitog zdravstvenog stanja proizvoljnim korišćenjem medikamenata, izbegavanjem terapije ili nepoštovanjem lekarskih zabrana, čak i kada su uključeni u proces lečenja. Iako realni obim lekarskih grešaka nije poznat, a razlozi velikog porasta nisu dovoljno jasni, očigledno je da se na ovom području sukobljava medicinska praksa sa pravom, što može dovesti do neujednačenih kriterijuma po kojima se u sudskoj praksi procenjuje postojanje odgovornosti lekara za slučaj lekarske, greške.

Ključni pojam na kome je bazirana procena stručne medicinske javnosti, ali i pravnih stručnjaka, o propustima u radu zdravstvenih radnika pri prevenciji, lečenju ili rehabilitaciji pacijenata koji bi mogli da budu razlog njihove pravne odgovornosti jeste tzv. lekarska greška, koja se tradicionalno i uporno vezuje za postupke lekara, iako ima mnogo šire značenje, jer se može odnositi na postupke bilo kog drugog zdravstvenog radnika. Jednim delom to je posledica činjenice da javnost u principu lekare smatra odgovornim za pacijente (pa tako i za slučaj pogoršanja njihovog zdravstvenog stanja), a drugim, zato što se u praksi sudski postupci najčešće pokreću protiv lekara.

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Lekarsku grešku uređuje i Zakon o zdravstvenoj zaštiti Republike Srbije [5]. U članu 40 je propisano pravo pacijenta na naknadu štete u slučaju da je stručnom greškom zdravstvenog radnika ili zdravstvenog saradnika uzrokovana šteta na telu pacijenta ili pogoršanje njegovog zdravstvenog stanja, dok se u drugim članovima propisuju dužnosti zdravstvenih radnika i zdravstvenih saradnika. Zakon, istovremeno, upućuje na opšta pravila obligacionog prava, po kojima se ostvaruje pravo na naknadu štete kako materijalne, tako i nematerijalne. Pravo na naknadu štete ne može se unapred isključiti ili ograničiti, što, čak i da nije naglašeno u članu 40. stav 2. Zakona o zdravstvenoj zaštiti, proizlazi iz odredaba obligacionog prava.

Pravna priroda odnosa između lekara i pacijenta

Savremeno društvo i savremeni pravni sistemi gotovo svih država u svetu u prvi plan stavljaju lična prava pojedinca. Značajno je pojačan stepen zaštite lica, na primer dece, koja nisu sama u stanju da vode brigu o svojim pravima i interesima. U tom smislu, izmenjen je tradicionalni odnos između lekara i pacijenta koji se zasnivao na bezuslovnom verovanju pacijenta u mogućnosti i sposobnosti lekara. Upravo je Hipokratovom zakletvom ozvaničen tradicionalni i paternalistički odnos između lekara i pacijenta. Savremeni pristup ovom problemu potpuno je drugačiji. Uvažavajući lična prava, pacijent ima pravo na zaštitu svog zdravlja i odgovornost za svoje zdravlje. Novouspostavljeni odnos između lekara i pacijenta ima sve odlike partnerskog odnosa u kome pacijent izražava volju i daje saglasnost na predlog lekara u pogledu potrebnog lečenja. U skladu sa takvim promenama i u članu 31 Zakona o zdravstvenoj zaštiti navodi se da pacijent ima pravo da slobodno odlučuje o svemu što se tiče njegovog života i zdravlja, osim u slučajevima kad to direktno ugrožava život i zdravlje drugih lica i da se bez njegovog pristanka ne sme, po pravilu, nad njim preduzeti nikakva medicinska mera. Ipak, taj u osnovi partnerski odnos u kome pacijent odlučuje da staranje o svom zdravlju prepusti lekaru, licu koje ima profesionalno znanje i koje râdi u ustanovi koja raspolaže potrebnim medicinskim sredstvima koja omogućavaju sprovođenje medicinskih postupaka i terapija kojima direktno utiču na ozdravljenje pacijenta, prelazi u mnogim segmentima u odnos u kome dominantnu ulogu ima lekar. Njegovo znanje, stručnost i obučenost u rukovanju medicinskim sredstvima, vode pacijenta kroz medicinske procedure i tretmane, a sve u cilju ozdravljenja pacijenta i povratka njegovog telesnog i psihičkog zdravlja [6].

Lekareve obaveze prema pacijentu u odnosu na njegovo telesno i mentalno zdravlje, kao i socijalno blagostanje, možemo posmatrati iz tri ugla: profesionalnog ili stručnog, moralnog i pravnog. Profesionalna ili stručna odgovornost procenjuje se prema standardima ponašanja. Za razliku od opštih pravila odgovornosti za prouzrokovanu štetu, gde se kao standard ponašanja uzima ponašanje razumnog i pažljivog čoveka, kod profesionalne odgovornosti zahteva se kao standard ponašanja, ponašanje razumnog i pažljivog stručnjaka. Moralne dužnosti lekara proizilaze iz njegove profesionalne obaveze da savesno i odgovorno obavlja dužnost radi koje je i stupio u odnos sa pacijentom. Pravne obaveze lekara zasnivaju se ugovorom i tiču se pružanja usluge lečenja (u skladu i propisima a prema procedurama u postupku lečenja koje određuju medicinska nauka, praksa i etičke norme). Upravo Zakon o zdravstvenoj zaštiti Srbije imperativnim odredbama u članu 169 stav 1 i 2 zahteva da svi zdravstveni radnici obavljaju zdravstvenu delatnost u skladu sa važećom doktrinom i u skladu sa kodeksom zdravstvene etike, s tim da, u suprotnom slučaju, zdravstveni radnici mogu biti pozvani na etičku, kaznenu i materijalnu odgovornost.

Lekarska greška kao uslov odgovornosti

Kao u svakom poslu, tako i u medicini postoji mogućnost pogreške koju možemo pripisati zdravstvenim radnicima, odnosno njihovom nemarnom ili namerno pogrešnom odnosu u pogledu lečenja nekog lica. „Pojam stručne lekarske greške nastao je veoma davno, ali se u literaturi pojavio prvi put sredinom devetnaestog veka“ [7], a prvi je na jasan način definisao nemački klasik medicine i političar Rudolf Virchow. Za njega je stručna greška lekara: „kršenje opšteprizнatih pravila veštine lečenja, usled odsustva potrebne pažnje i opreznosti“ [8]. Nakon ovako postavljene definicije otvorilo se dosta pitanja i nedoumica na koje sama definicija nije dala jasan i nedvosmislen odgovor. Jedno od najvažnijih pitanja bilo je da li pojam lekarske stručne greške obuhvata sve nepravilne medicinske radnje ili samo neke od njih. Ostalo je nejasno i šta je to greška (za koju bi zdravstveni radnici trebalo da su odgovorni), a šta lekarska omaška, koja se ne može isključiti u svakom slučaju, ali je lakše prirode i ne dovodi do odgovornosti.

Posebno, nezavisno od bilo kakvih omaški i grešaka lekara ili drugih zdravstvenih radnika i pored preduzetog lečenja u skladu sa pravilima struke, može doći do pogoršanja zdravstvenog stanja pacijenta ili smrti. Zbog toga, lekari i pravnici posebno insistiraju na razlici između stručnih lekarskih grešaka i tzv. nesrećnih slučajeva u medicini. Insistiranje na razlici utemeljeno je pre svega na tome što, za razliku od lekarskih grešaka, nesrećni slučajevi u medicini ne predstavljaju izraz nepropisnog lečenja i ne povlače odgovornost lekara. U savremenoj medicini i pravu, pojam lekarske greške označava postupanje lekara protivno pravilima vlastite struke (*contra legem artis*) [9]. Na isti način se ovaj pojam shvata i u pravnim pravilima u kojima se propisuje odgovornost lekara i zdravstvene ustanove u slučajevima pogrešnog lečenja.

O lekarskim greškama, propustima i nesavesnom radu zdravstvenih radnika zaposlenih u zdravstvenim ustanovama javnost najčešće saznaje preko medija. Smrt, kao najgora posledica, lekarske greške gotovo uvek inicira istragu o lečenju i posledicama koje su dovele do smrtnog ishoda kod nekog pacijenta. Lekarske greške koje su izazvale manje posledice uglavnom ostaju neotkrivene, tako što se opravdavaju dopunskim stručnim nalazima ili okolnošću da je to u datim uslovima bilo neizbežno rešenje, iako je preduzeto sve, pa i više od onog što je u datom trenutku bilo moguće. Nepoznavanje medicinskih procedura i načina lečenja uopšte, kod pacijenta stvara nerealnu predstavu da su njegov lekar i svi

zdravstveni radnici koji su učestvovali u njegovom lečenju učinili sve što je u konkretnom slučaju bilo moguće. Jedino ekstremne pogreške kao što su izrazita naruženost, fizička disfunkcija nekog dela tela posle operacije ili nakon lečenja, na primer, „otvaraju" oči pacijentima i svedoče o lekarskoj grešci u toku lečenja.

Same lekarske greške mogu se razvrstati prema različitim kriterijumima. Prema jednoj podeli možemo ih razvrstati na: dijagnostičke greške, taktičke greške, tehničke greške, greške u vođenju medicinske dokumetacije, greške u organizaciji, i greške u ponašanju medicinskog osoblja u zdravstvenim ustanovama [10].

Definicija lekarske greške sadržana je u članu 197, stav 4, Zakona o zdravstvenoj zaštiti Republike Srbije. Tako se „pod stručnom greškom u smislu ovog zakona, podrazumeva nesavesno lečenje, odnosno zanemarivanje profesionalnih dužnosti u pružanju zdravstvene zaštite, odnosno nepridržavanje ili nepoznavanje utvrđenih pravila i profesionalnih veština u pružanju zdravstvene zaštite, koje dovode do narušavanja, pogoršanja, povrede, gubitka ili oštećenja zdravlja ili delova tela pacijenta". Ta zakonska norma predstavlja osnov za procenu moguće etičke, ali i pravne odgovornosti lekara usled čije greške je došlo do narušenja zdravlja pacijenta ili do njegove smrti.

Osnov i vrste pravne odgovornosti lekara i ustanove

Još u rimskom pravu uslov za uspostavljanje odgovornosti lekara u slučaju smrti njegovog pacijenta bila je lekarska greška, jer se neumešnost lekara u lečenju izjednačavala se krivicom (*imperitia culpae adnumeratur*). Od tada do danas, u pravu se postupno uobličava odgovornost lekara za slučaj greške, proširuje na druge zdravstvene radnike i saradnike i na zdravstvenu ustanovu u kojoj rade.

Opšte pravilo o odgovornosti za štetu u srpskom zakonodavstvu sadržano je u Zakonu o obligacionim odnosima u članu 154 i odgovara davno postavljenom načelu u pravu da je onaj ko drugome prouzrokuje štetu dužan da je naknadi [11]. Pri tome, kada govori o osnovama odgovornosti, zakonodavac ne prihvata ideju o jedinstvenom pravnom osnovu odgovornosti, nego ideju o pluralitetu ili množini osnova [12–15]. Zato, postoji više osnova odgovornosti: krivica (kao osnov subjektivne odgovornosti štetnika koji postupa suprotno opštem pravilu i drugome nanosi štetu), prouzrokovanje štete (kao osnov tzv. objektivne ili odgovornosti bez krivice), pretpostavljena krivica (kao osnov odgovornosti poslodavca za štetu nastalu radnjama zaposlenog). Iz toga proizilazi mogućnost da se uspostavi obaveza naknade štete kako fizičkog, tako i pravnog lica, te za štetu može da odgovara ne samo onaj ko je neposredno svojim radnjama štetu pričinio (u našem primeru lekar ili drugi zdravstveni radnik), nego i pravno lice (zdravstvena ustanova) u kojoj je lekar ili zdravstveni radnik radio i u vezi sa radom pacijentu prouzrokovao štetu.

Odgovornost lekara za štetu pričinjenu pacijentu usled lekarske greške uvek se zasniva na krivici. Generalno gledano, lekar odgovara samo u slučaju kad je njegova medicinska intervencija bila pogrešna i kad je kriv zbog toga. Ovaj princip postojanja krivice važi za sve pripadnike tzv. slobodnih profesija: lekara, advokata, arhitekata, inžinjera i slično. „Radi osiguranja njihove slobode delanja i odlučivanja, svi oni moraju biti slobodni od odgovornosti ukoliko se pridržavaju pravila svoje struke i postupaju pažljivo" [16]. Ali, ako se sa nepažnjom odnose prema svojim profesionalnim obavezama, pa time drugome prouzrokuju štetu, biće obavezni da tu štetu nadoknade.

Kako se vidi, pretpostavka odgovornosti lekara za štetu je u svakom slučaju ustanovljena lekarska greška u konkretnom slučaju, a dovoljno je da je ona nastala nenamerno i iz nehata jer je lekar preduzeo neko pogrešno činjenje ili je propustio dužno činjenje. To znači da je lekar prilikom lečenja pacijenta učinio propuste (tako što je primenio pogrešna sredstva i način lečenja ili što nije primenio ona koje je bilo neophodno koristiti u konkretnom slučaju) i to zato što iz nepažnje nije poštovao pravila struke i medicinske etike. Da je pokazao dužnu pažnju, kakva se mogla očekivati u datim okolnostima od prosečno razumnog i pažljivog medicinskog stručnjaka njegovog profila, ne bi došlo do takvih propusta kojima je prouzrokovano narušenje zdravlja pacijenta. Pri tome „merilo po kome se prosuđuje krivica (nebrižljivost) lekara nije ono što je u domenu njegovih mogućnosti, nego ono što se moglo osnovano očekivati od razumnog i pažljivog lekara u datim okolnostima. Merilo je apstraktno i objektizovano, ono je postavljeno iznad konkretnog lekara, a ne u okviru njegovih mogućnosti" [17]. To merilo se procenjuje prema pravilima struke, u skladu sa protokolima lečenja, pri čemu za postojanje odgovornosti nisu od značaja neke druge okolnosti, na primer pitanje da li lekar ima ili nema dovoljno znanja ili radnog iskustva (na primer, da se nalazi na specijalizaciji) i slično. Za utvrđivanje lekarske greške koja se može dovesti u vezu sa nastalom štetom nužno je u sudskom postupku odrediti veštačenje veštaka medicinske struke koji treba sudiji koji nema znanje iz oblasti medicine i medicinske nauke da objasni kojih se pravila lekar morao pridržavati, da li postoji njegov propust koji se može smatrati stručnom lekarskom greškom i da li je taj propust uzrok nastale štete za pacijenta.

Pojedini pravni teoretičari, sa kojima autori dele mišljenje, smatraju da „unošenje pojma lekarske greške u sistem deliktne odgovornosti označava udvostručavanje subjektivnog elementa koji bi se jednom označavao kao krivica, a u nekim drugim slučajevima kao stručna greška lekara (lekarska greška)" [17]. Zato ustanovljena lekarska greška u konkretnom slučaju po pravilu dovodi istovremeno do etičkog prekora koji lekaru upućuje Lekarska komora i do uspostavljanja pravne odgovornosti za štetu (a ponekad i odgovornosti za krivično delo).

Određene najgrublje stručne greške, kako lekara, tako i drugih kategorija zdravstvenih radnika, kojima se prouzrokuje pogoršanje zdravstvenog stanja nekog lica mogu biti smatrane radnjom krivičnog dela iz člana 251 Krivičnog zakonika Republike Srbije (nesavesno pružanje lekarske pomoći). Radnju izvršenja pri pružanju lekarske pomoći predstavlja primena očigledno nepodobnog sredstva ili očigledno nepodobnog načina lečenja ili neprimenjivanje odgovarajuće higijenske mere ili uopšte neki drugi očigledno nesavesni postupak koji se može dovesti u uzročnu vezu sa posledicom

(pogoršanjem zdravstvenog stanja nekog lica). Grube stručne greške drugih zdravstvenih radnika koje su u članu 251 stav 2 propisane kao očigledno nesavesni postupci učinjeni pri pružanju medicinske pomoći, nege ili pri vršenju druge zdravstvene delatnosti, predviđene su kao druga radnja izvršenja ovog krivičnog dela. Za pogrešno lečenje lekar može da snosi krivičnu odgovornost samo u onim slučajevima kada njegovi postupci pri lečenju predstavljaju toliko veliko odstupanje od opšteprihvaćenih pravila savremene medicinske nauke i struke da se takva greška nikako, ni sa medicinskog stanovišta ne bi mogla tolerisati (a time se otvara i pitanje krivice lekara za krivično delo). Krajnje apstraktni kriterijumi sadržani su u članu 67 Zakona o zdravstvenoj zaštiti koji obavezuje zdravstvene radnike da u prevenciji, dijagnostikovanju, lečenju i rehabilitaciji primene samo naučno dokazaju, proverenu i bezbednu zdravstvenu tehnologiju, kao i da koriste dozvoljene metode i postupke tradicionalne medicine.

I kada su u pitanju krivična dela, lekar najčešće odgovora zbog svog nepažljivog, nemarnog odnosa prema posledici (pogoršano zdravlje pacijenta), kada kažemo da postupa iz nehata, ali se postojanje nehata u krivičnom pravu drugačije ceni u slučaju odgovornosti za štetu. Najpre se, na isti način kao u građanskom pravu, objektivno prema *lex artis* procenjuje da li je lekar u konkretnom slučaju poštovao standarde profesionalne dužne pažnje ili ne. „Prilikom ocene krivice ne vodi se računa o ličnim sposobnostima i znanju tuženog lekara, nego se sudija uvek pita kako bi se na mestu tuženog ponašao iskusan i savestan lekar odgovarajuće struke“ [18]. Zbog rizične prirode svog zanimanja, lekar mora pri lečenju da pokaže pojačanu pažnju koja se očekuje od stručnjaka, a to znači da se od njega zahteva da savesno obavlja lečenje uz poštovanje pravila svoje struke. U slučaju da se ustanovi da je postojalo kršenje tih standarda, ceni se dalje da li je lekar prema svojim subjektivnim svojstvima mogao da bude pažljiviji, u čemu odlučujući značaj mogu imati njegovo životno i stručno iskustvo, zdravstveno i psihičko stanje u vreme preduzimanja radnje izvršenja krivičnog dela i slične okolnosti, jer, pravo ne može ni od koga da zahteva više od njegovih individualnih sposobnosti. Prema tome, konačno „utvrđivanje krivice vrši se prema subjektivnom merilu za pažnju. Krivim se smatra samo onaj lekar koji je prema svojim ličnim sposobnostima i individualnom znanju bio u stanju da se ponaša onako kako se od njega i očekuje, tj. kao prosečno sposoban i savestan lekar određene struke. Nije, dakle, merodavna objektivno potrebna nego subjektivno moguća pažnja“ [19].

Za razliku od lekara ili drugog zdravstvenog radnika koji po osnovu krivice odgovaraju za vlastite propuste kojima su drugom pričinili štetu (ili krivično delo), zdravstvena ustanova, kao pravno lice, može odgovarati za nepravilan rad svog zaposlenog koju on prouzrokuje trećem, što uključuje i odgovornost za nepravilan rad lekara i drugih zdravstvenih radnika. Odgovornost pravnog lica za štetu koju njegov zaposleni pričini trećem licu u radu ili u vezi sa radom temelji se na odredbama članova 170 do 172 ZOO, od kojih se u članu 172 propisuje odgovornost države za štetu koju učini njen organ, pa bi ta odredba došla u obzir kada bi se postavilo pitanje odgovornosti Vojnomedicinske akademije, koja delatnost vrši u okviru ministarstva nadležnog za poslove odbrane. Kako se vidi, odgovornost zdravstvene ustanove je uvek subjektivne, a ne objektivne prirode i vezuje se za pretpostavljenu krivicu. Pretpostavka krivice je oboriva, jer odgovornost zdravstvene ustanove može da postoji „samo ako se dokaže da je šteta pacijentu pričinjena zato što lekar i zdravstveno osoblje medicinske ustanove nisu postupali onako kako je trebalo (u skladu sa pravilima medicinske nauke i sa odgovarajućom pažnjom)“ (iz obrazloženja presude Vrhovnog suda Srbije, Rev. 1659/84). Zato, „kad je utvrđeno da je hirurška intervencija bila nužna i izvedena po svim pravilima medicinske nauke, onda zdravstvena organizacija u kojoj je vršena hirurška intervencija nije u obavezi da oštećenom isplati naknadu štete, koju je ovaj pretrpeo kao posledicu hirurške intervencije“ (presuda Vrhovnog suda Srbije, Rev. 1832/85).

Međutim, može se dogoditi da stručne lekarske greške pri lečenju nije bilo, ali da pacijent ipak trpi štetu, a kada bi zdravstvena ustanova mogla biti odgovorna za štetu. Taj uslov je ispunjen u slučaju da se nastanak štetnih posledica može uzročno povezati sa nekim drugim propustom zaposlenih u ustanovi, a ne sa greškom lekara. Jer, „zdravstvena ustanova, koja vrši medicinsku intervenciju, može odgovarati samo za one posledice intervencije koje nastanu usled nestručnog, nepažljivog i nepropisnog rada njenih radnika, dakle, za posledice koje se ne mogu pripisati u krivicu lekarima i drugom medicinskom osoblju zbog postupanja koje nije bilo u skladu sa pravilima medicinske struke” (presuda Vrhovnog suda Srbije, Rev. 2066/80). Upravo u ovakve primere spada slučaj u kome se Vrhovni sud Srbije izjašnjavao u dve presude (Rev. 2714/92 od 8.4.1993. i Rev. 1821/05 od 19.05.2005. godine) i koji je detaljno analiziran u radu autora Mujović-Zornić i Petrović [20].

Propust zdravstvene ustanove u navedenom slučaju bio je vezan za činjenicu da tužilji nisu pre operacije predočene potpune informacije o tome kakve sve mogu biti posledice eventualnih komplikacija i neuspešnog ishoda zahvata, što je moglo odlučujuće uticati na njeno opredeljenje da se podvrgne operaciji. Nižestepeni sudovi su u svojim presudama polazili od stava da je pacijentkinja, pristajući na operaciju koja joj je sugerisana iz estetskih razloga, „prećutno“ pristala da snosi rizik od nastupanja retke komplikacije, do koje je u njenom slučaju i došlo. Međutim, Vrhovni sud Srbije je u obrazloženju prve svoje presude kojom je ukinuo nižestepene presude istakao da „pored ostalih obaveza zdravstvenih radnika, koje im nalažu pravila medicinske nauke, stoji i dužnost obaveštavanja pacijenta radi njegove lične sigurnosti i samoodređenja u odnosu na sopstveno telo. U zavisnosti od ovog obaveštenja je i pristanak pacijenta na određenu intervenciju, koji je punovažan samo ako pacijent zna suštinu i značaj onoga sa čime se saglašava. Obaveštavanje pacijenta ne podrazumeva iznošenje tehničkih detalja, ali u svakom slučaju pacijent bi trebalo stekne osnovna saznanja o onome šta će se sa njim događati ako na intervenciju ne pristane, kao i da mu bude razjašnjena delotvornost predloženog zahteva. To, pre svega, podrazumeva ukazivanje na mogući neuspeh zahvata i kad se on obavi kako treba, što znači da oba-

veštenje mora da obuhvati sve činjenice koje su bitne za pacijentovu odluku" (iz obrazloženja presude Vrhovnog suda Srbije, Rev. 2714/92).

Drugi, interesantan primer se tiče odgovornosti zdravstvene ustanove zbog štete koja je pacijentu naneta povredom čuvanja profesionalne tajne, a to je dužnost koja je zdravstvenim radnicima imperativno propisana u članu 37 Zakona o zdravstvenoj zaštiti. Od tužilje koja je bila pred kraj trudnoće uzeta je krv radi kontrole i praćenja trudnoće. Nakon porođaja, u tuženi Zdravstveni centar je stigao laboratorijski nalaz u kome je navedeno da je u njenoj krvi utvrđeno prisustvo virusa HIV uz uputstvo da se ponovo izvrši analiza krvi porodilje i njenog novorođenog deteta, koja je opet dala pozitivni rezultat, i tek je naknadnom analizom koju je vršio Klinički centar Srbije utvrđeno da u krvi porodilje i deteta nije bilo virusa HIV. U međuvremenu, lekar tuženog Zdravstvenog centra obavestio je porodilju i njenog muža o pozitivnim rezultatima analize krvi, a pored medicinskog osoblja koje je neposredno radilo na ovom slučaju, o laboratorijskim nalazima obavešteni su i drugi lekari i sestre u dečjem dispanzeru tuženog zdravstvenog centra, radi preduzimanja mere prevencije. „Istovremeno vest o prisustvu virusa HIV u krvi tužilje ubrzo se proširila po celom gradu u kome tužioci žive. Tužioce su počeli da izbegavaju meštani, prijatelji i rodbina. Deca su izbegavala da se igraju sa njihovom decom sada maloletnim tužiocima, nazivajući ih sidašima i bežali su od njih. Tužilac, suprug tužilje imao je problema i na radnom mestu jer niko od radnika nije hteo da radi sa njim, da se sa njim hrani i pije vodu, pa je poslodavac gde je tužilac bio zaposlen morao da ga raspoređuje na poslove na kojima je mogao da radi sam. Do prijema konačnog laboratorijskog nalaza o stvarnom zdravstvenom stanju, tužioci, kao bračni par, pretrpeli su duševne bolove jakog intenziteta zbog saznanja da boluju od teške i neizlečive bolesti i zbog odbacivanja od uže i šire socijalne sredine i promena u svakodnevnom porodičnom životu, a duševne patnje su pretrpeli i maloletni tužioci, odnosno njihova deca, zbog toga što su bila izvrgnuta porugama i izbegavanju od strane druge dece. Kod ovako utvrđenog činjeničnog stanja, nižestepeni sudovi primenili pravilno su materijalno pravo kada su obavezali tuženi zdravstveni centar da im nadoknadi nematerijalnu štetu u vidu pretrpljenih duševnih bolova zbog povrede ugleda i prava ličnosti", navodi se u obrazloženju presude Vrhovnog suda Srbije (Rev. 392/03 od 15. maja 2003. godine).

Zdravstvena ustanova može biti odgovorna da naknadi štetu pacijentu i po članu 177 stav 2 ZOO, kada je dovoljno dokazati da je pacijent pretrpeo štetu od opasne stvari u zdravstvenoj ustanovi u kojoj se nalazio na lečenju. Recimo, kada se tužilja nalazila u banjskom lečilištu u kome je imala svakodnevnu terapiju kupanjem u bazenu, pa se okliznula na klizavim pločicama i teško telesno povredila (presuda Okružnog suda u Čačku, Gž. 818/2009 od 3. 6. 2009. godine). U ovom slučaju odgovornost ustanove je objektivna (za sve štete koje budu pričinjene opasnom stvari kakvom se smatra i bazen) i ustanova će biti obavezna da isplati naknadu štete, osim ako se ne dokaže da je do štete došlo isključivo radnjom tužilje.

Pošto zdravstvene ustanove spadaju u organizacije kojima su poverena javna ovlašćenja na njih se ne primenjuje zakonodavstvo o odgovornosti pravnih lica za krivična dela, koje je u Republici Srbiji uvedeno 2008. godine.

„Lekarska greška" u uporednom pravu

Kao u zakonodavstvu Republike Srbije, tako se i u uporednom pravu o tzv. lekarskoj grešci, kao uzroku štete u medicinskom pravu posebno vodi računa. U evropskom pravu se tradicionalno razlikuju dva pravna sistema: kontintentalni i anglosaksonski (ili *common law* sistem). Zakonodavstvo Republike Srbije spada u kontinentalni tip i pokazuje izrazite sličnosti sa zakonodavstvima germanske podvarijante i zbog toga će u ovom radu biti istaknuta neka uporedna rešenja iz tih zemalja, a dopunski su navedeni primeri iz engleskog i ruskog prava i iz zakonodavstava osamostaljenih država iz sastava nekadašnje Socijalističke Federativne Republike Jugoslavije (SFRJ).

Švajcarsko pravo, povodom lekarskih grešaka daje jedinstvenu definiciju kojom se pod lekarskom greškom smatra „kršenje opštepriznatih pravila medicinske nauke i prakse usled nedostatka potrebne pažnje i opreznosti" [21]. Jasno je iz definicije da sam pojam lekarske greške podrazumeva krivicu lekara. Od 1987. godine švajcarski Savezni sud zauzeo je stav da ograničenje lekareve odgovornosti na grubu grešku predstavlja privilegiju lekara koja nema oslonac u zakonu te zbog tog stava lekar treba da odgovara za svako nemarno postupanje [22]. Ovo rešenje je vrlo slično onome koje se primenjuje u srpskom pravu.

U nemačkom pravu umesto termina „stručna lekarska greška" uobičajeno se koristi izraz „greška u lečenju ili tretmanu." Pojam podrazumeva ogrešenje o standard medicinske struke odnosno „svaku lekarevu meru koja je prema aktuelnom stanju medicinske nauke nepodesna" [23]. Radnja preduzeta prema pacijentu može podrazumevati činjenje, nečinjenje, preduzimanja identifikovanog ili neidentifikovanog postupka u lečenju i druge greške do kojih dođe prilikom uzimanja anamneze, postavljanja dijagnoze, preduzimanja mera profilakse, terapija ili naknadnog staranja o pacijentu [24].

Ruska pravna doktrina deli lekarske greške na greške koje su nastale savesnim ili nesavesnim radom lekara. Sam pojam lekarske greške još daleke 1941. godine dao je ruski lekar i akademik Davidovskij (И. В. Давыдовский). Prema njemu, lekarska greška predstavlja svesnu zabludu lekara koja se temelji na nesavršenosti stanja medicinske nauke i njenih metoda ispitivanja ili su izazvane osobenostima toka bolesti određenog pacijenta ili se objašnjavaju nedostatkom znanja ili iskustva lekara [25, 26]. Ruski pravnici lekarsku grešku definišu kao pogrešno činjenje ili nečinjenje lekara pri izvršavanju svojih dužnosti koje se tiču dijagnostike, organizacije i sprovođenja terapijsko profilaktičih mera koje u zavisnosti od realnog ishoda, može biti ocenjeno ili kao prekršaj ili krivično delo [27, 28]. Pored lekarske greške, u ruskom pravu i medicini prihvaćen je i pojam nesrećnog slučaja u medicini koji, ukoliko se desio, za sobom ne povlači nikakvu odgovornost lekara. Bez obzira što se pojam „lekarske greš-

ke“ može razmatrati i sa medicinskog i sa pravnog stanovišta, preovladava razmišljanje da se ipak radi o pravnom pojmu [29].

U engleskom pravu još u XIX veku kroz pojedine sudske slučajeve (sistem pravnih precendenata) razvila se odgovornost lekara za štetu učinjenu pogrešnim lečenjem. To pravo je imalo uticaja i na sva područja nekadašnje Britanske imperije, što utiče na generalne sličnosti između engleskog, američkog i prava drugih država koje pripadaju tzv. Komonveltu. Kada se radi o šteti koja nastaje kao posledica pogrešnog lečenja, onda se u engleskom pravu tradicionalno pravi razlika u vezi sa tim da li pacijent dobija privatno uslugu ili je ostvaruje na osnovu zdravstvenog osiguranja. U prvom slučaju se smatra da postoji zaključeni ugovor između pacijenta i lekara koji je izvor obaveze lekara. Međutim, kada se pacijent leči u državnoj ustanovi, pa pretrpi štetu usled lekarske greške, onda se smatra da je šteta nastala vanugovorno, preduzimanjem nedopuštene radnje lekara (engl. *tort*), a odgovornost za štetu se procenjuje prema pravilima deliktnog odštetnog prava. Privatno angažovani lekar ima striktne obaveze poštovanja ugovora, jer je pacijent njega izabrao, dok se lekar saglasio da ga leči na određeni način. To pretpostavlja odnos *intuitu personae* i razvoj poverenja između lekara i pacijenta, pa pacijent sa pravom može očekivati da se lekar ozbiljnije i efikasnije pozabavi njegovim slučajem, nego kada bi lečio pacijenta koji koristi usluge državnog fonda zdravstvenog osiguranja [30]. No i tada važe argumenti lepo obrazloženi u sudskoj odluci u slučaju *Thake and Another v Maurice* da opšte iskustvo svedoči o tome da rezultati medicinskog tretmana nisu uvek predvidljivi i da je uspešnost nekog tretmana povezana sa specijalnim karakteristikama pacijenta. Zato, razuman čovek ne može od odgovornog lekara očekivati da mu dâ bilo kakve garancije u pogledu lečenja jer medicina, iako visokostručna profesija, ipak nije egzaktna nauka. Prema tome, razumni čovek najviše može da očekuje da hirurg pokaže odgovarajuću pažnju i sposobnosti koje se očekuju od lekara takve specijalnosti, ali ne i da mu se garantuje 100% uspeh hirurške intervencije[*].

Kada medicinsku uslugu pacijentu pruža lekar prema osnovu državnog zdravstvenog osiguranja, pretpostavlja se da između njih postoji kratkotrajni kontakt koji ne omogućava uspostavljanje poverenja. Lekar nedovoljno poznaje pacijenta i njegovo ukupno zdravstveno stanje da bi mogao u svakom slučaju da izabere najpovoljniji način lečenja. Tada se odgovornost lekara procenjuje po kriterijumu povrede dužne pažnje. Računajući s tim da su medicinske usluge delatnosti koja stvaraju neminovan rizik po život i zdravlje ljudi, sudovi imaju zadatak da opredele predvidivi rizik (onaj sa kojim se moglo računati u konkretnom slučaju), što se čini po tačno utvrđenim kriterijumima: stepen rizika, težina posledica do kojih dovede materijalizacija rizika, teškoće preduzimanja predostrožnih mera i njihovi troškovi i ponašanje oštećenog [4]. Da bi se uspostavila odgovornost za delikt učinjen nepažnjom pri lečenju potrebno je da se utvrde četiri elementa: standard dužne pažnje sa kojom je lekar morao da postupa u konkretnom slučaju, kršenje tog standarda pri lečenju, uzročna veza između postupka lekara i štetnih posledica koje pacijent doživi i činjenica nastanka štete po pacijenta. Sa druge strane, standardizovani protokoli lečenja pomažu da se objektivizira procena da li jeste ili nije u konkretnom slučaju prekršen standard dužne pažnje: ako je lekar postupao u svemu prema protokolu, smatra se da je standard poštovan i da se nastanak štetnih posledica ne može pripisati njemu u krivicu.

Zdravstvene ustanove sa svoje strane u obavezi su da obezbede minimalne uslove za adekvatno zdravstveno zbrinjavanje pacijenta, a povreda te obaveze procenjuje se prema standardima zdravstvene zaštite koje utvrđuje Nacionalna zdravstvena služba (za Englesku i Vels). U sudskoj praksi se pretežno radi o štetama zbog odbijanja pružanja medicinske pomoći i zakasnele medicinske intervencije (na primer, za slučaj porođaja ili srčanih smetnji).

Polazne premise odgovornosti lekara za štetu učinjenu pacijentu vanugovorno preduzimanjem pogrešne radnje procenjuju se u američkom pravu na isti način kao i u engleskom. Prvenstveno se mora ustanoviti dužnost profesionalne pažnje, koju lekar ne mora da pokaže onda kada se smatra da nema uspostavljenog odnosa između lekara i pacijenta. Ali, lekar mora da postupa sa razumnom dužnom pažnjom ne samo pri lečenju „svojih“ pacijenata, nego i onda kada, na primer, zamenjuje kolegu, pruža besplatne usluge klinici koja medicinski zbrinjava siromašne ili hitno ukazuje pomoć povređenima u nesrećama i kada nisu na dužnosti. Kako se odgovornost lekara za stručne greške uređuje zakonodavstvima država-članica, moguće je da u tim zakonodavstvima, da bi se motivisali lekari da volontiraju u ustanovama za lečenje siromašnih ili onda kada nisu na dužnosti ukazuju hitnu pomoć u slučaju nesreća, budu propisani uslovi po kojima se sužava odgovornost lekara u ovakvim slučajevima. Jedina situacija u kojoj lekar ne bi morao da pokaže dužnu pažnju je onda kada ne postupa na profesionalnoj dužnosti: kada drugome pruža prijateljski uslugu van zdravstvene ustanove.

Sa svoje strane, pacijent kao tužilac mora se pozivati na to da je lekar prekršio odgovarajući standard (razumne) stručne zdravstvene nege, koji se opet različito definiše u zakonodavstvima država-članica. Najčešće se povreda tog standarda utvrđuje veštačenjem, osim kod očiglednih grešaka (recimo kad je operacija izvedena na pogrešnom ekstremite-

[*]Ovo je jedan u nizu sudskih slučajeva dosuđenih naknada za neželjenu trudnoću i rođenje deteta zbog propusta lekara pri sterilizaciji pacijenata. Tužilac je bio podvrgnut vazektomiji jer nije želeo više dece, budući da ih je imao petoro. Nije bilo lekarske greške pri hirurškoj intervenciji, ali je lekar propustio tužioca da obavesti da postoji izvestan mali rizik da ponovo postane plodan, tako da je supruga tužioca ostala u drugom stanju, ali je ignorisala početne znake trudnoće, pa nije na vreme izvršila pobačaj. Dete je prihvaćeno kao željeno. Ipak, supruzi su tužili lekara za štetu nanetu kršenjem ugovornih obaveza (u odnosu na muža) i građanskim deliktom (kad se radi o ženi). Dosuđena je naknada štete, zbog propusta lekara da pacijenta obavesti o svim rizicima zahvata, dok je supruga tužioca u žalbenom postupku naknadno ostvarila naknadu štete za nelagodnosti i patnje u trudnoći [Thake v Maurice (1986) QB 644]. U drugom poznatom slučaju u kome je tužena zdravstvena ustanova propustom lekara pri sterilizaciji pacijenta omogućeno je rođenje deteta sa kongenitalnim nenormalnostima. Neželjena trudnoća je fikcijom podvedena pod telesnu povredu i dosuđena je naknada štete, a odvojeno je odmerena naknada za izdržavanje deteta [Emeh v Kensington and Chelsea and Westminster Area Health Authority (1985) QB 1012, (1985) 2 WLR 233, (1984) 3 All ER 1044].

tu). U pogledu utvrđivanja uzročnosti dozvoljeno je da pacijent dokaže da je povredom dužne pažnje lekar mogao izazvati štetne posledice kakve je on pretrpeo. Naknada za pretrpljenu štetu uobičajeno se obračunava u novcu u skladu sa visinom pretrpljene štete [31, 32].

U osamostaljenim republikama iz sastava nekadašnje SFRJ pojam lekarske greške i pravni mehanizmi otklanjanja njenih štetnih posledica i utvrđivanja pravne odgovornosti štetnika i zdravstvene organizacije se u suštini uređuju na način sličan kao u Srbiji. Tako se u članu 45 slovenačkog Zakona o zdravstvenoj delatnosti, članu 120, stav 3, hrvatskog Zakona o zdravstvenoj zaštiti i članu 137, stav 3, Zakona o zdravstvenoj zaštiti Federacije Bosne i Hercegovine, izričito ističe dužnost zdravstvenih radnika da pri pružanju zdravstvene usluge postupaju u skladu sa medicinskom doktrinom, pravilima struke i medicinske etike, dok se u okviru prava pacijenta ponekad pominje pravo na naknadu štete (na primer u zakonodavstvu Federacije Bosne i Hercegovine u članu 27), ali to nije neophodno jer se obezbeđuje po opštim propisima. U Zakonu o zdravstvenoj zaštiti Republike Srpske, poseban član 32 odnosi se na stručnu grešku zdravstvenog radnika ili zdravstvenog saradnika. U istom članu se garantuje pravo na naknadu štete pacijentu kome se usled te greške pogorša zdravstveno stanje. Regulisan je i postupak ispitivanja lekarske greške koji preduzima nadležna zdravstvena komora na zahtev direktora zdravstvene ustanove. U Zakonu o zdravstvenoj zaštiti Republike Crne Gore nije posebno istaknuto pravo pacijenta na naknadu štete zbog lekarske greške niti obaveza zdravstvenih radnika da postupaju u skladu sa medicinskom doktrinom, medicinskom deontologijom i etikom, nego se, slično kao što postoji i u drugim zakonima, u članu 26 predviđa obaveza zdravstvene ustanove da primenjuje proverene i dokazane medicinske metode i postupke u prevenciji, dijagnostikovanju, lečenju i rehabilitaciji obolelih. Nasuprot tome, u modernom makedonskom Zakonu o zdravstvenoj zaštiti iz 2012. na nivo načela je podignuta odredba kojom se osiguranicima garantuje kvalitetan i bezbedan tretman (čl. 11), a zatim se to načelo konkretizuje kroz odredbe člana 27, stav 1, o obavezi zdravstvenih radnika i saradnika da svoje delatnosti vrše saglasno stručnim medicinskim pravilima zasnovanim na savremenoj svetskoj medicinskoj praksi, koje propisuje nadležno ministarstvo za zdravlje. U svim državama nekadašnji jugoslovenski Zakon o obligacionim odnosima postao je osnova za nova obligaciona pravila, tako da opšti uslovi za naknadu štete ne odstupaju od rešenja prihvaćenih u srpskom pravu. Ono što se čini najvažnijom razlikom jeste to što pojedine republike na savremeniji način regulišu naknade štete koja nastane iz lekarske nepažnje, jer je Slovenija, kao članica Evropske unije već uvela obavezno osiguranje lekara od profesionalne odgovornosti (postupajući prema pravu Evropske unije iz 2002. godine), dok je u Hrvatskoj uvedeno dobrovoljno osiguranje od takve odgovornosti, koje će biti zamenjeno obaveznim [33]. U ostalim republikama se u tom smislu preduzimaju tek prve aktivnosti.

Zaključna razmatranja

Obaveza naknade štete koju pacijent pretrpi tokom lečenja u zakonodavstvu Srbije predviđena je zakonom koji reguliše zdravstvenu zaštitu, a ostvaruje se opštim pravnim režimom obligacionog prava. Naknada, koja se pruža u takvim slučajevima, treba da obezbedi uspostavljanje ranijeg imovinskog statusa oštećenog i, kada se radi o nematerijalnoj šteti, svojevrsnu moralnu kompenzaciju radi uspostavljanja poremećene psihičke ravnoteže pacijenta zbog štetnih posledica pogrešnog lečenja [34]. Razlog za pravno uobličavanje odgovornost lekara i zdravstvene ustanove je lekarska greška čije se postojanje utvrđuje na objektivan način, dakle, procenjivanjem postupaka i procedura koje bi primenio lekar prosečnog znanja i prosečne profesionalne sposobnosti. Procena stepena krivice lekara temelji se na težini greške i činjenici koliko je velik stvoreni rizik od pogoršanja zdravlja pacijenta.

Međutim, klasični mehanizmi sudske zaštite prava pacijenta, kroz uspostavljanje pravne odgovornosti lekara ili zdravstvenih ustanova za slučaj lekarske greške i propusta u lečenju u današnje vreme se pokazuju skupim, nedovoljnim i neefikasnim. Više odgovaraju „kulturi sramoćenja“ (tzv. *blame culture*) u kojoj je važnije uputiti etički prekor i utvrditi pravnu odgovornost lekara (i za nepažnju sa kojom dođe do lekarske greške), nego što se dopušta mogućnost da se iz te greške nešto nauči. Lekar svakako ne može izbeći socioetički prekor ako nepažljivo učini najgrublju lekarsku grešku, ali bi u mnogobrojnim lakšim slučajevima bilo dovoljno na osnovu osiguranja od profesionalne odgovornosti (po modelu *non-fault compensation*) omogućiti obeštećenje pacijenata za sve štete koje im budu pričinjene tokom lečenja, bez obzira na krivicu lekara.

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CASE REPORTS

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Orthodontic-surgical treatment of the skeletal class III malocclusion: a case report

Ortodontsko-hirurško lečenje malokluzije III skeletne klase

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Abstract

Background. Class III malocclusions are considered to be ones of the most difficult problems to treat. Their causes are multifactorial and include genetic and/or environmental factors. Class III malocclusions are generally classified into 2 categories: skeletal and dental. The diagnosis is important due to the different treatment approaches. Generally a dental class III can be treated with orthodontics alone, while a true skeletal class III requires a combination of orthodontics and surgery. **Case report.** We presented a female patient with skeletal Class III malocclusion. The treatment was complete with positive overbite and acceptable occlusion using a combination of fixed orthodontic appliance treatment as well as the surgical operation. The patient was happy with her new appearance and function. **Conclusion.** Class III discrepancy should be diagnosed and classified according to its etiology and treated with appropriate surgery, including, if necessary, not only mandibular, but also maxillary surgery, in order to achieve a normal facial appearance. In any case, as the field of orthodontics continues to develop technologically and philosophically, we can expect that advances in diagnosis and treatment planning are imminent and inevitable.

Key words:
malocclusion; orthodontics, corrective; oral surgical procedures; treatment outcome.

Apstrakt

Uvod. Malokluzije III klase smatraju se među najtežim za lečenje. Faktori koji dovode do njihovog formiranja su različiti, počev od naslednih do onih koji se javljaju tek posle rođenja. Ove malokluzije se inače dele na dve velike grupe: dentoalveolarne i skeletne. Zbog različitih pristupa samom lečenju kako dentoalveolarnih, tako i skeletnih oblika ove malokluzije, najvažnije je postaviti tačnu dijagnozu. Dentoalveolarni oblici III klase mogu se lečiti samo ortodontski, dok teži slučajevi skeletnih oblika moraju da kombinuju ortodontsko-hirurško lečenje. **Prikaz slučaja.** U ovom radu prikazana je bolesnica sa malolkuzijom III skeletne klase. Lečenje je završeno sa pozitivnim zadovoljavajućim preklopom i okluzijom ortodontskim prehirurškim lečenjem, kao i hirurškim zahvatom. Bolesnica je bila zadovoljna novim promenama kako intraoralnim, tako i ekstraoralnim, uočljivim na samom licu kao i postignutom funkcijom. **Zaključak.** Mimoilaženje vilica III klase neophodno je dijagnostikovati i svrstati prema poreklu i uzroku i lečiti primenom odgovarajuće hirurgije uključujući, prema potrebi, ne samo hirurgiju mandibule, već i maksile. U svakom slučaju, možemo očekivati stalno usavršavanje u postavljanju dijagonoze i lečenju s obzirom na činjenicu da se ortodoncija razvija i tehnološki i filozofski.

Ključne reči:
malokluzija; ortodoncija, korektivna; hirurgija, oralna, procedure; lečenje, ishod.

Introduction

A developing skeletal class III malocclusion is one of the most challenging problems confronting the practicing orthodontists [1–3]. Compared to class II and class I, a true class III malocclusion is rare. This type of malocclusion is a growth-related problem that often becomes severe if left untreated, and should be corrected as soon as its initial signs are recognized, such as edge to edge bite or cross bite [4]. Jaw growth is a slow and gradual process, and in some individuals, the upper and lower jaws may grow at different rates affecting chewing, speech, long-term oral health, and appearance [5].

Skeletal class III malocclusion is characterized by mandibular prognathism, maxillary deficiency or both and has a significant genetic component [1–5]. Clinically, these patients have a concave facial profile, with a retrusive nasomaxillary

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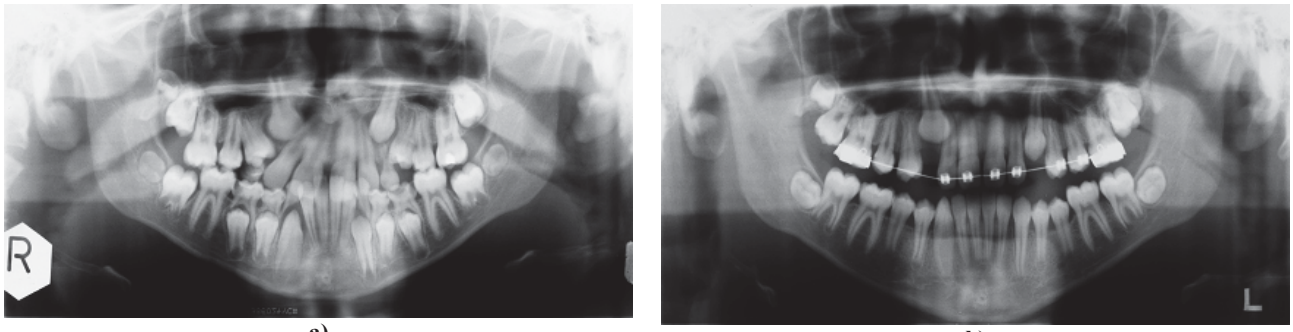
area and a prominent lower third of the face and often the lower lip is protruded relative to the upper lip. Usually the upper arch is narrower than the lower one, and the overjet and overbite can range from reduced to reverse [6]. Also, this profile is associated with functional and esthetic problems. Since the lower incisors are located in front of the upper incisors, they can erupt to unattractive lengths. This type of profile is also known as a "prognathic", or "strong chin" appearance [7, 8].

To obtain the best results in the treatment of patients with angle class III malocclusion, the etiology of malocclusion should first be clarified [9–14]. Cephalometric analysis is still the best way to approach the definition of phenotypes within the class III population. The goal of early orthodontic treatment is to correct the existing or developing skeletal, dentoalveolar and muscular imbalance and to improve the oral environment [9].

There are three main treatment options for skeletal class III malocclusion: growth modification, dentoalveolar compensation (orthodontic camouflage), and orthognathic surgery [10]. Growth modification should be commenced before the pubertal growth spurt. After this spurt, only the latter two options are possible. However, how should clinicians determine whether or not patients are suitable for surgery? Decision to reposition the mandible posteriorly or the maxilla anteriorly in the treatment of class III malocclusions depends upon multiple clinical, cephalometric, and biomedical considerations. In each case the decision must be made on the basis of frontal and profile treatment objectives, occlusion, and the needs of the patient. In many instances, depending upon the magnitude of the disharmony, the treatment plan will be based upon the clinical judgment and experience of the surgeon and orthodontist. Surgery for class III patients is both predictable and stable, in proportion to how much maxilla or mandible has been moved [15–20].

Treatment of the presented case was undertaken using a combination of a fixed orthodontic appliance treatment and a surgury.



a) b)

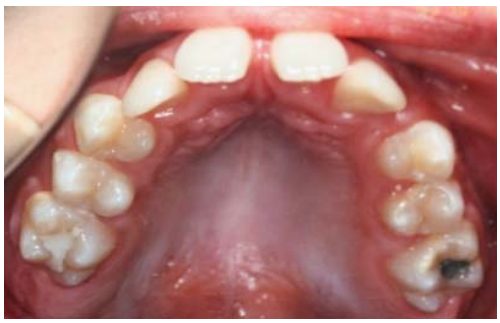
Fig. 1 – a) The panoramic radiography reveals the opening of the spaces for both maxillary canines and their eruption; b) Initial intraoral photoimage of the occlusal aspect of the maxillary and mandibular dental arch

Case report

At the beginning of the treatment a 12-year-old female had a long problem list: impacted upper right and left canines, class III mandibular prognathism and a skeletal anterior and posterior crossbite on the right and left side and her chief complaint was "teeth do not come together, jaw protruding, and trouble chewing“. A panoramic radiograph showed that all teeth were present with all the third molars. There were no supernumerary teeth. The crown-root ratios were normal with good alveolar bone levels, no bone pathology, and mandibular condyles, nasal floor and maxillary sinuses appeared normal. The patient's periodontal status was healthy, with no bleeding on probing and generalized gingival recession was found throughout the mouth, however, with thin periodontal tissues.

The treatment goals for the patient were: to eliminate the CR-CO discrepancy (centric occlusion – centric relation) and anterior crossbite; to establish class I canine relationships; to eliminate maxillary and mandibular arch length discrepancies; to align the arches; to align the midlines; to correct the right/left posterior crossbite and to finish with about 2 mm of overbite and 2 mm of overjet; to provide an aesthetic smile.

In view of the fact that this was a patient with class III malocclusion, the orthodontic treatment was planned in two presurgical and one postsurgical step: the first presurgical treatment was undertaken only in the maxilla (Figures 1a, b). The second one was performed two years after the first treatment had ended, but that time in both jaws. During the initial phase of fixed appliance treatment, the upper right and left canines needed to be extruded. Firstly, it was necessary to provide the spaces, which was achieved in three month’s time using pendulum appliance. Extrusion of the canines into a correct relationship with the adjacent teeth required an additional six months (Figures 2a–d). The second fixed appliance treatment, undertaken in both jaws, required 9 months. When the second phase of fixed appliance treatment was finished, all erupted teeth were bonded with brackets for the final presurgical preparation (Figures 3a, b). Both presurgical treatments had moved the teeth into a new position, so that they fitted together properly when the lower jaw was surgically repositioned – orthognathic surgery involved a mandibular setback. Correction of skeletal and dental problems allowed the occlusal, functional and aesthetic goals to be achieved. Class I canine relationships were established with



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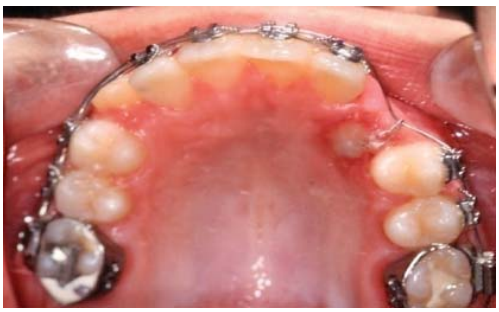

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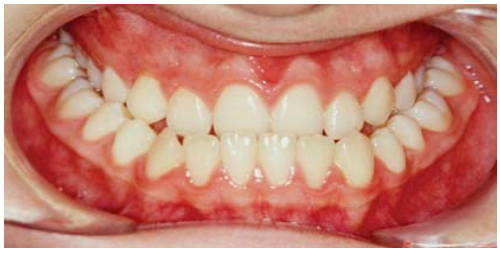

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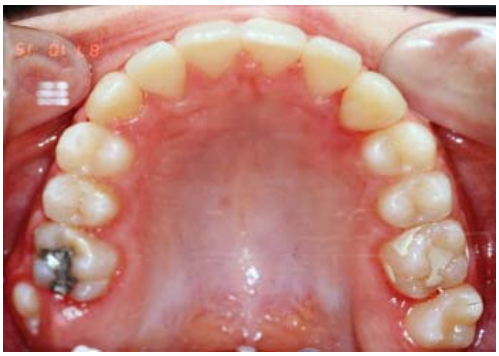
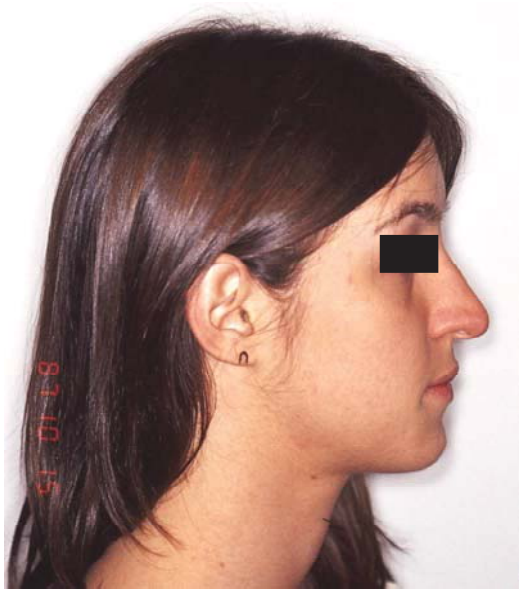


d)

Fig. 2 – a) A progress occlusal view shows an adequate space created in the maxillary canine regions and their eruption; b) Initial intraoral photoimage of the maxillary dental arch occlusal aspect; c, d) A maxillary occlusal perspective at the end of the first step of the orthodontic treatment shows a generally good dental arch form



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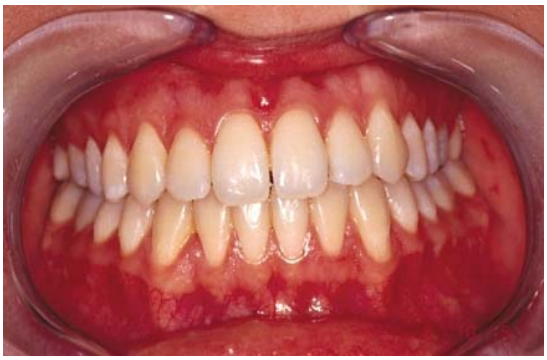
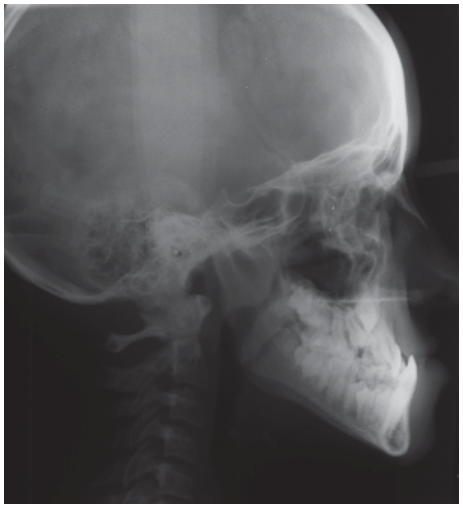


b)

Fig. 3 – a) A post-treatment profile shows the patient`s good facial balance and esthetics following the whole treatment (The prognatic mandible and concave profile type improved significantly); b) The maxillary and mandibular dental intercuspation occurred efficient with a good control of the overall dental arch form

good alignment of the teeth. A positive overjet was established and the overbite was somewhat reduced. Good torque control was maintained while the mandibular incisors were retracted resulting in better incisal inclination after orthodontic and surgical treatment. The maxillary incisors were proclined significantly resulting in better upper lip prominence and an improved facial profile (Figures 3c, d). Correction of malocclusion was accomplished with dental movement as well as with surgical operation. On completion of active treatment, further occlusal adjustment was performed: maxillary and mandibulary fixed retainers were inserted (Figures 4a–f). Final cephalometric analysis demonstrated a change in values of the ANB angle (anterior posterior angle of the maxilla with the mandible) from -4˚ to ideal 2˚ (Table 1).



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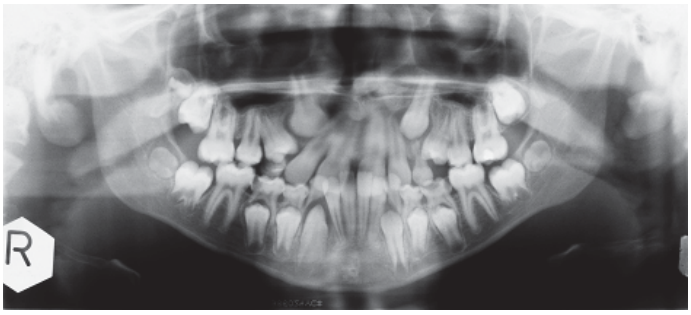

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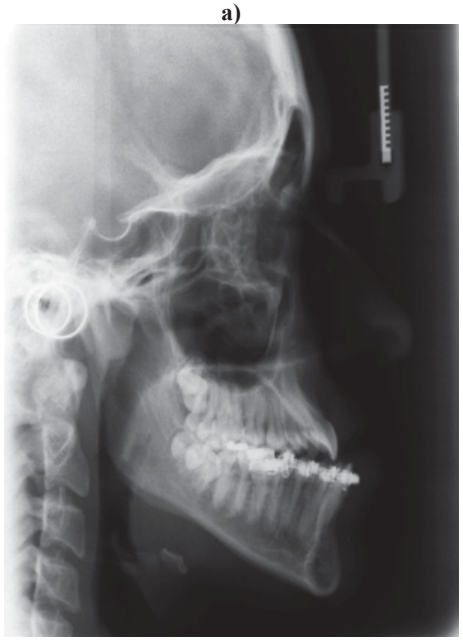

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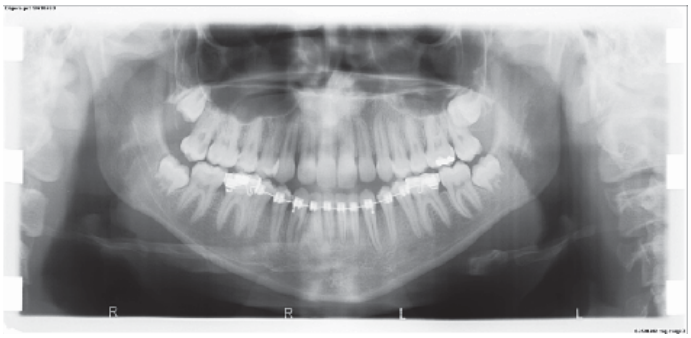

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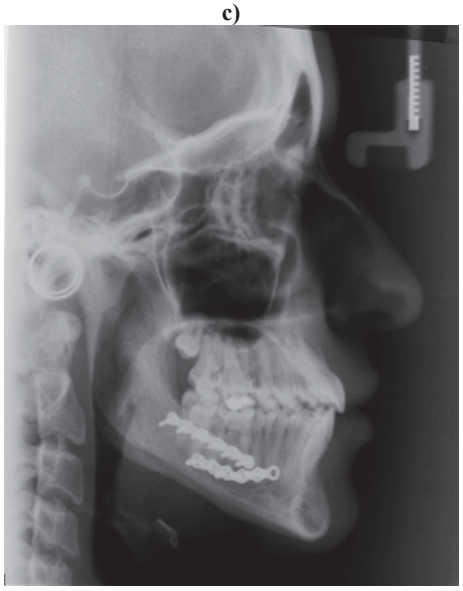

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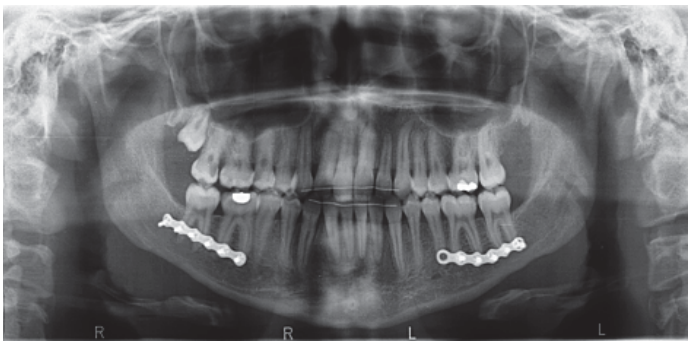

f)

Fig. 4 – The panoramic radiography: a, b) at the beginning of the whole treatment; c, d) at the beginning of the second orthodontic presurgical treatment; e, f) after the whole treatment

Table 1

The values of the SNA, SNB and ANB angles before and after the whole treatment

Angles	The values before and after the treatment	The referent values
SNA	$80^0 \rightarrow 80^0$	82^0
SNB	$84^0 \rightarrow 78^0$	80^0
ANB	$-4^0 \rightarrow 2^0$	2^0

SNA – position of the maxilla (normal, prognathic, retrognathic); SNB – position of the mandible (normal, prognathic, retrognathic); ANB – skeletal relationship between the maxilla and the mandible

Discussion

Every orthodontic treatment aims to achieve an adequate occlusion thus ensuring satisfactory and healthy functioning of the stomatognathic system's physiological routine, an optimal facial, oral and dental aesthetics, resulting in a long-term stability [21].

Skeletal class III malocclusion is a classic example of "easy to be recognized but difficult to treat", the situation where sometimes orthodontic possibilities are limited and need support from other specialties, particularly surgery [22–24]. However, the key to a successful treatment lies in understanding and integrating these two specialties in seeking the best alternatives and procedures, as it was in our case where the treatment was carried out through orthodontic preparation and orthognathic surgery. The surgical correction of class III malocclusion can be undertaken in a variety of ways, by a bilateral sagittal split osteotomy to retract the mandible or by the Le Fort I procedure to advance the maxilla, or a combination of these. Before and after surgical correction of the skeletal discrepancy, the occlusion startes and finishes orthodontically to class I relationship [25–27].

The presented case, with a skeletal class III malocclusion actually had two presurgical orthodontic treatments, firstly only in the upper jaw and second by in both jaws. Why was it in two phases? The answer is very simple. Since the patient was only 12 years old, we had plenty of time for the treatment, and on the other hand there were many more problems in upper jaw, and that is why we began the first phase of treatment only in maxilla. The result of both treatments was the correction of malocclusion but only with dentoalveolar changes, while the mandible was still prognathic. After surgical correction of mandibular setback, the occlusion was finished orthodontically to class I relationship, with a positive overbite and overjet.

Conclusion

Class III discrepancy should be diagnosed and classified according to its etiology and treated with appropriate surgery, including, if necessary, not only mandibular, but also maxillary surgery, in order to achieve a normal facial appearance. In any case, as the field of orthodontics continues to develop technologically and philosophically, we can expect that advances in diagnosis and treatment planning are imminent and inevitable.

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CASE REPORT

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Nasal septum extramedullary plasmacytoma

Ekstramedularni plazmocitom nosnog septuma

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Abstract

Introduction. Plasmacytomas are malignant tumors characterized by abnormal monoclonal proliferation of plasma cells. They originate in either bone – solitary osseous plasmacytoma, or in soft tissue – extramedullary plasmacytoma (EMP). EMP represents less than 1% of all head and neck malignancies. **Case report.** We presented a case of EMP of the nasal septum in a 44-year-old male who had progressive difficulty in breathing through the nose and frequent heavy epistaxis on the right side. Nasal endoscopy showed dark red, soft, polypoid tumor in the last third of the right nasal cavity arising from the nasal septum. The biopsy showed that it was plasmacytoma. Bence Jones protein in the urine, serum electrophoresis, bone marrow biopsy, skeletal survey and other screening tests failed to detect multiple myeloma. This confirmed the diagnosis of EMP. The mass was completely removed *via* an endoscopic approach, and then, 4 week later, radiotherapy was conducted with a radiation dose of 50 Gray. No recurrence was noted in a 3-year follow-up period. **Conclusion.** EMP of the nasal cavity, being rare and having long natural history, represents a diagnostic and therapeutic challenge for any ear, nose and throat surgeon. Depending on the resectability of the lesion, a combined therapy is the accepted treatment.

Key words:
plasmacytoma; nasal septum; diagnosis; diagnosis, differential; otorhinolaryngological surgical procedures; radiotherapy; treatment outcome.

Apstrakt

Uvod. Plazmocitomi predstavljaju maligne tumore koji se karakterišu abnormalnom proliferacijom plazma ćelija poreklom iz jednog klona. Nastaju ili u kostima – usamljeni koštani plazmocitom, ili u mekim tkivima – ekstramedularni plazmocitom (EMP). Manje od 1% svih malignih tumora glave i vrata čini EMP. **Prikaz bolesnika.** U radu je prikazan muškarac star 44 godine sa EMP, koji se javio na pregled zbog progresivnog otežanog disanja na nos i čestih epistaksi iz desne nosne šupljine. Endoskopijom nosa uočen je tamnocrven, mek, polipoidni izraštaj u zadnjoj trećini desne nosne šupljine, polazišta sa nosne pregrade. Patohistološki (PH) nalaz ukazao je na plazmocitom. U urinu nije bio Bence-Jonesovih proteina, elektroforeza seruma, biopsija koštane srži, RTG nalazi skeleta i ostali skrining testovi nisu pokazali da se radi o multiplom mijelomu, što je potvrdilo dijagnozu EMP. Tumor je kompletno uklonjen endoskopskim pristupom, a 4 nedelje nakon operacije, sprovedena je zračna terapija, radijacijskom dozom od 50 Gray-a. Tokom 3-godišnjeg praćenja bolesnika nije ustanovljen recidiv tumora. **Zaključak.** Zbog svog rariteta i nespecifičnih simptoma, koji mogu dugo da budu neprepoznati, EMP nosa predstavlja i dijagnostički i terapijski izazov za svakog otorinolaringologa. U odnosu na operabilnost tumora, kombinovan pristup (hirurgija i radioterapija) predstavlja prihvatljiv tretman.

Ključne reči:
plazmocitom; nos, septum; dijagnoza; dijagnoza, diferencijalna; hirurgija, otorinolaringološka, procedure; lečenje, ishod.

Introduction

Plasmacytomas are malignant tumors characterized by abnormal monoclonal proliferation of plasma cells. They originate in either bone (solitary osseous plasmacytoma) or in soft tissue (extramedullary plasmacytoma – EMP) [1], and could be either primary (without evidence of disease in other foci) or a part of a systemic process during the course of multiple myeloma. The etiology of EMP is unknown.

The first case of EMP was reported in 1905 by Schridde. [2] Alexiou et al. [3] reviewed all previous reports of EMP and found 869 cases; 714 (82.2%) of them had occurred in the upper aerodigestive tract. The most frequently affected areas in the upper aerodigestive tract were the nasal cavity or paranasal sinuses (43.8%), followed by nasophar-

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ynx (18.3%), oropharynx (17.8%), and larynx (11.1%) [3]. Wax et al. [4] report that 75% of extramedullary plasmacytomas occur in the sinonasal/nasopharyngeal area, 12% in the oropharynx, 8% in the larynx. Other sites in the head and neck that have been reported include the tongue, minor salivary glands, thyroid, parotid, orbit and temporal bone [4]. EMP affects men 3–4 times more often in women and typically occurs in the 6th to 7th decade, with over 95% of cases occurring in patients above 40 years of age [5]. EMP is a destructive tumor and, beside the tendency for local recurrence [5, 6], has the ability to spread to regional lymph nodes and ability for distant metastasis with progression to multiple myeloma. EMP is rare tumor and represents less than 1% of all head and neck malignancies [7, 8] and it accounts for 4% of all nonepithelial tumors of the nasal cavity, nasopharynx, and paranasal sinuses [9].

The rarity of this tumor and its long natural history represent a diagnostic and therapeutic challenge for any ear, nose and throat (ENT) surgeon. To exclude multiple myeloma or plasmacytoma of the bone, a systemic work-up and follow-up of the patient are mandatory, including serum protein electrophoresis, urin analysis for the Bence-Jones protein, skeletal survey and bone marrow biopsy [10, 11]. The optimal management of EMP is controversial. However, based on the well-known radiation sensitivity of plasma-cell tumors, radiotherapy is generally, accepted as the treatment of choice for EMP, while the role of chemotherapy in these tumors is not well-defined [12]. Surgery can achieve high rates of local control in certain situations. However, radical excision is often impossible due to the size or the location of the tumor [13].

Most reports in the literature, relating to EMP, are descriptions of individual cases. In this paper we described an interesting case of EMP of nasal septum.

Case report

A 44-year-old male was admitted to the ENT Clinic, Clinical Center in Kragujevac, due to progressive difficulty in breathing through the nose at the right side in the last year and frequent heavy epistaxis from the same side. Because of that, in the past month, the patient was admitted to anterior nasal packing three times. The patient was in good physical condition, not consuming alcohol nor cigarettes, and not suffering from any chronic disease.

Nasal endoscopy showed dark red, soft, polypoid tumor, slightly bleeding to touch, in the right nasal cavity, approximately 2.5 cm in diameter, arising from the last third of the nasal septum and provoking obstruction almost in the entire nasal cavity, especially in its last third. The tumor extended toward the choana and obstructed it.

Computed tomography of the nasal cavities and paranasal sinuses with intravenous contrast showed a heterogenous soft tissue mass, dimensions of approximately 3.5 × 2.0 × 1.5 cm, which arised from the last third of nasal septum, filling the right nasal cavity with signs of mass effect of the medial wall of the right maxillary sinus, locally thinned with no signs of erosion. In the right maxillary sinus there was a content of thicker consistency (Figure 1).

No cervical lymph nodes were palpable. Ultrasonography of the neck and abdomen showed normal findings, and lung X-ray did not show the presence of secondary deposits.

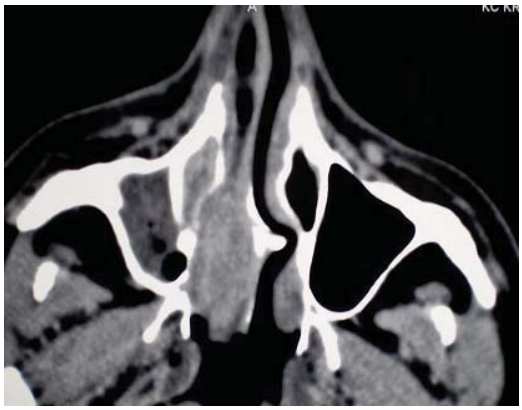

Fig. 1 – Computed tomography of paranasal sinuses (axial view) shows a heterogenous soft tissue mass in the right nasal cavity which arised from the last third of the nasal septum and obstructing the choana

We performed biopsy of the tumor endoscopically. Histopathologic findings, complemented by immunohistochemical examination, fully stressed the intermediate differentiation (Gr. 2 – intermediate grade) extramedullary tumor originating from plasma cells (tumor cells were clearly positive for CD79α, MUM-1, CD138, CD38 and lambda) (Figure 2).

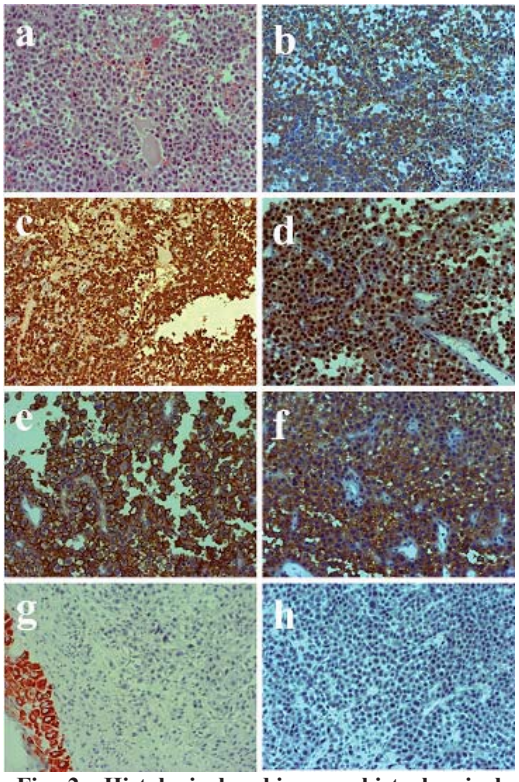

Fig. 2 – Histological and immunohistochemical characteristics of plasmacytomas (original magnification, × 200)

a) HE staining techniques – the tumor is easily constructed from polymorphic cells, high N/C ratio, large roundish nuclei, prominent nucleoli, basophilic cytoplasm easy; immunohistochemically, the cells were plasmacytomas: b) lambda positive, c) intensively express CD79α, d) MUM1, e) CD138 and f) CD38, while negative for: g) Ae1/Ae3 and h) CD20.

After this histopathologic findings, a systemic work-up to exclude (or confirm) multiple myeloma was performed. Renal and liver function were normal. Measurements of hematocrit, white blood cells, blood urea, serum creatinine, serum electrolytes, serum calcium, serum protein electrophoresis and immunoelectrophoresis were within normal limits. Bone marrow biopsy and complete skeletal radiographic survey (skull, AP and profile of cervical, thoracic and lumbar spine, chest, pelvis and long bones of extremities) were negative. Bence Jones protein was absent in the urine.

Since the possibility of multiple myeloma was excluded, the diagnosis of solitary extramedullary plasmacytoma of the nasal septum, stage 1 according to Wiltshaw, was established. Because of the size and location of the tumor, we decided to combine therapeutic approach. The patient underwent complete surgical removal of the tumor *via* transnasal endoscopy. Four week later, the patient received radiotherapy with a radiation dose of 50 Gray in 25 fractions.

No recurrence was detected at clinical examination or at CT scan performed during the first (Figure 3), second and third year after the treatment.

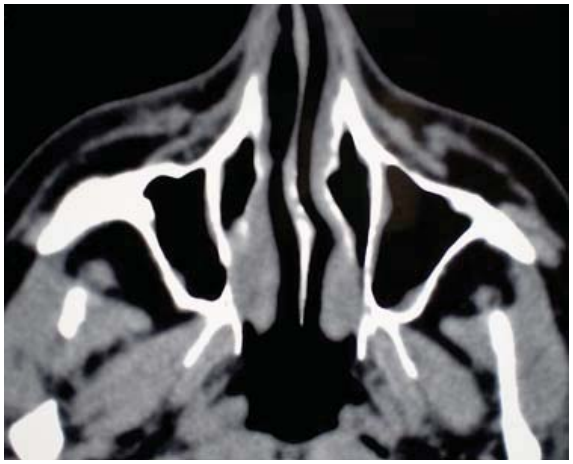

Fig. 3 – Computed tomography of paranasal sinuses (axial view) a year after the treatment, shows the normal anatomy

Discussion

It is important for otolaryngologists to recognize EMP since 80% of EMP occur in the head and neck as single lesions and 10%–20% of cases may present with multiple lesions [6]. This tumor is characterized by a slow clinical course, atypical symptoms, noncharacteristic clinical picture and untimely recognition. The diagnosis is somewhat difficult to made because of nonspecific CT and MRI features of solitary EMP of the sinonasal tract [14]. Although several reports regarding EMP in the nasal cavity have been published so far, a finding of such a lesion in the nasal septum is quite rare. For all these reasons, initially we were not thinking of EMP in the presented patient.

Physical examination usually reveals submucosal, dark red to grayish red, sessile or polypoid tumor, which bleeds easily with manipulation. The mucosa is typically intact but ulceration and necrosis may occur in advanced cases. Clinical presentation is primarily a function of the mass effect and varies according to the site of involvement. Because most of these lesions arise in the sinonasal or nasopharyngeal area, the most common symptoms are nasal mass, nasal obstruction, and epistaxis [15]. In Kapadia et al. [5] series of 20 patients of EMP of the head and neck, 80% of patients presented with the complaint of a mass or swelling, 35% of patients complained of airway obstruction, 35% complained of epistaxis, 20% of localized pain, 15% with proptosis, 10% with nasal discharge, 10% with regional lymphadenopathy, and 5% with a VI nerve palsy.

The diagnosis of EMP usually follows histologic examination. Immunohistochemical staining will demonstrate the monoclonal nature of plasma cells and confirm the neoplastic nature of the lesion. In addition, immunohistochemical study, too, is used to differentiate EMP from other malignant disorders, such as undifferentiated carcinoma, melanoma, and esthesioneuroblastoma [15, 16]. When plasmacytoma is confirmed histologically, secondary diagnostic procedures must be carried out to exclude systemic involvement. A systemic work-up including complete blood profile, renal and liver function, calcium, serum and urinary protein electrophoresis, serum immunoglobulin level, complete skeletal radiographic survey and bone marrow biopsy.

After the diagnosis have been confirmed, EMP can be staged as follows: stage 1 – limited to an extramedullary site (localized and controllable disease), stage 2 – involvement of regional lymph nodes or local extension, and stage 3 – multiple metastasis (although it is no longer a solitary plasmacytoma). In our case the finding confirmed the diagnosis of stage 1 extramedullary plasmacytoma.

The treatment of localized EMP of the head and neck somewhat is controversial. Some authors advocate radiation therapy alone and others advocate surgery alone. Based on the documented radiation sensitivity of plasma cell tumors, the accepted treatment is radiotherapy, but radiotherapy does not always reduce the size of the tumor, perhaps because of an abundant deposition of amyloid within the mass [17–19]. Therefore, most clinicians recommend a combined approach (surgery and radiotherapy) for the management of nasal cavity extramedullary plasmacytoma [6, 19–22]. In fact, a combination treatment may provide the best results. Alexiou et al. [3] in their review of 714 cases of upper aerodigestive tract extramedullary plasmacytomas reported between 1905–1997, found that the median overall survival or recurrence free survival was longer than 300 months for patients who underwent combined surgery and radiotherapy as compared to a median survival rate of 144 months for patients who underwent only radiotherapy and 156 months for only surgically managed patients. Chemotherapy may be considered for patients with refractory or relapsed disease.

The median survival of patients varies from 4–10 years. Local recurrence has been reported to occur in 8%–30% of adequately treated EMP in the upper aerodigestive tract [6, 23]. Because of the tendency of EMP to progress into disseminated multiple myeloma (conversion of EMP to multiple myeloma has been reported in 8%–36% of cases within 3 to 61 months), [24] a lifelong follow-up of these patients is rec-

ommended. CT and measurements of serum immunoglobulin and urinary Bence Jones protein levels may be useful in detecting recurrence or conversion to multiple myeloma [25].

Conclusion

Extramedullary plasmacytoma of the nasal cavity is rare and should be considered in the differential diagnosis of nasal cavity masses. A multidisciplinary approach (haematologist, otorhinolaryngologist, pathologist, radiation oncologist, radiologist) is required for the optimal diagnosis and management of EMP. It is essential to exclude any systemic involvement before arriving at a diagnosis of solitary EMP. Depending on lesion resectability, a combined therapy (surgery and radiotherapy) is the treatment of choice.

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CASE REPORT

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Malignant stromal tumor of the stomach with giant cystic liver metastases prior to treatment with imatinib mesylate

Maligni stromalni tumor želuca sa ogromnim cističnim metastazama u jetri pre lečenja imatinib mesilatom

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Abstract

Introduction. Gastrointestinal stromal tumors (GISTs) are rare and account for 0.1%–3% of all gastrointestinal neoplasms. GISTs are most commonly located in the stomach (60%) and 20%–25% are malignant, with metastases involving the peritoneum or the liver. Cystic liver metastases are extremely rare. Only two previous cases of patients with cystic liver metastases, prior to treatment with imatinib mesylate, have been described so far. **Case report.** We reported a 52-year-old woman presented with a history of abdominal fullness and discomfort. Clinical examination revealed two palpable masses, first in the right upper abdomen and second left to the umbilicus. Examinations revealed 4 cystic metastases in the liver, 3 in the right lobe (including a huge one measuring 20.5 × 16 cm), and 1 in the left lobe, together with a primary tumor on the greater curvature of the stomach. Gastric tumor was removed with a Billroth II gastrectomy. Partial excision of the largest liver metastasis was performed for histopathology. Immunohistochemistry confirmed the diagnosis of a GIST in both tissue samples. After an uneventful recovery the patient was commenced on imatinib mesylate therapy. The patient remainsed symptom-free at 24 months follow-up. **Conclusion.** This was the third reported case of gastric GIST with giant cystic liver metastases present prior to treatment with imatinib mesylate. Although extremely rare, GISTs may present with cystic liver metastases prior to treatment with imatinib mesylate, and should be considered in the differential diagnoses of patients with liver cysts of uncertain aetiology.

Key words:
gastrointestinal stromal tumors; stomach; diagnosis, differential; liver; neoplasm metastasis.

Apstrakt

Uvod. Gastrointestinalni stromalni tumori (GIST) su retki tumori koji čine ukupno 0,1–3% svih gastrointestinalnih neoplazmi. Najčešća lokalizacija GIST je u želucu (60%). Oko 20–25% ovih tumora je maligno, dok su metastaze najčešće lokalizovane po peritoneumu ili u jetri. Pojava cističnih metastaza GIST u jetri je veoma retka i do sada su u literaturi opisana samo dva bolesnika sa ogromnim cističnim metastazama GIST u jetri pre lečenja imatinib mesilatom. **Prikaz bolesnika.** Prikazana je bolesnica stara 52 godine, koja se javila lekaru zbog osećaja napetosti u trbuhu. Kliničkim pregledom nađena su dva palpabilna tumefakta ispod desnog rebarnog luka i paraumbilikalno sa leve strane. Kompletnom radiološkom pretragom utvrđeno je postojanje stromalnog tumora velike krivine želuca i četiri krupne cistične promene u jetri, tri u desnom i jedna u levom lobusu jetre. Najveća od ovih promena bila je dimenzija 20,5 × 16 cm. Bolesnica je operisana kada je urađena resekcija želuca tipa Billroth II i parcijalna ekscizija najveće promene iz desnog lobusa jetre. Patohistološka i imunohistohemijska analiza potvrdile su dijagnozu GIST u želucu i jetri. Nakon uspešnog postoperativnog oporavka ordinirana je terapija imatinib mesilatom na koju je bolesnica dobro reagovala. Dve godine nakon operacije bolesnica je dobro i bez znakova recidiva bolesti. **Zaključak.** Ovo je tek treći opisani bolesnik sa gastričnim GIST, sa ogromnim cističnim metastazama u jetri pre lečenja imatinib mesilatom. Mada veoma retko, GIST se mogu prezentovati sa cističnim metastazama u jetri pre lečenja imanitib mesilatom i treba da budu razmotreni u diferencijalnoj dijagnozi bolesnika sa cistama u jetri nepoznate etiologije.

Ključne reči:
gastrointestinalni stromalni tumori; želudac; dijagnoza, diferencijalna; jetra; neoplazme, metastaze.

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Introduction

Gastrointestinal stromal tumors (GISTs) are rare and account for 0.1%–3% of all gastrointestinal tumors, and 5.7% of gastrointestinal sarcomas [1, 2]. They are mesenchymal in origin, defined as c-kit (CD 117) positive tumors, and have a characteristic set of histological features including spindle or epitheloid cells [3].

Around 20%–25% of gastric GISTs are malignant and frequently metastasize to the liver and peritoneum. Liver metastases are typically solid, and cystic lesions are extremely rare in these patients at presentation [4, 5]. Cystic liver metastases appear in colorectal, ovarian and pancreatic mucinous adenocarcinomas, usually as a result of an accumulation of mucin/serous fluid produced by the tumor itself or from cystic degeneration consequent to ischemic necrosis or infarction of the tumor mass [4]. Solid GIST liver metastases can also develop cystic changes following targeted therapy with imatinib mesylate, which inhibits c-kit and causes tumor shrinkage [6].

Only two patients with cystic GIST liver metastases prior to treatment with imatinib mesylate have been described so for. This is the third patient with gastric GIST and huge cystic liver metastases.

Case report

A 52-year-old woman with no significant past medical history presented to our unit with a 2-month history of fullness and discomfort in the right upper abdomen. On examination the patient had a large spherical, painless, fluctuant mass palpable in the right upper abdomen, extending up behind the right costal margin. Another, smaller, hard, painless and mobile mass was also found to the left of the umbilicus. There was no splenomegaly or ascites, and there were no dilated veins on the abdominal wall. The patient had an elevated white blood cells (WBC) (18.9 × 109/L), mild anemia and mild thrombocytosis (516 × 109/L). Blood proteins were elevated (93 g/L) as were fibrinogen (9.0 g/L), alkaline phosphatase (199 u/L) and γ-GT (120 u/l). All other laboratory data including tumor markers (ALP, CA 19-9, CA 72-4, CEA) were within normal limits.

Ultrasonography showed that much of the right lobe of the liver was taken up by 3 cystic lesions. The largest of these measured 20.5 cm × 16 cm and contained several tissue layers (Figure 1); two smaller cysts measuring 5.7 cm × 6.3 cm and 6.7 cm × 8 cm were located in segments VII and VIII of the liver. A fourth cyst measuring 3 cm in diameter cm was present in the left liver lobe. A gastric tumor measuring 9.5 × 7.5 cm × 6.5 cm was present in the left mesogastrium. Computed tomography scan confirmed the ultrasonography findings (Figures 2, 3 and 4).

We performed the operation throuph right subcostal laparotomy. A firm, mobile, rough tumor was found attached to the greater curvature of the antral part of the stomach. The tumor had not created any defect of the gastric mucosa and was removed by way of a Billroth II gastrectomy. Frozen section histology of the tumor was suggestive of GIST. The

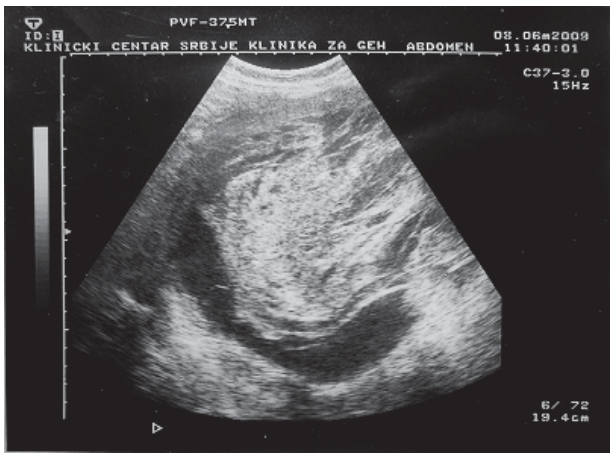

Fig. 1 – Ultrasound showing a giant cyst within the right lobe of the liver containing several tissue layers

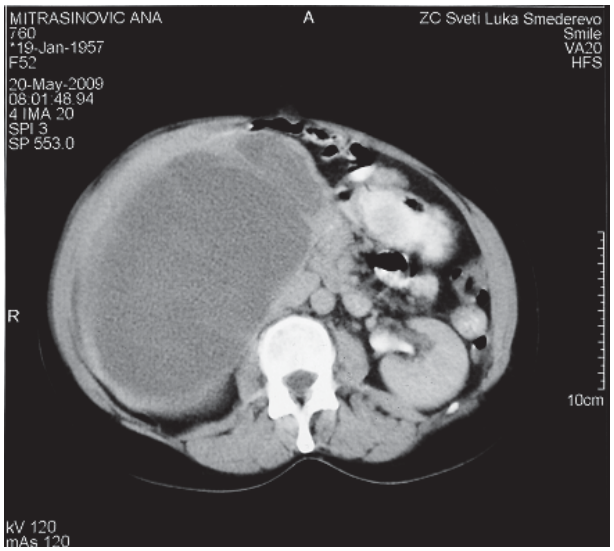

Fig. 2 – Computed tomography scan showing a giant cyst of the right lobe of the liver, and a part of the segment VIII smaller cyst

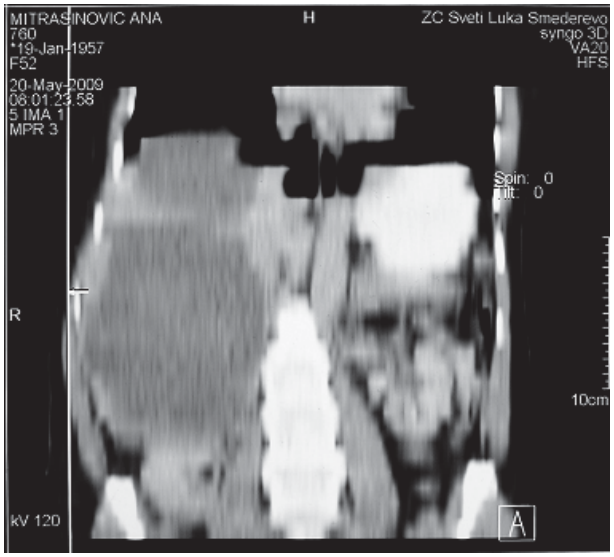

Fig. 3 – Computed tomography scan showing a frontal section of the segment VII and giant liver cysts

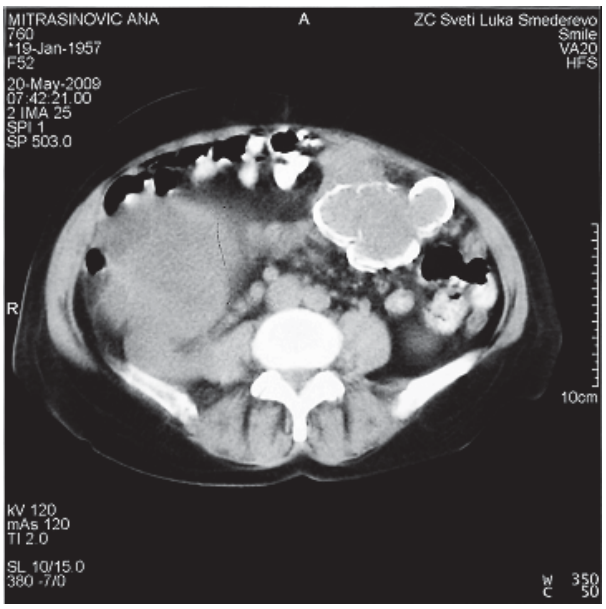

Fig. 4 – Computed tomography scan showing a cyst like gastric gastrointestinal stromal tumors in the left mesogastrium

mal neoplastic proliferation which was mostly hypercellular and epithelioid, but with many additional microcystic and pseudohemangiomatous areas (Figure 5a). The periphery contained the areas of mixed spindle and epithelioid cells with a vacuolar and sclerosing appearance, suggestive of gastric GIST. The liver cyst specimen had a very thin rim of neoplastic tissue at its periphery, which was sharply demarcated from hepatic parenchyma (Figure 5b). Its histology was identical to that the gastric tumor confirming that it was a liver metastasis.

Immunohistochemistry showed strong PDGFRA immunostaining of the epitheliod areas but only focal and weak "dot-like" cytoplasmic CD117 immunostaining in the mixed spindle and epithelioid areas, in both the primary and secondary lesions (Figure 5c and d). Vimentin immunostaining was also positive, however CD34, desmin, αSMA, S-100 protein, WT-1, calretinin and markers for other epithelial, neuroendocrine and other non-mesenchymal markers were all negative.

The patient's postoperative recovery was uneventful. Following histological confirmation of a malignant GIST of

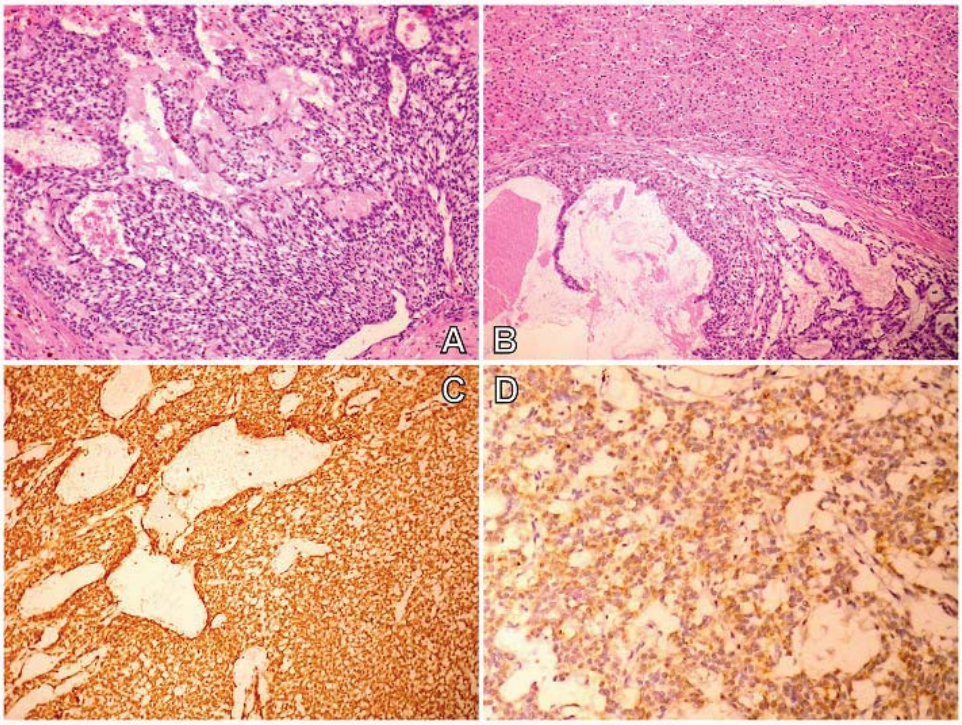

Fig. 5 – Microcystic pseudohemangiomatous appearance of the predominantly epithelioid gastric gastrointestinal stromal tumors (A) and its cystic liver metastasis (B), which revealed diffuse strong PGFRA immunoexpression (C) and weak focal CD117 immunostaining (D)

largest of the previously identified cystic lesions of the liver contained old blood and a jellylike material. The well of the cyst wall was excised and sent for histology.

The antral gastric tumor hads predominantly extramural growth, and measured up to 95 mm in diameter. Macroscopically, it appeared pseudocystic as most of the central areas were necrotic and hemorrhagic. This soft, fragile internal tissue was surrounded by a more firm, partly sclerotic and calcified pseudocapsule. Histology revealed mesenchy-

the stomach with cystic liver metastases the patient was commenced on imatinib mesylate treatment, and remainsed clinically well at 24 months.

Disussion

GISTs encompass a wide spectrum of tumors with varying locations and biological potentials. Previously classified as leiomyomas, leiomyosarcomas, neurofibromas and

schwanomas, GISTs are now known to be quite different both histologically and immunohistochemically [6]. They predominantly affect middle-aged and older patients (median age of 55–60 years) [3], and while they can occur in different parts of gastrointestinal tract, they most commonly occur in the stomach (60% of cases) and small intestine (35%); they can also occur in the rectum and other parts of gastrointestinal tract, and in those tissues in close proximity to them [1, 3, 4]. GISTs are believed to originate from the intestinal cells of Cajal or related stem cells, and can be divided histologically into 4 spindle cell (70%) and 4 epitheloid variant (30%) subgroups [3].

A significant proportion of GISTs are malignant, however differences exist according to primary tumor location [7, 8]; 20%–25% of gastric and 40%–50% of small intestine GISTs are malignant [3]. Metastases frequently occur in the peritoneum and liver, and only rarely involve the bones, soft tissues and skin; lymph node and lung metastases are extremely rare. Metastases can develop up to 10–15 years after primary surgery, and thus a life-long clinical follow-up is mandatory [3].

GIST liver metastases are typically solid, and cystic lesions are extremely rare in these patients at presentation [4, 5]. In 2003 Zonios et al. [5] reported a 73-year-old woman with gastric GIST who presented with low grade fever, weight loss, left-quadrant pain and multiple cyst-like hepatic metastases, while in 2009 Jain et al. [4] presented a 50-year-old man with gastric GIST who had a 12 cm × 9 cm cystic liver metastasis. Both these patients had cystic GIST liver metastases prior to any treatment.

Solid GIST liver metastases are known to develop cystic changes as a result of targeted therapy with imatinib mesylate, which inhibits c-kit and causes tumor shrinkage [6]. Indeed these morphological changes are now regarded as a positive prognostic sign, as the overall survival is significantly better in those patients developing these cystic changes following imatinib mesylate treatment [1].

This is only the third documented case of gastric GIST with cystic metastases present prior to any treatment. The presented patient had multiple cystic liver metastases and the largest such reported lesion.

Conclusion

Although extremely rare, GISTs may present with cystic liver metastases prior to treatment with imatinib mesylate, and should be considered in the differential diagnoses of patients with liver cysts of uncertain etiology.

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CASE REPORT

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Metastatic malignant ovarian melanoma – a case report

Metastatski maligni melanom ovarijuma

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Abstract

Background. Malignant melanomas of the female reproductive system are rare. These are biologically highly aggressive tumors with poor prognosis. Preoperative establishment of the diagnosis is practically impossible. Therapeutic approach and treatment of patients with metastatic ovarian melanoma are highly dependent on precise histological analysis. **Case report.** A woman aged 48 was admitted to the clinic for occasional pains in the lower abdomen and suspected myomatous changes of the uterus. The patient underwent surgery for melanoma on her right arm five years ago. Classic hysterectomy with bilateral adnexectomy with infracolic omentectomy and selective iliac lymphadenectomy were performed. Macroscopic examination revealed an oval tumefaction on the left ovary sized 12.5 x 10 x 3.5 cm of solid structure. Tumor tissue was yellowish-brown colored, of solid structure and mostly localized subcortically with central edema. Microscopic examination showed positive reaction for HMB-45, anti-Melan-A and S-100 protein, but negative immunoreactivity for estrogen and progesterone receptors. Malignant disease caused death after a 4-year follow-up period following gynecological operation. **Conclusion.** The previous diagnosis of skin melanoma is also indicative of metastatic ovarian tumor, while immunohistochemical analyses confirmed the histopathological diagnosis.

Key words:
ovarian neoplasms; melanoma; neoplasm metastasis; diagnosis; gynecologic surgical procedures; treatment outcome.

Apstrakt

Uvod. Maligni melanomi ženskog reproduktivnog sistema su retki. To su biološki veoma agresivni tumori sa lošom prognozom. Postavljanje dijagnoze pre operacije je gotovo nemoguće. Terapijski pristup i lečenje bolesnika sa metastatskim ovarijalnim melanomom veoma zavisi od precizne histološke dijagnoze. **Prikaz bolesnika.** Žena, stara 48 godina, primljena je na kliniku zbog povremenih bolova u donjem delu abdomena i zbog sumnje na miomatozno izmenjenu matericu. Pet godina ranije imala je operaciju melanoma desne ruke. Urađena je klasična histerektomija sa obostranom adneksektomijom i parcijalnom resekcijom omentuma sa selektivnom ilijačnom limfadenektomijom. Makroskopski, opisan je ovalni tumefakt levog ovarijuma 12,5 x 10 x 3,5 cm solidne strukture. Tumor je bio žućkastobraon boje, uglavnom lokalizovan supkortikalno sa centralnim edemom. Mikroskopsko ispitivanje je pokazalo pozitivnu reakciju na HMB-45, anti-melan-A and S-100 protein, ali negativnu imunoreaktivnost na estrogenske i progesteronske receptore. Progresija maligne bolesti dovela je do smrti posle 4-godišnjeg praćenja bolesnice nakon ginekološke operacije. **Zaključak.** Prethodna dijagnoza melanoma kože upućuje na metastatski ovarijalni tumor, dok imunohistohemijske analize potvrđuju histopatološku dijagnozu.

Ključne reči:
jajnik, neoplazme; melanom; neoplazme, metastaze; dijagnoza; hirurgija, ginekološka, procedure; lečenje, ishod.

Introduction

It has been known that different gynecological malignancies extend toward the ovary by direct invasion as well as that gastrointestinal adenocarcinoma and breast cancer are the most frequent non-gynecological malignancies metastasizing to the ovaries. Ovarian localization of secondary deposits of extraovarian malignancies is relatively frequent and it accounts for approximately 10% of all ovarian tumors [1].

Malignant melanomas of the female reproductive system are rare and they account for 3%–7% of all melanoma localizations. Less than 50 cases have been described in the literature so far. These are biologically highly aggressive tumors with poor prognosis and most of the patients die within initial two years after verification of the tumor. Primary malignant melanoma of the ovary is exceptionally rare in gynecological oncology. It may develop as a result of malignant transformation of melanocytes in mature cystic ovarian teratoma [2, 3].

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Preoperative establishment of the diagnosis is practically impossible since occasionally the tumor is not clinically manifested as adnexal or ovarian mass, although history of previous treatment of melanoma of other localization may rise suspicion [4–6].

Therapeutic approach and treatment of patients with metastatic ovarian melanoma are highly dependent on precise histological analysis. Establishment of the accurate diagnosis is a prerequisite and imperative for the therapy [7–9].

We presented a case with secondary malignant melanoma of the ovary following previously treated melanoma on the arm.

Case report

A woman aged 48 was admitted to the clinic for occasional pains in the lower abdomen and suspected myomatous changes of the uterus.

The patient had no specific gynecological history, had two vaginal deliveries and two artificial abortions. Five years ago the patient underwent surgery for melanoma on her right arm while two years ago she had gallbladder surgery.

Clinical gynecological examination revealed solid, uneven uterus with palpable tumefaction on the left side, adjacent to the uterus of approximately 10 cm in diameter. Ultrasound examination of the small pelvis evidenced uterus size of 11 x 7 x 5 cm, with subserous myoma arising from the fundus sized 10 x 8 cm. Pathologic adnexal findings were defined neither on the right nor on the left side.

Preoperative results of blood analyses were normal, including blood count and liver and kidney functions, while erythrocyte sedimentation was slightly increased. Tumor markers CEA, CA 15-3 i CA 19-9 in the serum were within the normal range, while CA 125 was discreetly increased. Lung and heart x-rays as well, echosonography of the abdomen were normal.

In the course of laparotomy, a small quantity of ascites was aspirated. The left ovary with tumefaction sized 10 x 8 cm was in the immediate contact with the uterus, twice torqued around its axis. Surface of the tumefaction was roughly nodular and uneven. The right ovary and fallopian tube as well as the uterus appeared normal. The abdominal organs accessible to examination were free of any visible pathological changes.

Classic hysterectomy with bilateral adnexectomy with infracolic omentectomy and selective iliac lymphadenectomy were performed. Surgical material undergone histopathological analysis.

Macroscopic examination revealed an oval tumefaction on the left ovary sized 12.5 x 10 x 3.5 cm of solid structure and whitish, roughly nodular surface (Figure 1). On the section, tumor tissue was yellowish-brown colored, of solid structure and mostly localized subcortically with central edema. The uterus, left ovary, fallopian tube, omentum and lymph nodes were normal.

Microscopic examination revealed the presence of tumor in the left ovary, composed of solid islets, nests or band-like formations. Tumor cells were polygonal, occasionally fusiform with oval, pleomorphic, hyperchromic nuclei. Mitoses numbers were moderate (Figure 2). The tumor did not penetrate the ovarian capsule or spread to the contralateral ovary, uterus, omentum or two analyzed lymph nodes.

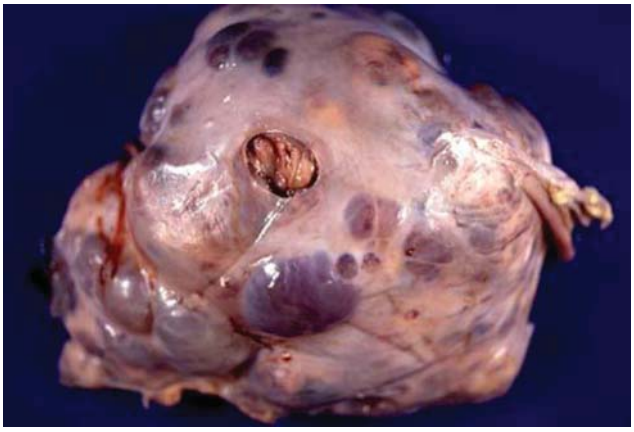

Fig. 1 – Macroscopic finding of ovary with malignant melanoma

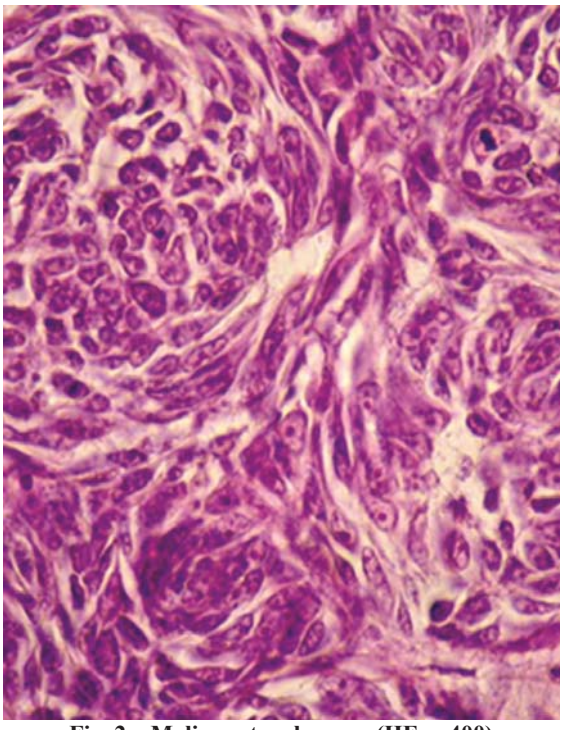

Fig. 2 – Malignant melanoma (HE, x 400)

The tumor cells showed marked cytoplasmic immunoreactivity to HMB-45 (Figure 3) and anti-Melan-A (Figure 4), as well as to S-100 protein (Figure 5), however with somewhat lower intensity of staining. Immunohistochemical reaction to estrogen and progesterone receptors was negative.

Accordingly, the diagnosis of malignant metastatic ovarian melanoma was established.

The postoperative course was uneventful. Progression of the malignant disease caused death of the patient after a 4-year follow-up period following gynecological operation, nine years after the surgery for primary malignant melanoma on the right arm.

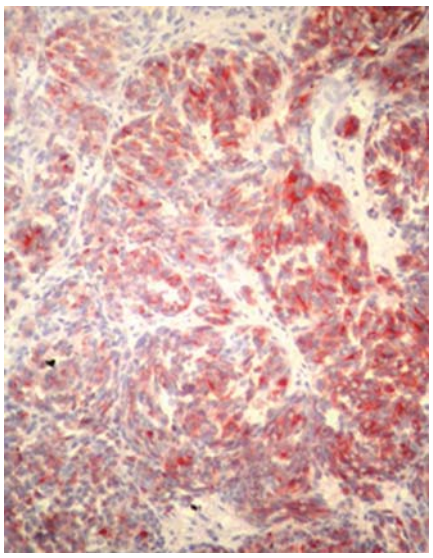

Fig. 3 – Diffuse expression of HMB-45 antibodies

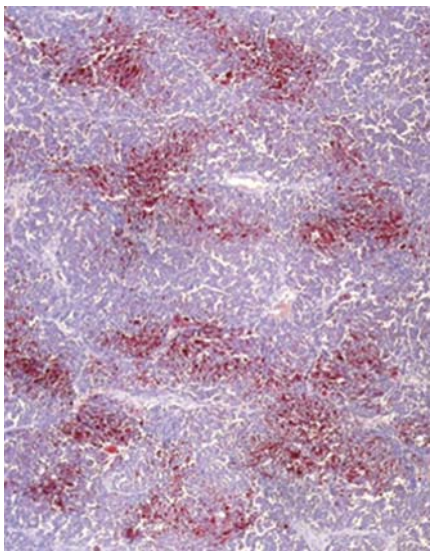

Fig. 4 – Focal expression of anti-Melan-A antibodies

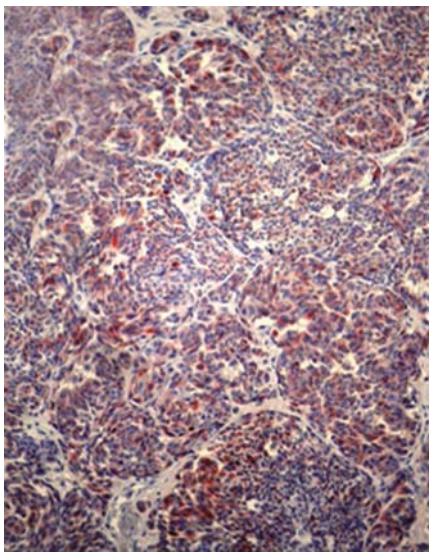

Fig. 5 – Diffuse expression of S-100 protein

Discussion

Melanomas of the female genital tract are known to be rare and they are most commonly biologically highly aggressive and lethal tumors [10]. The interval between primary melanoma and ovarian metastasis is about 78 months [2], but in our case report it was 60 months.

This ovarian neoplasm is, as a rule, unilateral, and it is mostly detected in postmenopausal women averagely aged 53 years. Based on the reference data, the patients present due to the abdominal bloating and occasional pains in the small pelvis. Gynecological palpation reveals tumor masses, however the findings are occasionally inconclusive and also indicative of benign lesions of the genital organs, as it was the case with our patient. If the disease is accompanied by dysuria and loss of body weight or signs of acute abdomen, more detailed investigations and treatment are indicated in order to establish the exact diagnosis [11, 12].

Occasional abdominal pain was the major symptom in the reported patient. Ultrasound examination of the pelvis failed to evidence possible presence of a malignant process while tumor change was interpreted as subserous myoma of the uterus. It has been known that the presence of melanin in the course of magnetic resonance imaging may show changes in signal acquisition. Our patient did not undergo magnetic resonance imaging since malignant tumor in the small pelvis was not suspected.

More detailed clinical examination of the skin and visible mucosal membranes failed to evidence melanoma of any other localization or other secondary deposits and based on the history, pigmented skin tumor was confirmed. It is assumed that spontaneous regression of the primary lesion sometimes occurred [10].

The tumor was unilateral, which is a frequent characteristic of secondary melanoma, its structure was solid, yellowish colored and it infiltrated extensively the ovarian tissue, without penetration of the capsule. No residues of the teratomatous component were found in the tumor, which was important for determination of the primary nature of melanoma [2, 7].

In the reported case, the tumor was subjected to detailed histopathological examination and no teratomatous elements were detected, while previously diagnosed skin melanoma was suggestive of the metastatic nature of the tumor.

Establishment of the accurate histopathological diagnosis and evaluation of differential diagnostic possibilities of other ovarian neoplasm require verification based on immunohistochemical demonstration of S-100 proteins i HMB-45 and negative staining for keratin and other antigens [7, 12]. In case of our patient, histopathological diagnosis was confirmed immunohistochemically by positive tumor cell staining for HMB-45, anti-Melan-A and S-100 protein.

Reductive surgery is currently considered to be the most important approach to treatment of malignant ovarian melanoma, although different additional protocols of chemotherapy and radiotherapy are also available, as well as attempted treatments based on application of immunotherapy. The treatment of the patient included total hysterectomy and

bilateral adnexectomy with selective iliac lymphadenectomy and infracolic omentectomy [3, 5].

Conclusion

The presented patient shows that seemingly insignificant and occasional painful sensations in the small pelvis may be the result of a highly malignant process, which may occasionally lead to misdiagnosis of primary benign tumor owing to its localization and structure. The absence of teratomatous component in malignant ovarian melanoma evidenced by histopathological analysis is indicative of secondary deposits. The previous diagnosis of skin melanoma was also indicative of metastatic ovarian tumor, while immunohistochemical analyses confirmed the histopathological diagnosis.

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Alexander P. Borodin (1833–1887) – great composer, army physician and distinguished scientist-chemist

Aleksandar P. Borodin (1833–1887) – veliki kompozitor, vojni lekar i priznati naučnik-hemičar

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Key words:
physicians; military personnel; science; biochemistry; music.

Ključne reči:
lekari; vojni kolektiv; nauka; biohemija; muzika.

Introduction

Alexander Porfiryevich Borodin (Figure 1) was born on November 12, 1833, in the capital of the Imperial Russia St. Petersburg, as a rejected and illegitimate son of 62-year-old Georgian prince Luka Gedevanishvili (according to one historical source) or prince Luka Stepanovich Gedeonov (second historical source) and a 37 years younger Russian peasant Evdokia Konstantinovna Antonovna. The father forgot Borodin's name and enrolled him in the birth registry as the son of a room made, Jevdokia, and an indoor servant, Borodin Porfiryevich. On his deathbed in 1843 Luka Gedeonov admitted Alexander Borodin as his son and released him of obligations as servant [1].

Fig. 1 – Portrait of Alexander P. Borodin

Interest in music

The father's aristocratic position allowed Borodin to gain good education in his childhood, including instructions on how to play the piano and cello. Later on Borodin surmounted the technique of playing the flute oboe. By the way, he was also occupied with literature. This talent for music was noticed at the age of nine when his first compositions appeared [2]. Although being skinny and in poor health he successfully attented the lessons of foreign languages in adolescent years delivered by his energetic and wise mother, although she had a very modest education.

Shortly after his father's death, Borodin's mother married a military doctor Kleinek. Later on, he helped Borodin to enroll the study of medicine [3]. In the years to come, Borodin acquired culture and fine manners of aristocratic society where he belonged to [4]. At that time, Borodin was dreaming and planning about the study of chemistry due to his interest in the chemistry of fireworks. Passion and commitment to chemistry obscured his musical skills and creativity when for a time he neglected composing and performing.

As his mother was dissuading him from chemistry at his puberty age, she persuaded her husband, Borodin's stepfather, to help her. Finally, Borodin enrolled medicine study in 1850 [5]. Another reason to give his assent for the medicine study was as follows: receiving university diploma in chemistry was a condition to obtain diploma in any biomedicine study. Upon the next five years of successful studies in Peters-

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burg at the Military Academy of Medicine and Surgery (Military Academy of Surgery or Military Medical Academy as stated by various literature data) Borodin graduated in 1855 [5].

Employment

After graduation, in the 1856 Borodin worked as family doctor at a military hospital. At the same time, he became more and more engaged in experimental medical chemistry at the Academy. This well-equipped institution was the Russian center of natural sciences allowing him a wide field of work and open up his mind to researching and achievements that followed up [5].

Borodin spent some time as an intern at the Academy in the rank of physician-surgeon. There he met a young guard officer, lieutenant Modest Mussorgsky, made friends with him and worked on musical cooperation forming a famous circle of musicians, the so-called "Mighty Five" [6].

There was a lot of work in the crowded Russian military hospitals with sick soldiers and wounded in those years after big losses during the Russo-Turkish war in Crimea (1853–1856). It was noted that Borodin had selfless kindness, compassion and tenderness for patients at ambulance work in the next three years. He was often on duty as a volunteer due to the large influx of warriors from the Crimean front. However, at that internship time Borodin got a "coordinator" role at military hospital. Once Borodin almost fainted during examination and treatment of serious wounds of Crimean wounded. From then on, he realized that medical practice was too hard and emotional work for him (Borodin always felt a slight dizziness when in contact with blood or bloody interventions) [2]. Borodin began more and more to move away from clinical practice of medicine and in time moved to the Department of Anatomy and Patohistology to work as a preparator. At the same time, he began to care for medical chemistry from laboratory and scientific aspects of biochemical research that would later bring him a great fame [5].

A career in science – chemistry

As renowned expert in four languages, Borodin attended International Congress of Ophthalmology and Optics (1857) as the Russian government delegate. During those months, he intensively conducted research in the pharmaceutical laboratory and received advanced training, studied and travelled to professional meetings.

After specialization in medical chemistry at the "Institute for Youth doctors" in 1858, he attented practical courses in organic chemistry (biochemistry). Next year (1859), he published an article in the field of balneology and balneomedicine [7].

Borodin conferred a doctorate in the field of toxicology ("Analogy in the action of arsenic and phosphoric acid in the chemical and toxicological sense") on May 15th 1858. It was the first written and defended PhD in Russian language in former Russia at the "Military Medical Academy", while all previous ones were in Latin. St. Petersburg Academy of that time was a glorious institution where the famous Nobel Prize winner, Ivan Pavlov, one of the founders of modern human physiology, soon began to work.

Shortly after that, Borodin began specialization in medical (physiological) chemistry. He rearranged the apartment in a small laboratory and focused researching on toxicology and organic chemistry. Besides, he used to teach at the University School of Medicine that made him more exhausted. Many historians recorded that his excessive experimentation in the laboratory placed within the house produced heavy mist of chemicals threating with fire all the time.

After diploma promotion at the end of 1859 Borodin went to the Western Europe as a state scholarship and gained further education. This was to thank to his teacher, Nikolai Nikolayevich Zinin, who payed much respect to Borodin. Zinin enabled him postdoctoral scholarship in Prussia because at that time Russian medical profession was the youngest in Europe with modern medicine still in its infancy compared with the Western European one [8]. Zinin used to chide him many times in a friendly way for his excessive dedication to music instead to laboratory research for which he was believed to possess a great talent especially in the field of applied chemistry in medicine. Since then, Borodin used to compose and perform music "in the underground".

Filled with energy and enthusiasm, Borodin went to the Prussian Heidelberg in autumn 1859 to do his postdoctoral studies in the laboratory of a famous Emil Erlenmeyer up to the 1862. Here he also collaborated with the renowned chemist Bunsen [8] and compatriots Mendeleyev and Sechenoff, the scientists already recognized at the time [1].

The scholarship was extended to him for two years due to the prolific scientific work. Borodin moved to Italy because of his fiancé illness (tuberculosis). Actually, pianist Ekaterina Protopopova, his "dear Katyusha" was advised by doctors for hot climate treating. Beloved Ekaterina will be taken as wife in 1863 in Petersburg. Leisure time Borodin spent in Viareggio, the village near Pisa, where used to work in the chemical research laboratory "Lucca and Tasiperi", the property of Sebastian di Lucca, where studied mostly organohalogens. During that period Borodin composed little in rare spare time spent at home nurturing the sick wife.

Mastery of the English, French, German and Italian allowed him to travel in the next few years to professional meetings and for medical research projects across Europe (Pisa, Paris, Berlin, Karlsruhe, Frankfort, Jena, Belgium, Switzerland etc).

Returning to Russia in the autumn of 1862, the famous Borodin became associate professor of organic chemistry at the Military Medical Academy in St. Petersburg where kept on hard scientific research [1]. At that time he established "Medical school for women". This was a very important event for Russian medicine since it was the first time in Russia (St. Petersburg) that women were allowed to be educated for the health care profession and physicians [1]. This type of training Borodin would repeated in the period from 1872 to 1887 organizing the multiple "courses of medicine for women" [3].

Due to the dedicated work at the Academy and the scientific achievements, Borodin was announced in 1864 as full professor. Then he met a famous composer Mily A.

Balakirev and his music colleagues, and made friends with him. Hence, he became the member of the so-called "Big Five" ("Mighty Handful"), later on the famous independent group of composers. Modest Musorgsky who left military service for the sake of love or music joined the "Mighty Five" and got down to hard work to create and express "Russian" versus "German" art music. They wanted to prevent the enormous impact of the West, mainly German, composers and create independent Russian art music folk songs and folklore of the big Russia. Doing so, they were gradually very successful in years to come [3, 9].

Borodin created the first symphony under the Balakirev's influence but written in four years because torn up by family and professional responsibilities. He experienced its successful performance not until 1869.

Researhes in the field of biochemistry, toxicology and organic chemistry

At the same time Borodin realized many organic analytic and synthetic reactions in the field of biochemistry applied to laboratory tests, medical and pharmaceutical industry. Much effort Borodin invested in organohalogens synthesis (up to that time the first synthetic organic compounds of fluorine). He published a paper on fluorine compounds (fluorbenzen) in the German magazine "Chemical annals" from Liebig. His notable studies on amides as well as those related to polymerization, addition and condensation of aldehydes were presented in 21 scientific articles. The fundamental discovery was his aldol-addition achieved in 1872 that soon led to the synthesis of the first sedatives. His successfully published work "The effect of ethyl iodide to hydrobenzamide and amarine" was notable. Having early realized the harmfulness of adverse effects of opioids (in the middle of the 19th century it came from Afghanistan), Borodin conducted the tests on the medicinal properties of Bukhara opium with high content of morphine that he announced in the article in 1876. By the way he examined the synthesis of fatty acids and the effect of antiseptic means (1878) and tea (1883) in human medicine and published it too [2]. Borodin was competing for superiority in the field of substitution and the addition of halogen elements in cyclic hydrocarbons to the famous chemist Kekulé. He also worked on the organohalogens synthesis important for medical industry of that time: methyl chloride (kryoanesthetic, degreasing mean), chloroform (inhalation drug, penicillin extractor, an organic solvent), ethyl chloride (inhalation anesthetic), iodine (antiseptic), war poisons (mustard gas, vesicants, phosgene), vinylbromide (component of rubber for dental prosthesis) and many other substances [10, 11]. He did and described the aldol synthesis at the same time as Wurtz did (1872) although this reaction was named only by the German author ("Wurtz's synthesis"). It is explained by the strong influence of German lobby in the European Chemists' Association, as well as the first previously published statement issued by the author Wurtz.

Due to excessive effort in the field of medicinal chemistry in 1874, Borodin was appointed the head of chemical laboratories at the Military Medical Academy.

The last completed Borodin study was to investigate the amount of urea in urine. Specifically, in 1876 for a purpose Borodin invented a nitrogenous method and apparatus for quantitative determination of urea by measuring the quantity of elemental nitrogen extracted from urine. He published detailed article about this method in the same year. This easy and precise method soon became widely accepted in biochemical and clinical laboratories [1]. Borodin also continued to investigate the toxicological aspects of phosphoric and arsenic acid started as early as during his PhD researches.

Overall, Borodin presented on life over forty and published around 20 papers of high quality in Russian and foreign journals. Actually, on that way Borodin fought for supremacy in many fields of toxicology, biochemistry and organic chemistry with German and French researchers.

Because of dedicated work, the Academy promoted him to title of academician in 1877.

Borodin did not reach the old age but died of massive heart attack in 1887 at his own admission, the masked ball in the lobby of the Academy. Historical sources indicate different causes of death: ..."rupture of blood vessels of the heart" [12], …"congestive heart disease" [9] or …"aortic aneurysm" [13].

Thousands of people were in the parade to pay a final tribut to him, when the young ones carried the coffin on their hands to the grave drenched with the most sincere tears of all those who loved him and knew his creativity, endless kindness, spirituality and goodness [3]. He was buried with great honour next to Modest Mussorgsky in the St. Petersburg laura of the Alexander Nevsky Monastery.

Instead of conclusion – in praise of great composer and scientist

Borodin became famous in music for life attained the fame noticed by a musical genius of that time, Franc List, whom he met once in 1877. List organized for him with great compliment the performance of the First Symphony in 1880 in Germany with the advice …"not to change anything in so original style of composing…" Belgian Countess Mercy-Argento propagated his music across France and Belgium, what consequently quickly spread Borodin's glory and across the Atlantic.

It is known that the Borodin's music influenced French composers Debussy and Ravel. The latter one paid homage to him in 1913 in the piano piece.

Evoking memories to Borodin, Robert Wright and George Forest created the work "Kismet" ("Fate"), an adaptation of his compositions in 1953, most pronounced in the song "Stranger in Paradise". Realizing the size of Borodin music through the presentation of this compilation, he was posthumously awarded by the "Tony Award" recognition in 1954 [12, 13].

It can be said that the popular arias from "Polovetzian dances", the unfinished opera "Prince Igor", which he used to make intermittently over 17 years, represent the pearl of evergreen music that popularizes the best authentic Russian

music arose from an open soul and the heart of Russian peasants.

However, due to many duties at the Academy, mentoring and nurturing a seriously ill spouse, lack of continuous spare time necessary for the serious business of music and weak concentration unfavorable for composing, Borodin failed to create a large opus of work. Besides, he was ill himself often. He barely attained to compose something meaningful on Sundays he called hence himself "a weekend composer" [13].

However, for a few compositions of the small opus he created (about 20), one can say to be the unrepeatable masterpieces of art music of a specific and unique expression of full flavor and taste of great Russia with nuances of oriental music. Borodin wove in a specific way lyricism and harmony derived from Russian folk songs.

How deeply ambivalent figure Borodin was the next quotation could explain ... "I am ashamed of own musical activities because I believe that music just relaxes me. I feel the passion to my main profession as research in the field of medical chemistry and biochemistry"... [1, 11].

This quote from a letter confirms Borodin's agonizing in many fields: ... "I compose during the winter when my illness does not allow lectures and work in the lab. Then I am forced to stay at home until my head bursting with pain and tears filled my eyes. That is why my music fans do not want me to be healthy but ill... "[14, 15].

In his best creative years Borodin left great works unfinished (opera "Prince Igor" and scientific publications), completed by his students and his colleagues (Alexander Glazunov and Rimsky-Korsakov). The successor of Borodin's chemical laboratory research was his brother in law, young A. P. Dianin.

Homage to this great researcher of medical chemistry presents the chemical reaction of halogen substitution in cyclic hydrocarbons that is well-known as "Hunsdicker's reaction". This reaction was published in Western Europe not until 1939, but performed as early as 1862 by Borodin. The Chemists' Association of Russia demonstratively but with full right named the substitution reaction as "Borodin's reaction". "Borodin's silver-decarboxylation reaction" was also named after him [14].

Although under-reported in the areas dealt with, overexerted by family obligations and professional activities, Borodin left an indelible stamp on art music of the national romanticism of the 19th century.

Borodin's researches in toxicology, biochemistry, organic and physiological chemistry altogether used as basic achievements led to the synthesis of important pharmacological substances that are being applied in modern medicine. In this manner, Borodin gave great impetus to the development not only of Russian but also of European medicine in the second half of the 19th century, otherwise some branches of up-to-date medicine could not exist.

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IZVEŠTAJ SA STRUČNOG SKUPA

41. simpozijum – Stremljenja i novine u medicini

The 41st Symposium – Aims and Inovations in Medicine

Redovni godišnji simpozijum Medicinskog fakulteta Univerziteta u Beogradu, „Stremljenja i novine u medicini“ u čast Dana fakulteta (9. 12) održan je 3–7. decembra 2012. u svečanoj sali Dekanata Medicinskog fakulteta.

Podeljen na brojne mini simpozijume posvećene različitim oblastima medicine, biologije, molekularne biologije, farmakologije, 41. simpozijum izazvao je posebnu pažnju svih onih koji pišu svoje naučne radove za medicinske časopise, svih onih koji rade u medicinskoj nauci i struci i najzad, iako ne najmanje važno, svih onih koji jezički uređuju naučne radove za objavljivanje u medicinskim časopisima svojim Mini simpozijumom „Medicinski jezik juče, danas, sutra“. Organizator ovog Mini simpozijuma, prof. dr Sofija Mićić, je profesor engleskog jezika na Medicinskom i Stomatološkom fakultetu (Doktorske studije).

Uzimajući u obzir činjenicu da je nagli i nezaustavljivi razvoj svih oblasti nauke i tehnologije, medicine, a naročito molekularne biologije i genetike, uglavnom u anglosaksonskim zemljama, izazvao i nastanak brojnih novih reči, naziva i izraza, moglo bi se reći da je angloglobalizacija sasvim normalan sled toga. Tako, engleski jezik ne samo da više nije vlasništvo jedne zemlje, ni samo pravi jezik sporazumevanja već je postao takozvani odomaćeni strani jezik u jezicima sveta, pa tako i u srpskom jeziku.

Engleski jezik uopšteno, a naročito jezik struke kao važni segment nauke i obrazovanja, naime kao *lingua franca* za medicinske akademske svrhe predstavlja veoma plodno područje proučavanja u svetu. Medicinski fakultet Univerziteta u Beogradu ima dugogodišnju tradiciju izučavanja i nastave medicinskog engleskog. Svoju studiju pod naslovom „Stavovi studenata prema učenju engleskog jezika medicine i prilagođavanje nastave potrebama studenata“ profesor Sofija Mićić i assist. Danka Sinadinović ilustrovale su upitnicima koje su popunili studenti pokazujući svoju potpunu svest o neophodnosti poznavanja ovog specijalizovanog jezika i pozitivan stav prema nastavi engleskog jezika.

Da neodgovarajuće preuzimanje anglicizama i njihovo mešanje sa srpskim jezikom može biti jezički nezgrapno i neprihvatljivo upozorio je dr Zoran Radovanović u svojoj studiji „Anglicizmi u našem narodnom zdravlju“, i ustvrdio da su oni znak nepoštovanja socijalnih i kulturnih vrednosti i nebrige za jezik. Za „nasilje nad jezikom“ autor posebno krivi „zdravstvene vlasti“ koje „uprkos upozorenju lingvista i jezički obrazovanih lekara“ nameću, na primer, sintagmu 'javno' umesto utemeljenog 'narodno' zdravlje, 'kontrolu' umesto 'suzbijanje' bolesti. Isto tako rogobatno izgledaju i kovanice „Nacionalni centar za farmakovigilancu, data centar, sentinel nadzor, subjunit vakcina i mnoge druge“. Kao dobar primer zabrinutosti zbog ove vrste bahatosti i nastojanja da se ona zaustavi, autor navodi zajedničku raspravu srbista, anglista i lekara na skupu pod nazivom „Jezik u medicini“ na Medicinskom fakultetu u Nišu u septembru 2012.

Prof. dr Goran Belojević u svom izlaganju „Nazivi bolnica u Srbiji“ otkrio je razloge za svakodnevni dolazak brojnih pacijenata u Institut za higijenu i medicinsku ekologiju, Klinički centar Srbije u kome on radi, iako su imali upute za doktora za uho ili doktora za kožne bolesti: nazivi susednih klinika, Klinika za otorinolaringologiju i Klinika za dermatovenerologiju izazivaju pometnju kod 25% funkcionalno nepismenih stanovnika. To nije slučaj sa nazivom „Dečja univerzitetska klinika“. Upotreba srpskih reči i standardizacija srpskog (ovde medicinskog) jezika zaista je neophodna.

Jezik predstavlja uslov za svaku dobru komunikaciju! Poznato je, takođe, da je dobar odnos između lekara i pacijenta veoma važan za uspešnu prevenciju, dijagnostiku, lečenje i rehabilitaciju i da se može uspostaviti samo preko jezika medicine. Autori rada „Lekarska tajna – bioetički aspekti“, dr Karel Turza, assist. dr Sandra Radenović i sar. dr Vida Jeremić podsećaju da je viševekovni paternalistički koncept odnosa lekar-pacijent bio gruba prepreka za razgovore lekara sa pacijentima. Upućivanje pacijenata u njihovo sopstveno stanje zdravlja, medicinske postupke ili moguće opasnosti nije bilo uobičajeno u svakodnevnoj lekarskoj praksi. U drugoj polovini 20. veka jezik medicine doživljava temeljitu promenu, svest o značaju mišljenja pacijenta toliko narasta da je informisani pristanak široko prihvaćen u medicinskoj etici, bioetici i pravnosankcionisan princip odnosa lekar-pacijent.

Neki kažu da „*Non est medicina sine lingua Latina*“. Nastavnik latinskog jezika, mr. Vera Marković, u saopštenju „Grčko-latinska terminologija kao osnov za međunarodni medicinski jezik“ ističe činjenicu da je u osnovi medicinskog jezika starogrčki jezik transkribovan u latinski! Jezik medi-

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cine stekao je status međunarodnog jezika pošto mu je u osnovi grčko-latinska terminologija. Medicinske reči koje se navode kao latinske potiču iz starogrčkog jezika „što je razumljivo ako imamo na umu da je starogrčka medicina predstavljala vrhunac medicine u starom veku“ istinoljubivo podseća mr. Vera Marković.

Ovu „vavilonsku“ pometnju valjalo bi ispraviti za vreme koje dolazi i olakšati složene vidove komuniciranja među ljudima, uopšteno govoreći, pa i u odnosu lekar-pacijent. U svom radu „Verbalna vs neverbalna komunikacija u medicini“ assist. dr Danijela Tiosavljević Marić i doc. dr Gordana Nikolić Balkoski mudro savetuju da lekar stalno radi na sebi „u cilju adekvatnog odgovora na raznovrsne potrebe obolele osobe“.

Upoređujući upotrebu termina za anatomske strukture u anatomskim atlasima, udžbenicima i naučnim radovima na srpskom i na engleskom jeziku, nastavnik engleskog jezika Biljana Vučković Lacković u „Srpska Nomina Anatomica: današnji zadatak za budućnost“ ukazuje na neophodnost „standardizacije latinskih i srpskih anatomskih termina“.

Još mnogo toga ostaje da se izvrši standardizacija jezika medicine kao važnog segmenta nauke i obrazovanja. Mnogo više napora potrebno je za ponovno uspostavljanje poštovanja prema vekovnoj tradiciji srpskog jezika i jezika struke kao važne podgrupe za posebne, akademske svrhe, jer pokidane su bezbrojne niti koje su nas spajale sa tim lepim i egzaktnim srpskim jezikom. To je bio jezik koji su svojom kulturom i učenošću raskošno obogaćivali Dositej Obradović, Matija i Ljubomir Nenadović, Jovan Jovanović Zmaj, Isidora Sekulić, Milan Rakić, Desanka Maksimović, Milutin Milanković, Miloš Crnjanski, Miloš N. Đurić, Mihajlo Đurić, Radomir Konstantinović, Laza K. Lazarević, Milan Jovanović Batut, Aleksandar Đ. Kostić, Vladan Đorđević – da spomenemo samo neke od čitave plejade velikana srpskog jezika i nauke. Pomenimo i veliki napor i trud koji je uložila prof. dr Sofija Mićić u višegodišnje istraživanje i prikupljanje građe za Medicinski rečnik u kome je sačuvala srpsku leksiku i srpski jezik tako što je standardizovala srpske reči umesto tuđica kad god je to bilo moguće. Objavila je monografiju „Nazivi bolesti i poremećaja u engleskom i srpskom“, a zatim je usledila knjiga „Studije o jeziku medicine u engleskom i srpskom“ koja predstavlja celokupan osvrt na ovu oblast jezika za akademske i stručne namene.

Dragana Mučibabić
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jezički redaktor časopisa Vojnosanitetski pregled

ERRATA

1. *Miroslav Kojić, Dragan Mikić, Darko Nožić, Lidija Zolotarevski*
Atypical form of cat scratch disease in immunocompetent patient
Periferna osteotomija sa karnojevim rastvorom kao racionalan pristup lečenju odontogene keratociste: prikaz bolesnika sa 5-godišnji praćenjem
Vojnosanit Pregl 2013; 70(1):IV

Erratum in: Vojnosanit Pregl 2013; 70(2): 239.
Miroslav Kojić, Dragan Mikić, Darko Nožić, Lidija Zolotarevski
Atypical form of cat scratch disease in immunocompetent patient
Atipična forma bolesti mačjeg ogreba kod imunokompetentne bolesnice

2. Vojnosanit Pregl 2013; 70(1): CXX
Janković Snežana.....743

Erratum in: Vojnosanit Pregl 2013; 70(2): 239.
Janković Snežana.....707,743

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Poziv za reklamiranje u 2013. godini

U prilici smo da vam ponudimo mogućnost oglašavanja i reklamiranja proizvoda i usluga u časopisu „**Vojnosanitetski pregled**“ (VSP). To je sigurno najbolji vid i najzastupljeniji način upoznavanja eventualnih korisnika sa vašim uslugama i proizvodima.

Časopis „**Vojnosanitetski pregled**“, zvanični organ lekara i farmaceuta Vojske Srbije, naučno-stručnog je karaktera i objavljuje radove iz svih oblasti medicine, stomatologije i farmacije. Radove ravnopravno objavljuju stručnjaci iz vojnih i civilnih ustanova i iz inostranstva. Štampa se na srpskom i engleskom jeziku. Časopis izlazi neprekidno od 1944. godine do sada. Jedini je časopis u zemlji koji izlazi mesečno (12 brojeva), na oko 100 strana A4 formata, a povremeno se objavljuju i tematski dodaci (suplementi). Putem razmene ili pretplate VSP se šalje u 23 zemlje sveta. Radove objavljene u VSP-u indeksiraju: *Science Citation Index Expanded (SCIE)*, *Journal Citation Reports/Science Edition*, *Index Medicus (Medline*), *Excerpta Medica (EMBASE), EBSCO* (preko ove baze VSP je *on line* dostupan od 2002. godine u *pdf* formatu) i *Biomedicina Serbica*.

Cene reklama i oglasa u časopisu „Vojnosanitetski pregled“ u 2012. godini su:

1.	Oglas u crno-beloj tehnici A4 formata za jedan broj	20 000,00 dinara
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Od 1. januara 2012. godine Vojnosanitetski pregled prešao je na e-Ur: Elektronsko uređivanje časopisa.

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http://aseestant.ceon.rs/index.php

U VSP-u se objavljuju **uvodnici**, **originalni članci, prethodna** ili **kratka saopštenja**, revijski radovi tipa **opšteg pregleda** (uz uslov da autori navođenjem najmanje 5 autocitata potvrde da su eksperti u oblasti o kojoj pišu), **aktuelne teme** ili **metaanalize**, **kazuistika**, članci iz **istorije medicine**, lični stavovi, naručeni komentari, pisma uredništvu, izveštaji sa naučnih i stručnih skupova, prikazi knjiga, referati iz naučne i stručne literature i drugi prilozi. Radovi tipa originalnih članaka, prethodnih ili kratkih saopštenja, metaanalize i kazuistike **objavljuju se uz apstrakte na srpskom i engleskom jeziku**.

Rukopis se piše sa proredom 1,5 sa levom marginom od **4 cm**. Koristiti font veličine 12, a načelno izbegavati upotrebu **bold** i *italic* slova, koja su rezervisana za podnaslove. Originalni članci, opšti pregledi i metaanalize ne smeju prelaziti 16 stranica (sa prilozima); aktuelne teme – osam, kazuistika – šest, prethodna saopštenja – pet, a pisma uredniku, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina.

Za obradu teksta koristiti program **Word for Windows** verzije 97, 2000, XP ili 2003. Za izradu grafičkih priloga koristiti standardne grafičke programe za **Windows**, poželjno iz programskog paketa **Microsoft Office** (**Excel, Word Graph**). Kod kompjuterske izrade grafika izbegavati upotrebu boja i senčenja pozadine.

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Priprema rada

Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** i **literatura.**

1. ***Naslovna strana***

a) Naslov treba da bude kratak, jasan i informativan i da odgovara sadržaju rada. Podnaslove treba izbegavati.

b) Ispisuju se puna imena i prezimena autora.

c) Navode se puni nazivi ustanove i organizacijske jedinice u kojima je rad obavljen i mesta u kojima se ustanove nalaze, sa jasnim obeležavanjem odakle je autor, koristeći standardne znake za fus-note.

2. ***Apstrakt i ključne reči***

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

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Zaključak** može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije. U **uvodu** ponovo napisati naslov rada, bez navođenja autora. Navesti hipotezu (ukoliko je ima) i ciljeve rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo strogo relevantne podatke iz literature i ne iznositi opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost etičkog komiteta.

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

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

Literatura se u radu citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i dodaje et al. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa *Interneta* citiraju se uz navođenje datuma.

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Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u desnom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fusnoti, ne u zaglavlju. Za fus-notu koristiti sledeće simbole ovim redosledom: ***, †, ‡, §, ||, ¶, **, ††, ...** Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

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Koristiti samo standardne skraćenice, izuzev u naslovu i apstraktu. Pun naziv sa skraćenicom u zagradi treba dati kod prvog pominjanja u tekstu.

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Preparation of manuscript

Parts of the manuscript are: **Title page; Abstract with key words; Text; References**.

1. ***Title page***

a) The title should be concise but informative. Subheadings should be avoided;

b) Full name of each author;

c) Name and place of department(s) and institution(s) of affiliation, clearly marked by standard footnote signs.

2. ***Abstract and key words***

The second page should carry a structured abstract with the title for original articles, metanalyses and case reports. The abstract should state the purposes of the study or investigation, basic procedures (selection of study subjects or laboratory animals; observational and analytical methods), main findings (giving specific data and their statistical significance, if possible), and the principal conclusions. It should emphasize new and important aspects of the study or observations. S t r u c t u r e d abstract should contain typical subtitles: *background/aim, methods, results* and *conclusion*. The abstract for metaanalyses and obrginal papers should have up to 450 words, and up to 150 words for case reports (with subtitles *background, case report, conclusion*). Below the abstract authors should provide, and identify as such, 3–10 key words or short phrases that will assist indexers in cross-indexing the article and will be published with the abstract.

3. ***Text***

The text of original articles is divided into sections with the headings: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

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

Discussion is to emphasize the new and important aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

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References should be superscripted and numbered consecutively in the order in which they are first mentioned in the text. **The references must be verified by the author(s) against the original document.** List all authors, but if the number exceeds 6, give 6 followed by et al. Do not use abstracts, secondary publications, oral communications, unpublished papers, official and classified documents. References to papers accepted but not yet published should be designated as ”in press“. Information from manuscripts not yet accepted should be cited in the text as ”unpublished observations“. References are cited according to the ***International Committee of Medical Journal Editors.*** *Uniform Requirements for Manuscripts Submitted to Biomedical Journals. Ann Intern Med 1997; 126: 36–47. Updated October 2001.*

Examples of references:

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

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.

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

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Each table should typed double-spaced on a separate sheet, numbered in the order of their first citation in the text in the upper right corner and supplied with a brief title each. Explanatory notes are printed under a table, using the following symbols, in this sequence: ***, †, ‡, §, ||, ¶, **, ††, ...** Each table has to be mentioned in the text. If you use data from another source, acknowledge fully.

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Figures are submitted as photos which should be sharp. Letters, numbers, and symbols should be clear and even throughout and of sufficient size that when reduced for publication, each item will still be legible. Each figure should have a label on its back indicating the number of the figure, author's name, and top of the figure. If a figure has been published, acknowledge the original source.

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Use only standard abbreviations. Avoid abbreviations in the title and abstracts. The full term for which an abbreviation stands should precede its first use in the text.

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