YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2018; November Vol. 75 (No. 11): p. 1061–1150.

Vojnosanitetski Pregled 2018 November Vol. 75 (No. 11): p. 1061–1150.

vol. 75, br. 11, 2018

VOJNOSANITETSKI PREGLED

Prvi broj *Vojnosanitetskog pregleda* izašao je septembra meseca 1944. godine

Časopis nastavlja tradiciju *Vojno-sanitetskog glasnika*, koji je izlazio od 1930. do 1941. godine

IZDAVAČ
Univerzitet odbrane, MO Republike Srbije

ISSN 0042-8450
eISSN 2406-0720
Open Access
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Adresa redakcije: Univerzitet odbrane, Institut za naučne informacije, Crnotravska 17, 11 040 Beograd, Srbija.
Informacije o pretplati: Tel.: +381 11 3608 997. E-mail (redakcija): **vsp@vma.mod.gov.rs**

Radove objavljene u „Vojnosanitetskom pregledu“ indeksiraju: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), EBSCO, Biomedicina Serbica. Sadržaje objavljuju Giornale di Medicine Militare i Revista de Medicina Militara. Prikaze originalnih radova i izvoda iz sadržaja objavljuje International Review of the Armed Forces Medical Services.

Časopis izlazi dvanaest puta godišnje. Pretplate: Žiro račun br. 840-19540845-28, poziv na broj 122742313338117. Za pretplatu iz inostranstva obratiti se službi pretplate na tel. +381 11 3608 997. Godišnja pretplata: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za pretplatnike iz inostranstva. Kopiju uplatnice dostaviti na gornju adresu.

Štampa Vojna štamparija, Beograd, Resavska 40b

vol. 75, No. 11, 2018

VOJNOSANITETSKI PREGLED

The first issue of *Vojnosanitetski pregled* was published in September 1944
The Journal continues the tradition of *Vojno-sanitetski glasnik* which was published between 1930 and 1941

PUBLISHER
University of Defence, Ministry of Defence of the Republic of Serbia, Belgrade, Serbia

ISSN 0042-8450
eISSN 2406-0720
Open Access
(CC BY-SA)

Editorial Office: University of Defence, Institute for Scientific Information, Crnotravska 17, 11 040 Belgrade, Serbia.
E-mail: **vsp@vma.mod.gov.rs**

Papers published in the Vojnosanitetski pregled are indexed in: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), EBSCO, Biomedicina Serbica. Contents are published in Giornale di Medicine Militare and Revista de Medicina Militara. Reviews of original papers and abstracts of contents are published in International Review of the Armed Forces Medical Services.

The Journal is published monthly. Subscription: Giro Account No. 840-19540845-28, refer to number 122742313338117. To subscribe from abroad phone to +381 11 3608 997. Subscription prices per year: individuals 5,000.00 RSD, institutions 10,000.00 RSD, and foreign subscribers 150 €.

Printed by: Vojna štamparija, Beograd, Resavska 40b

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The exhibit at the Military Museum in Belgrade
Eksponat u Vojnom muzeju u Beogradu.

'Serbian barrel' was a simple, but effective device in the fight against the typhus epidemic in the First World War. It consisted of a wood barrel with a lattice floor, placed on an iron bin in which water was heated. The resulting hot water vapor flowed through the laundry and the clothes that were placed in the barrel and destroyed lice.
In this issue of the Vojnosanitetski pregled there is an article by Goran Čukić entitled *`Serbian, the first phase` of the suppression of epidemics in 1914 and 1915*, describing the method of fighting the spread of the typhus epidemic in the first years of the Great War (see pp. 1143–8).

'Srpsko bure' bilo je jednostavna, ali efikasna naprava u borbi protiv epidemije tifusa u Prvom svetskom ratu. Sastojalo se od kazana u kome se zagrevala voda, a na koje se stavljalo drveno bure sa rešetkastim dnom. Nastala vrela para prolazila je kroz veš i odeću naslaganu u buretu, uništavajući vaške.
U ovom broju Vojnosanitetskog pregleda nalazi se članak Gorana Čukića *'Srpska, prva faza' suzbijanja epidemija 1914. i 1915. godine*, u kome je opisan način borbe protiv širenja epidemije tifusa u prvim godinama Velikog rata (vidi str. 1143–8).

ORIGINAL ARTICLES

UDC: 616.89-008.441.44:616.89-008.45
https://doi.org/10.2298/VSP161201023T

Relationship of depersonalization and suicidality in depressed patients

Povezanost depersonalizacije i suicidalnosti depresivnih bolesnika

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Abstract

Background/Aim. Depersonalization is considered to be the third leading symptom in psychiatric morbidity. The aim of this study was to investigate the correlation of depersonalization and different patterns of suicidal behaviour in patients suffering from depresssive disorder. **Methods.** The study included 119 depressed patients divided into two groups: the first group consisted of depressed patients with clinically manifested depersonalization according to the Cambridge Depresonalisation Scale presented score ≥ 70, and the second group consisted of the patients whithout clinically manifested depersonalization symptomatology, or, it was on the subsyndromal level. Subsequently, these two groups were compared regarding the suicidality indicators. **Results.** According to the Scale for Suicide Ideation of Beck, the depressed patients with depersonalization had statistically significantly higher scores regarding suicidal ideation, both active and passive, more often manifested suicidal desire, suicidal planning and overall suicidality ($p < 0.000$). Positive ideation, as a protective factor, was reduced in this group ($p < 0.000$). These patients had more previous suicide attempts ($p < 0.001$) and family history of suicides ($p = 0.004$). The depressed patients with depersonalization had 8 times more often active suicidal desire, 11 times more often passive suicidal desire and 5 times more often suicidal planning compared to patients without depersonalization. **Conclusion.** Suicidal potential, manifested in various patterns of suicidal behaviour among the patients suffering from depressive disorder with clinically manifested depersonalization is prominent. It is necessary to pay particular attention to depersonalization level during diagnostic and treatment procedure of the depressed patients having in mind that it may be associated with high suicidal potential.

Key words:
depression; depersonalization; suicide; risk factors; mental disorders; behaviour; suicide, attempted.

Apstrakt

Uvod/Cilj. Depersonalizacija, uz anksioznost i depresiju spada među tri najvažnija psihijatrijska simptoma. Cilj istraživanja bio je da se utvrdi povezanost depersonalizacije i različitih oblika suicidalnog ponašanja kod depresivnih bolesnika. M**etode.** Istraživanjem je obuhvaćeno 119 depresivnih bolesnika (21% muškog, 79% ženskog pola). U istraživanju su korišćeni: *Cambridge Depersonalization Scale* (CDS), *Scale for Suicide Ideation of Beck* (SSI) i *Positive and Negative Suicidal Ideation* (PANSI). Na osnovu skora na CDS skali bolesnici su podeljeni na grupu bolesnika sa depersonalizacijom (skor ≥ 70) i na grupu bolesnika bez depersonalizacije (< 70). Ove dve grupe su komparirane u odnosu na indikatore suicidalnosti. **Rezultati.** Oboleli od depresije sa depersonalizacijom imali su statistički značajno veći skor za suicidalne ideje po Bekovoj skali za suicidalne ideje, češće su manifestovali, kako aktivne, tako i pasivne suicidalne želje, suicidalne planove i globalnu suicidalnost ($p < 0.000$). Pozitivne ideje, kao protektivni faktor, bile su redukovane u ovoj grupi ispitanika (prva grupa) ($p < 0.000$). Ovi bolesnici imali su više ranijih pokušaja suicida ($p < 0.001$), kao i suicide u porodičnoj istoriji ($p = 0.004$). Depresivni bolesnici sa depersonalizacijom imali su osam puta češće aktivne suicidalne želje, jedanaest puta češće pasivne suicidalne želje i pet puta češće suicidalne planove u odnosu na bolesnika bez depersonalizacije. **Zaključak.** Suicidalni potencijal, manifestovan kroz različite obrazce suicidalnog ponašanja obolelih od depresivnog poremećaja sa visokom depersonalizacijom, je izražen. Neophodno je obratiti posebnu pažnju na nivoe izraženosti depersonalizacije tokom dijagnostičkih i terapijskih procedura depresivnih bolesnika, imajući u vidu da postojanje depersonalizacije može biti povezano sa visokim suicidalnim potencijalom.

Ključne reči:
depresija; depersonalizacija; samoubistvo; faktori rizika; psihički poremećaji; ponašanje; samoubistvo, pokušaj.

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Introduction

There is a number of identified risk factors that can provide clinicians with a suicide risk profile. Thus, health professionals who are familiar with these risk factors can indentify potential at risk patients for further assessment of suicidality and preventive measures [1].

Some studies showed that some psychiatric disorders and conditions are related to a high suicide risk, especially mood disorders, psychotic disorders, anxiety disorders, some personality disorders as well as substance abuse and dependence (particularly alcohol) [1–4]. Major depression is outlined as a particularly significant suicide risk factor because even 50% of those who attempted a suicide were suffering from this disorder. In addition to the diagnosis itself, the presence of specific symptoms occuring within the depressive syndrome may be associated with an increased suicide risk.

On the other hand, in terms of frequency depersonalization is a symptom considered to be at the third place on the scale in psychiatric morbidity (just after anxiety and depression) [5]. However, it is often not recognized. According to data from literature, there is relatively a high prevalence of depersonalization symptomatology in depressive disorder [6–8]. The depersonalization symptomatology within depressive disorder was found in 4% of patients in primarly care [9], 28% of outpatients [10] and even 60% of inpatients [11].

Due to very unpleasant experience, such as feeling that their own body, mental processes and environment are strange and changed or numbness of perceptive experience, patients with depersonalization are occassionally apt to self-injuring which shortly interrupts the horror of changed experience [12]. Also, the depersonalization is associated with an increase of suicidal ideation as well as suicidality in general. In the community-based survey with 5,000 participants, the authors found out that depersonalization and the Type-D personality are uniquely associated with suicidal ideation [13]. In a non-clinical sample of 7,905 participating surgeons, the presence of suicidal ideation was related with all 3 domains of burnout (emotional exhaustion, depersonalization and low personal accomplishment) and symptoms of depression [14].

One of the suicidality risk factors was previous suicide attempt. Some authors describe it as the most important risk factor [15].

Having in mind the aforementioned as well as the fact that depersonalization very often goes along, i.e., represents the associated symptom in depressive disorder, the aim of this study was to investigate the correlation of depersonalization and diferent patterns of suicidal behaviour in subjects suffering from depressive disorder.

Methods

The study included 119 patients, of both genders [25 (21%) males, 94 (79%) females] The inclusion criteria for our cross-section study were: diagnosis of depressive episode or recurrent depressive episode (F32.0-2, F33.0-2) according to ICD X, age 18–65, primary education minimum, the absence of cognitive impairment or organic couse of depression (F06.3), mental retardation, substance abuse disorders, a history of seizures, absence of serious medical (somatic) illnesses that were not considered well-controlled. Patients with psychotic feature, or hystory of (hypo)manic episodes, according to ICD X, were excluded from our investigation. All study patients were consecutively admitted to hospital treatment at the Psychiatry Clinic, Gornja Toponica, or treated as outpatients at the Clinic for Mental Health Protection, Niš. All patients who passed inclusion criteria were tested cross sectionally during treatment at mentioned psychiatric institutions. All psychological assessments were focused on the areas of depression, depersonalization and suicidality. Standard psychometric instruments were: the Cambridge Depersonalization Scale (CDS) [15] for measuring an intensity of depersonalization symptomatology (scores exceeding or equal 70 represent the indicators of clinically manifested depersonalization). This scale consists of 29 items, the Scale for Suicide Ideation of Beck (SSI) – suicidality assessment scale [16], comprised 19 items. We can obtain three subscales: active suicidal desire, passive suicidal desire and specific suicidal plan as well as a total score of suicidality, (higher scores indicate greater level of suicidality); Positive and Negative Suicidal Ideation (PANSI) [17] is the 14-item scale for assessing suicidal thoughts (data processing provides evidence about positive and negative suicidal thinking).

All examined patients also responded to the questionnaire items, devised by the authors .The questionnaire items focused on their sociodemographic characteristics as well as previous suicide attempts and family history of suicide.

All 119 depressed patients were divided into two groups: the first group consisted of depressed patients with clinically manifested depersonalization (according to CSD [15] presented score ≥ 70), and the second group of patients was without clinically manifested depersonalization symptomatology, or it was on the subsyndromal level. Based on these criteria, the group with depersonalization consisted of 50 patients and the group without depersonalization consisted of 69 patients. Subsequently, these two groups were compared on the basis of indicators of suicidality.

The study was approved by the Regional Ethical Committee, all patients gave written consent and the study was performed in full accordance with the Declaration of Helsinki (1965) and later revisions.

Within and between the groups comparison were performed using The Statistical Package for the Social Sciences, Version 17 (SPSS 17). Preliminary analysis was performed to ensure there was no violation of the assumptions of linearity and normality. In order to determine whether the data were normally distributed, we used the Kolmogorov-Smirnov test (KS-test). Data were expressed as mean ± standard deviation (SD), except for non-Gaussian parameters, which were presented as median (range). We used Student's *t*-test for parametric data. For nonparametric data, we used χ^2 test, Spearman's rho, Mann-Whitney *U*, Phi and odds ratio with confidence intervals. All reported *p*-values are exact two-sided significance levels. Statistical significance was defined at $p < 0.05$.

Results

Patients

Both groups did not significantly differ concerning gender, place of residence, age and level of education (Table 1). In both groups, the majority of patients were females and most of participants lived in urban environment (in town). The average age of patients in the group with clinically manifested depersonalization was 42.11 ± 11.82 years and in the group without clinically manifested depersonalization was 44.93 ± 11.20 years ($t = 1.188$, df = 117, $p = 0.237$). Most of the patients had a standing partner. The patients with the intermediate level of education dominated in both groups.

Suicidal ideation

Positive ideation, i.e., positive attitudes to life opposite to suicide was more intensive in the group of patients without depersonalization (mean rank 72.29). There was a statistically significant difference compared to the group with depersonalization where the mean rank was 43.04 (Mann-Whitney $U = 877.0$, $p < 0.0001$). Depersonalization score was in negative correlation with positive suicidal ideation and correlation was statistically significant (Spearman's rho = -0.452, $p < 0.0001$).

Suicidal desire

Suicidal desires distribution in examined patients in both groups is shown in Table 2.

Suicidal desire, both active and passive, was more often present among the depressed patients with depersonalization (the first group) (Table 2).

There was a highly significant association between the level of depersonalization and suicidal desire (active and passive).

Sucidal planning

Suicidal planing was more often reported by the patients from the first group (Table 2). The correlation between the level of depersonalization and suicidal planing was positive and statistically significant (Table 3).

Overall suicidality

Similar to previous results and in accordance with it, the presence of suicidality in general was more often reported within the group with depersonalization (Table 2). There was a significant association between depersonalization and suicidality (odds ratio 6.6) (Table 2). Correlation between level of depersonalization and general suicidality scores was positive and statistically significant (Table 3).

Table 1

Sociodemographic data of patients included in the study

Characteristics	Patients with depersonalization n (%)	Patients without depersonalization n (%)	χ^2	Df	*p*
Gender			1.303	1	0.265
female	42 (84)	52 (75.4)			
male	8 (16)	17 (24.6)			
Place of residence			0.531	1	0.767
town	26 (52)	40 (58)			
village	19 (38)	24 (34.8%)			
big village	5 (10)	5 (7.2)			
Partnership			2.628	1	0.165
with partner	35 (70)	57 (82.6)			
single	15 (30)	12 (17.4)			
Education			2.836	1	0.425
low (8 years)	6 (12)	13 (18.8)			
medium (12 years)	36 (72)	46 (66.7)			
higher (15 years)	4 (8)	2 (2.9)			
high (16–18 years)	4 (8)	8 (11.6)			
Previous suicide attempt			12.950	1	< 0.001
yes	25 (50)	13 (18.8)			
no	25 (50)	56 (81.2)			
Family history of suicide			8.881	1	0.004
yes	16 (32)	7 (10.1)			
no	34 (68)	62 (89.9)			

Table 2

Suicidal behavior and depersonalization

Suicidality (sub) scale	Patients with depersonalization n (%)	Patients without depersonalization n (%)	χ^2	df	*p*	Odds ratio	95% confidence interval
Active suicidal desire			24.585	1	< 0.001	8.0	3.350–19.188
yes	41 (82)	25 (36.2)					
no	9 (18)	44 (63.8)					
Passive suicidal desire			37.728	1	< 0.001	11.3	4.716–27.258
yes	40 (80)	18 (26.1)					
no	10 (20)	51 (73.9)					
Specific suicidal plan			16.189	1	< 0.001	5.0	2.224–11.239
yes	28 (56)	14 (20.3)					
no	22 (44)	55 (79.7)					
General suicidality			20.416	1	< 0.001	6.7	2.804–15.872
yes	41 (82)	28 (40.6)					
no	9 (18)	41 (59.3)					

Table 3

Association of suicidal behavior and depersonalization among patients with depersonalization

Variable	Phi	*p*
Active suicidal desire	0.455	< 0.001
Passive suicidal desire	0.532	< 0.001
Specific suicidal plan	0.369	< 0.001
General suicidality	0.414	< 0.01
Previous suicidal attempts	0.330	< 0.001
Family history of suicide	0.273	0.004

Previous suicide attempt

In our study, higher percentage of subjects who previously attempted suicide was in the group with depersonalization (even 50%), while in the group of patients without depersonalization disorder, it was almost two-thirds less (18.8%). After the statistical processing, we obtained statistically significant difference between the groups (Table 1).

Family history of suicide

Regarding the family history, there was a statistically significant difference between the groups (Table 1).

Discussion

Suicidal ideation refers to thoughts, fantasies, ruminations and preoccupations with death, self-harm and self-inflicted death [18]. Suicidal ideation is presented by two variables: positive and negative ideation. Our study showed that the depressed patients with high depersonalization (≥ 70, according to CDS), had significantly reduced positive thinking about life, therefore reduced positive ideation as an important suicide protective factor. At the same time, negative ideation significantly increased, reflecting a lack of motivation for life and giving advantage to suicide as a possible way of resolving the actual situation. Our results are in accordance with Yoshimasu et al. [19], in part that refers to male subjects. Based on Spearman's rho coefficients, increasing of depersonalization in depressive disorder, resulted in the reduction of intensity of positive ideation and increasing of negative ideation. Suicide ideas could be active, when a person clearly wishes to commit suicide, or passive, when a person does not try to protect himself/herself in situations potentially dangerous for their life. This pattern of suicide behaviour was significantly more expressed in the depressed patients with clinically relevant depersonalization, 80% vs. 20% (among the patients without depersonalization). The depressed patients with depersonalization compared with those without this disorder presented eight times more often active suicidal desire and 11 more often had passive suicidal desire (according to odds ratio), indicating the strong association between depersonalization and suicidal desire (active and pasive) as one of the suicide risk factors.

Our study patients who suffered from depression with concomitant depersonalization five times more often than patients without depersonalization had suicidal planing (according to odds ratio), indicating the suicidal intent (suicidal plan making) as a serious risk factor which was also strongly associated with depersonalization.

The presence of overall suicidality was in accordance with previous results, indicating that the depressed patients with depersonalization had five times more often then those without depersonalization any type of suicidality. The similar conclusion was derived from the results of the previous studies [18, 20], that reported the higher risk if suicidal thoughts were present longer and occurred more frequently [18, 20]. Our study results also indicated that, in order to assess suicidality, it is very important to establish not only the existance of suicidal thoughts, but also to determine their intensity as a prominent suicidal risk factor.

There are some other facts which indicate and raise suicidal risk. First of all, previous suicide attempt(s) is/are a bad prognostic sign(s) because of a great risk of reattempting or

commiting suicide [21, 22]. In our study almost triple number of patients with previous suicide attempt(s) were in the group with depersonalization which indicated that the combination of previous attempt(s) increases additionally the suicide risk and potential mortality. At the same time, the presence of family history regarding a suicide was also tripled in the group with depersonalization, so this was considered a significant risk factor by some authors [23, 24]. Previous suicidal attempts and family history of suicide had further negative impact on global suicidality pattern in depressed patients with concomitant depersonalization. The association between depersonalization and suicidality in depressive disorder was significant and may be considered as a bad prognostic sign.

However, there are some study limitations: small sample size, cross-section study design, the lack of explanation what kind of relationship it is – direct or indirect among depersonalization and suicidality. In order to find out an answer, we should perform further analysis that could uncover the main road of this association (such as mediation analysis) as well as conduct a research on a larger sample. Our findings are based on a limited number of patients which makes our data vulnerable to statistical biases and increases the threshold for obtaining statistical significance between groups. Data reaching statistical significance may therefore be viewed as highly indicative though not conclusive.

Conclusion

Suicidal potential in persons affected by depressive disorder with clinically manifested depersonalization is prominent. Concomitant pathological depersonalization among depressed patients was associated with the increase of suicidal ideas, active and pasive suicidal desire and suicidal planning. Suicide attempts as well as family history of suicide among depressed patients with depersonalization, additionaly increase a suicide risk. It is necessary to pay particular attention to depersonalization level, during diagnostic and treatment procedure od depressed patients, having in mind that it may be associated with high suicidal potential.

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Received on December 1, 2016.
Revised on January 17, 2017.
Accepted on January 19, 2017.
Online First February, 2017.

ORIGINAL ARTICLE

UDC: 616.9:578]:616.36-002.2-06
https://doi.org/10.2298/VSP161115031P

The occurrence of liver steatosis in patients with chronic hepatitis C – the experience of the Clinical Center of Vojvodina, Serbia

Pojava steatoze jetre kod bolesnika sa hroničnim hepatitisom C – iskustvo Kliničkog centra Vojvodine

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Abstract

Background/Aim. Hepatic steatosis in patients with chronic hepatitis C occurs in about half of the cases. Its occurrence is influenced by factors of the host and viral factors and its importance lies in the fact that it reduces the success of antiviral therapy based on interferon in the treatment of chronic hepatitis C and that, associated with other factors, exacerbates liver disease. The aim of this study was to determine the prevalence and severity of steatosis in patients with chronic hepatitis C and to determine the factors that affect its occurrence. **Methods.** The study included 123 patients with chronic hepatitis C with diagnosis of liver steatosis made by liver biopsy and histopathological examination according to which ≥ 5% of hepatocytes was affected by fatty change. Based on the presence of steatosis, the patients were divided into two groups: 43 patients with steatosis and 80 patients without steatosis. The influence of certain factors on the occurrence of steatosis was examined using standard statistical methods. **Results.** Liver steatosis was found in 34.96% of patients with chronic hepatitis C, and a majority of patients (76.74%) had mild steatosis. Of the examined predictive factors for the occurrence of steatosis, statistical significance in its occurrence was connected to elevated body mass index (BMI), genotype 3 hepatitis C virus (HCV) and HCV viremia. **Conclusion.** Hepatic steatosis often occurs in people with chronic hepatitis C, and most often it is mild. The occurrence of hepatic steatosis in our sample was most often affected by genotype 3 HCV and HCV viremia level. Hepatic steatosis can reduce the success of antiviral therapy based on interferon and negatively affect chronic liver disease course. Therefore, we need to recognize it, treat it and make it withdraw.

Key words:
fatty liver; hepatitis c; risk factors; genotype; hepacivirus; body mass index.

Apstrakt

Uvod/Cilj. Steatoza jetre se javlja kod oko polovine bolesnika sa hroničnim hepatitisom C. Na njenu pojavu utiču faktori domaćina i faktori virusa, a njen značaj je u tome što smanjuje uspeh antivirusne terapije za lečenje hroničnog hepatitisa C zasnovane na interferonu i što, udružena sa drugim faktorima, pogoršava bolest jetre. Cilj ovog rada bio je da se utvrdi prevalenca i težina steatoze kod obolelih od hroničnog hepatitisa C i da se utvrde faktori koji utiču na njenu pojavu. **Metode.** Istraživanjem su obuhvaćena 123 bolesnika sa hroničnim hepatitisom C, kod kojih je dijagnoza steatoze jetre postavljena biopsijom jetre i patohistološkim (PH) pregledom. Uslov za pozitivnu PH dijagnozu steatoze jetre bio je da ≥ 5% hepatocita bude zahvaćeno masnom promenom. Na osnovu prisustva steatoze bolesnici su bili podeljeni u dve grupe: 43 ispitanika sa steatozom i 80 bez steatoze. Ispitivan je uticaj pojedinih faktora na nastanak steatoze, uz korišćenje standardnih statističkih metoda. **Rezultati.** Steatoza jetre nađena je kod 34,96% ispitanika sa hroničnim hepatitisom C, a najveći broj ispitanika (76,74%) imao je blagu steatozu. Od ispitivanih prediktivnih faktora za pojavu steatoze, statističku značajnost u njenoj pojavi imali su povišen indeks telesne mase [*body mass index* (BMI)], genotip 3 hepatitis C virusa (HCV) i HCV viremija. **Zaključak.** Steatoza jetre se često javlja kod bolesnika sa hroničnim hepatitisom C i najčešće je blaga. Na pojavu steatoze jetre u našem uzorku najviše su uticali genotip 3 HCV i visina HCV viremije. Steatoza jetre može umanjiti uspeh antivirusne terapije zasnovane na interferonu i može pogoršati tok hronične bolesti jetre. Zbog toga je treba prepoznati, lečiti i ukloniti.

Ključne reči:
jetra, masna infiltracija; hepatitis c; faktori rizika; genotip; hepatitis c, virus; telesna masa, indeks.

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Introduction

Interconnection and association of chronic hepatitis C (CHC) and liver steatosis were observed before detecting hepatitis C virus (HCV) when the occurrence of steatosis in the histopathological liver examinations of the patients with hepatitis non-A non-B with characteristic changes of chronic hepatitis was found [1]. Hepatic steatosis and CHC are more often found associated than separated, and the prevalence of hepatic steatosis in the patients with CHC is around 55% [2, 3]. With the prevalence of hepatitis C in general population from 1.6% to 2.8% worldwide and non-alcoholic fatty liver disease (NAFLD) from 20% to 30% of adults in Western countries, a significant number of patients in the world is affected with these two diseases [4–7].

Hepatitis C is a liver inflammation caused by HCV which in 75%–85% of cases has a chronic course with persistent liver inflammation. In case of the presence of risk factors, such as excessive alcohol consumption, age greater than 40 years at the time of acquiring infection, male gender, coinfection with hepatitis B virus (HBV), human immunodeficiency virus (HIV), the simultaneous presence of steatohepatitis, insulin resistance, etc., it could result in further progression of the disease and development of liver cirrhosis in approximately 10%–20% of patients during 20–30 years after acquiring HCV infection [8, 9]. HCV has 6 genotypes, of which the most often genotypes in humans are genotype 1 and 3 which have a proven direct steatogenic effect on liver [10, 11]. Hepatic steatosis may be mild and stable disease, but, if accompanied with necroinflammatory changes in hepatocytes (steatohepatitis), it receives a progressive form of the disease which induces the development of fibrosis, the formation of liver cirrhosis and even hepatocellular carcinoma [12, 13]. Hepatic steatosis occurs in people with excessive alcohol consumption and it is also common in obese patients, in patients with diabetes mellitus, dyslipidemia, as a side effect of certain drugs (corticosteroids) and states (parenteral nutrition, starvation) [7, 14].

In case of liver steatosis occurrence in patients with CHC, the 'viral factors' such as HCV genotype and HCV viremia, and the 'host factors' such as obesity, diabetes mellitus, dyslipidemia, alcohol consumption and others, are concerned when it comes to the development of steatosis [15]. If host factors are dominating, we talk about metabolic steatosis which is based on insulin resistance and which carries the risk of developing diabetes and cardiovascular diseases [16, 17]. With the infection with genotype 3 HCV, structural proteins of the virus act directly with a steatogenesis on hepatocytes causing the accumulation of fatty particles in hepatocytes. Therefore, liver steatosis with genotype 3 HCV infection is significantly more common (70%–80% of cases), and among them, the degree of steatosis correlates with HCV viremia and withdraws after successful eradication of viruses with antiviral therapy [18, 19]. The infection with other HCV genotypes involves the interference of metabolism of lipids which the virus uses for its life cycle in hepatocytes causing the formation of insulin resistance by a joint action of viral and metabolic factors of the host [20, 21]. In these patients, it is necessary to eliminate metabolic factors causing the development of liver steatosis and that involves dietary measures, treatment of insulin resistance, diabetes, hyperlipidemia, the application of hepatoprotectives, antioxidants, and lifestyle changes [22, 23]. Clinical significance of liver steatosis in patients with CHC reflects in a rapid progression towards liver fibrosis, reduces the success of the standard double antiviral therapy with pegylated interferon and ribavirin and increases a risk of cardiovascular diseases and diabetes [24, 25]. In case of unsuccessful treatment and the existence of other adverse factors, through the development of liver cirrhosis, steatosis can lead to the development of hepatocellular carcinoma [26, 27]. CHC and NAFLD are among most often indications for liver transplantation in developed countries [28, 29].

The aim of the study was to determine the existence, frequency and degree of liver steatosis in patients with CHC, and to examine the influence of individual factors of the host and viruses on the emergence appearance of liver steatosis.

Methods

The research included 123 patients with CHC at the Clinic for Infectious Diseases of the Clinical Center of Vojvodina (CCV) in Novi Sad, Serbia. All respondents gave their consent to participate in the research by signing an informed consent and the Ethical Committee of CCV approved the examination. Based on the presence of liver steatosis, the respondents were divided into two groups: there were 43 patients with hepatic steatosis and CHC in the first one and 80 people with CHC without steatosis in the other one. The CHC diagnosis was based on the presence of elevated activity of alanine aminotransferase (ALT) and anti-CHC antibodies in blood, lasting longer than six months, a positive Polymerase chain reaction (PCR) test and histopathological examination of a liver tissue sample obtained by blind biopsy. Qualitative and quantitative PCR test was done at the Virology Laboratory of the Institute for Infectious and Tropical Diseases at the Clinical Center of Serbia in Belgrade using Cobas Amplicor HCV Test version 2.0 (Roche Diagnostics, Menheim), sensitivity: 50 IU/mL. HCV genotyping was done using the Linear Array HCV genotyping test (Roche Diagnostics). Histopathological liver biopsy examination was done at the Centre for Pathology and Histology of CCV. Determination of necroinflammatory activity and the degree of fibrosis was expressed according to Knodell modified numerical score. The presence of steatosis was stated on the basis of the percentage of hepatocytes affected by fatty changes of ≥ 5%. The degree of steatosis was expressed by Brunt system modified by Kleiner, by which mild steatosis has 5%–33% of hepatocytes affected by the fatty change, moderate (> 33%–66%) and severe (> 66%). Data about taking medications that can lead to liver steatosis (amiodarone, nifedipine, diltiazem, tamoxifen, glucocorticoids, synthetic estrogens, methotrexate), contact with hepatotoxic substances (organic solvents, phosphorus, fungi toxins) and excessive alcohol consumption six months prior to the examination (daily alcohol intake of more than 20 g/day for women and more than 30 g/day for men) were

collected from the respondents, and such respondents were not included in the study. People with HBsAg and anti-HIV seropositivity were also not included in the study. All respondents did anthropometric measurements of body height and weight to calculate the body mass index (BMI) representing the ratio of body weight and body height square expressed in meters (kg/m^2) as follows: BMI > 30.0 kg/m^2 – obese people, BMI 25–29.9 kg/m^2 – overweight, i.e., pre-obese people and BMI 18.5–24.9 kg/m^2 – normal weight individuals.

Statistical data processing was carried out by using the statistical package SPSS version 13.0. Testing statistical significance was determined for parametric data by analysis of variance test (ANOVA) and for non-parametric tests by χ^2 test, Fisher's or Mann Whitney's test. For all tests, the level of of statistical significance was established at 0.05.

Results

Demographic and clinical characteristics of patients are shown in Table 1. Of 123 patients with CHC, liver steatosis was found in 43 (34.96%) respondents. Thirty-three patients (76.74%) had mild degree steatosis, six of them (13.95%) had moderate steatosis, while only four (9.3%) patients had signs of severe hepatic steatosis. As for the host factors which can influence the occurrence of steatosis, measuring BMI determined that respondents with steatosis had a statistically significant higher BMI compared to the group without steatosis (t = -4.129, p < 0.05). The mean BMI in respondents with hepatic steatosis amounted to 25.53 kg/m^2, whereas in respondents without liver steatosis the mean BMI was 23.53 kg/m^2. According to the BMI, respondents with hepatic steatosis fell within the range of pre-obese people, while those without steatosis fell within normal weight people range. The mean value of triglycerides in the group of subjects with hepatic steatosis was 1.503 mmol/L, which was statistically significantly higher than in the group without steatosis where this value was 1.228 mmol/L (t = -2.286, p < 0.05). The mean value of the total cholesterol in the serum of the respondents with liver steatosis amounted to 5.00 mmol/L, which was within the limits of the normal range and it was no significantly different when compared to the respondents without steatosis, where this value was 4.74 mmol/L (t = -1.445, p > 0.05). The analysis of HCV genotypes in the respondents with CHC and liver steatosis showed the greater share of genotype 3 compared to other genotypes in the respondents with hepatic steatosis and those without steatosis, 21/43 (48.84%) vs. 20/80 (25%), which was statistically significant (Fisher test, p < 0.05). The share of certain HCV genotypes in the group of patients with and without liver steatosisis is shown in Figure 1. As to the correlation of share of certain HCV genotypes and the degree of liver steatosis, we found that the share of genotype 3 HCV increased with the degree of steatosis which was statistically significant (c^2 = 7.882, p < 0.05). Measuring the level of HCV RNA in serum of the respondents in the group with hepatic steatosis, we found the mean value of 6,728.968 IU/mL which was statistically significantly higher than the mean value of HCV RNA level of the respondents without liver steatosis which was 2,397.232 IU/mL (t = -2.978, p < 0.05). Analysis of the mean values of the level of HCV RNA viremia in the respondents without steatosis, with first-degree steatosis and those with second and third-degree steatosis together, showed statistically significant difference among the observed groups. The mean value of HCV RNA viremia (Kruskal-Wallis test, X^2 = 9.492, p <0.05) increased with the degree of steatosis. The ratio of HCV RNA viremia and the degree of steatosisis is shown in Figure 2. The mean value of genotype 3 HCV RNA viremia in the group of subjects with hepatic steatosis was 10,833.357 IU/mL, which was significantly higher than the average viremia of HCV genotype 3 in the group of respondents without steatosis, 3,091.118 IU/mL (t = 2.207, p < 0.05). It was not the case with non-3 genotype HCV RNA viremia where a small difference in the mean viremia was measured (2,811.141 IU/mL in the group with steatosis, 2,165.936 IU/mL in the group without steatosis) which did not prove itself to be statistically significant. The ratio of the number of viral particles in serum of the patients with genotype 3 HCV infection and the degree of steatosis showed that the level of viremia increased with the degree of steatosis. The correlation was statistically significant (ANOVA, F = 3.36, p < 0.05). The ratio of HCV RNA viremia and the degree of steatosisis is shown in Figure 2.

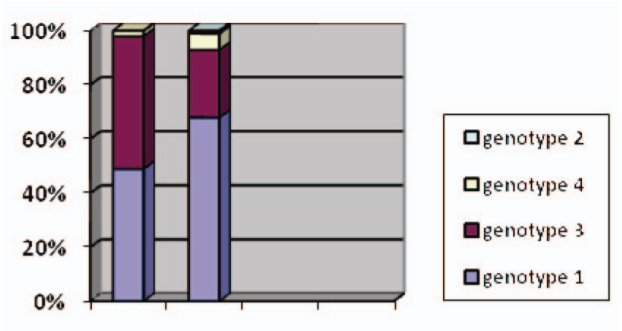

Fig. 1 – Hepatitis C virus (HCV) genotypes in patientes with without liver steatosis.
left – Steatosis; right – No steatosis

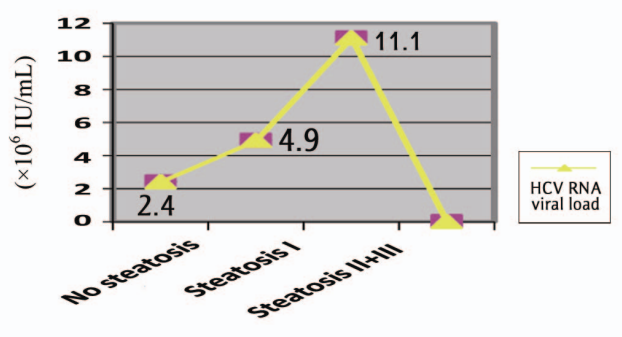

Fig. 2 – Hepatitis C virus ribonucleic acid (HCV RNA) viral load and the degree of liver steatosis.

Table 1

Demographic and clinical characteristics of patients

Characteristics	Steatosis (n = 43)	No steatosis (n = 80)	*p*
Gender, n (%)			
male	33 (76.7)	89 (72.4)	> 0.05
female	10 (23.3)	34 (27.6)	> 0.05
Age (mean ± SD; years)	35.8 ± 10.1	36.14 ± 11.6	> 0.05
BMI (mean ± SD; kg/m^2)	25.53	23.53	> 0.05
Glucosae (mean ± SD; mmol/L)	5.38 ± 1.42	5.03 ± 1.07	< 0.05
Triglycerides (mean ± SD; mmol/L)	1.503 ± 0.79	1.228 ± 0.527	> 0.05
Cholesterol (mean ± SD; mmol/L)	5.00 ± 1.16	4.74 ± 0.837	> 0.05
ALT (mean ± SD; IU/L)	116.26 ± 73.52	101.71 ± 74.38	> 0.05
AST (mean ± SD; IU/L)	59.67 ± 34.24	56.74 ± 38.13	> 0.05
GGT (mean ± SD; IU/L)	73.49 ± 53.12	70.21 ± 48.38	> 0.05
HCV genotype, n (%)			
1	21 (48.84)	54 (67.50)	> 0.05
2	1 (2.32)	1 (1.25)	> 0.05
3	21 (48.84)	20 (25.00)	< 0.05
4	0	5 (6.25)	> 0.05
HCV RNA viral load (×10^6 IU/mL), n (%)			
all genotypes	6.72	2.39	< 0.05
genotype 3	10.83	3.09	< 0.05
genotypes non-3	2.81	2.16	> 0.05

BMI – body mass index; ALT – alanine aminotransferase; AST – aspartate aminotransferase; GGT – gamma-glutamyl transferase; HCV – hepatitis C virus; RNA – ribonucleic acid; SD – standard deviation.

Table 2

Results of multivariate logistic regression predictive factors for the development of hepatic steatosis

Variable	Regression coefficient	OR	95% CI	*p*
BMI	0.364	1.439	1.175–1.761	0.000
HCV load	0.000	1.0	1.0–1.0	0.025
HCV genotip 3	0.043	1.113	1.053–1.100	0.010

BMI – body mass index; HCV – hepatitis C virus; OR – odds ratio; CI – confidence interval.

A summary of all the examined predictive factors for hepatic steatosis showed a statistical significance for elevated BMI, elevated triglycerides, genotype 3 HCV and HCV viremia. When all of the above factors were taken into account when calculating multivariate logistic regression, we found that elevated BMI, genotype 3 HCV and HCV viremia statistically significantly contribute to liver steatosis. Histology activity index (HAI) score in the respondents with hepatic steatosis was not significantly different compared to the respondents without liver steatosis (Mann-Whitney U = 1,687, $p > 0.05$). A statistically significant difference regarding the degree of liver fibrosis between the observed groups (Fisher test, $p > 0.05$) was not found (Table 2).

Discussion

The prevalence of steatosis in patients with CHC occurs in about half of the cases [2, 3]. In hepatitis C viral infection caused by genotype 3 HCV, steatosis prevalence is higher and more pronounced than in other genotypes. Liver steatosis with infection with other HCV genotypes (non-3 genotype) is associated with the metabolic factors of the host and the occurrence of insulin resistance. Accordingly, if the sample has more people infected with genotype 3 HCV, or if the sample has more people with pronounced metabolic factors such as obesity, hypertriglyceridemia, diabetes mellitus and hypertension, steatosis percentage is higher. Liver steatosis is concerned when more than 5% of hepatocytes is affected by fatty changes, which can be seen in the histopathological examination of liver biopsy, and radiological examinations when the accumulation of fatty particles in the liver is even more pronounced [30]. The overall prevalence of steatosis is largely affected by histopathological criterion (what the lowest percentage of hepatocytes affected by fatty change is required for the diagnosis of hepatic steatosis. For some, it is more than 0%, according to others it is more than 1%, somewhere it is more than 3%, but usually it is more than 5% of hepatocytes affected by the fatty change.

In people with CHC, our research determined the prevalence of steatosis of 34.96% (43/123). The resulting prevalence of steatosis was lower compared with the data of most authors, due to heterogeneous criteria, different demographic characteristics of the sample and different share of genotype 3 HCV. The closest prevalence of steatosis was obtained in the research of Greek authors (31.74%) and the research of Pakistani authors (39%) where there was a group of patients with very similar demographic and clinical characteristics and the same histological criteria for the diagnosis of steatosis [31, 32]. An interesting research was conducted by Pais et al. [33] who used steatotest as a noninvasive marker of steatosis

instead of liver biopsy and found that 43% of respondents with CHC had signs of steatosis, which was also similar to our result. In our neighborhood, a group of Hungarian authors [34], found steatosis in 64% of the respondents infected by genotype 1 HCV, but their sample also included the respondents with significant alcohol consumption, patients with diabetes and a significant number of obese patients with BMI > 30 kg/m^2, and these are all diseases and conditions which facilitate the development and incidence of liver steatosis; therefore, they obtained a significantly higher percentage of people with liver steatosis. A group of Romanian researchers [35] found liver steatosis in 76.59% of the respondents with CHC, which was significantly larger percentage than that of the respondents with other viral hepatitides. However, their work did not state histopathological diagnostic criteria for steatosis, did not include the share of genotype 3 HCV which is steatogenic and also did not include the demographic characteristics of the respondents such as obesity, patients habits (such as excessive alcohol consumption), or comorbidities that may cause steatosis. Our results showed that most respondents, 33 (76.74%), had signs of mild steatosis, 6 (13.95%) had signs of moderate steatosis while 4 (9.31%) of respondents had signs of severe steatosis. According to the literature data, steatosis in patients with CHC is usually mild, when fatty changes affect up to 33% of hepatocytes. Using the same histopathological criteria for determining the degree of steatosis, Irimia et al. [36] also found a major presence of mild steatosis in patients with CHC while they had more people with severe steatosis. This can be explained by the greater age of the respondents in their study, lack of data on BMI of their subjects, habits of patients regarding alcohol and drugs use that can lead to steatosis and the existence of comorbidity that facilitate the emergence of liver steatosis.

As for the factors that may affect the occurrence of steatosis in our research, it was found that elevated BMI, elevated triglycerides, the presence of genotype 3 HCV and the level of HCV RNA viremia were statistically significant. When all of the above factors are taken into account together when calculating the multivariate logistic regression, elevated BMI, presence of HCV genotype 3 and the level of HCV RNA viremia remained as statistically significant predictors of steatosis. Such a result was also obtained in earlier investigations of other authors [37–39]. All of these factors (dietary measures, lifestyle changes and habits which can lead to the reduction of overweight and lowering of the BMI to the value of normal weight as well as effective antiviral therapy that can lead to the eradication of HCV) could be eliminated, and if eliminated, signs of hepatic steatosis withdraw. It is primarily provided by a new antiviral therapy for the treatment of CHC based on direct acting antivirals, which unlike standard antiviral therapy based on a combination of pegylated interferon and ribavirin, have high efficiency and leads to the eradication of the HCV in 95% of infected individuals [40].

Conclusion

In our study, hepatic steatosis occurred in 34%–96% of patients with CHC and it was usually mild. The occurrence of hepatic steatosis was mostly affected by elevated BMI, genotype 3 HCV and the level of HCV RNA viremia. In patients with CHC and hepatic steatosis, genotype 3 HCV was present in half (48.84%) of the respondents, and its presence was growing with the increase of the degree of steatosis. In our sample, respondents with hepatic steatosis had a statistically significant higher HCV RNA viremia compared to those without steatosis and this viremia grew along with the degree of steatosis. Hepatic steatosis can reduce the success of antiviral therapy based on interferon and ribavirin, but with the emergence of more efficiently new antivirals, the impact of steatosis on treatment efficacy needs to be examined. Steatosis caused primarily by viral factors would be eliminated after a successful antiviral treatment, but the one primarily caused by host's factors, i.e., metabolic factors may adversely affect the future course of the liver disease.

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Received on November 15, 2016.
Revised on January 30, 2017.
Accepted on February 6, 2017.
Online First March, 2017.

ORIGINAL ARTICLE

UDC: 616.8-009.1-08
https://doi.org/ VSP160508039S

Continuous intrathecal baclofen delivery in severely disabling spasticity

Kontinuirana intratekalna primena baklofena kod teškog onesposobljavajućeg spasticiteta

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Abstract

Background/Aim. Spasticity is the consequence of several clinical conditions including cerebral palsy, brain injury, spinal cord injury, multiple sclerosis, aneurysm bleeding, and some other neurological disorders. The aim of this study was to determine the efficacy of intrathecal baclofen (ITB) treatment in medically intractable severely disabling spasticity and present the challenges encountered during pump implantation surgery on these patients. **Methods.** The patients who underwent intrathecal baclofen pump implantation surgery between the years 2012 and 2015 with minimum follow-up of six months were recruited from the clinic archives. Twenty two patients with severe spasticity who had Modified Asworth Spasticity Scale (MASS) score of 3 or 4 were enrolled in our series. Eight of twenty-two patients were at pediatric age and they all were non-ambulant before surgery. **Results.** All of the patients underwent programmable intrathecal baclofen pump implantation surgery. Catheters were placed via percutaneous technique into to the subarachnoid space in 18 patients while, we had to perform partial hemi-laminectomy in order to place the catheters in 4 patients. All the patients improved significantly and 5 began using upper extremities and 3 adults became ambulant following physical therapy. Mean of the MASS scores improved from 3.59 to 1.32 ($p < 0.001$). **Conclusion.** The ITB therapy obviously increased quality of life and functional outcome in patients with disabling spasticity. As a result, physical treatment was more useful for these patients. Although some spinal abnormalities due to spasticity may necessitate partial hemilaminectomy to implant the pump, patients with intractable spasticity should be given the chance of intrathecal baclofen treatment at the earliest period of their lifetime disability.

Key words:
baclofen; infusion pumps; anesthesia, spinal; catheters, indwelling; muscle spasticity; brain diseases; spinal cord diseases; prognosis.

Apstrakt

Uvod/Cilj. Spasticitet je posledica nekoliko kliničkih stanja kao što su cerebralna paraliza, povrede mozga, povrede kičmene moždine, multipla skleroza, ruptura neurizme sa krvarenjem, kao i neki drugi neurološki poremećaji. Cilj ove studije bio je da se utvrdi efikasnost primene intratekalnog baklofena (ITB) u lečenju upornog teškog onesposobljavajućeg spasticiteta, kao i izazovi sa kojima se susrećemo tokom hirurške ugradnje pumpe kod ovih bolesnika. **Metode.** Iz kliničkih protokola u periodu 2012–2015. godina izdvojili smo bolesnike kojima je ugrađena baklofenska pumpa intratekalno uz minimlno praćenje od šest meseci. Dvadeset dva bolesnika sa teškim spasticitetom koji su imali modifikovani Asworth skor spasticiteta (MASS) između 3 i 4 bili su uključena u studiju. Osam od 22 bolesnika bila su u dečjem uzrastu i svi su bili nepokretni pre hirurškog zahvata. **Rezultati.** Svi bolesnici bili su podvrgnuti hirurškoj implantaicji programabilne intratekalne baklofenske pumpe. Kateteri su postavljeni perkutano u subarahnoidni prostor kod 18 bolesnika, dok smo parcijalnu hemilaminektomiju primenili za ugradnju katetera kod četiri bolesnika. Kod svih bolesnika javilo se značajno poboljšanje, 5 bolesnika počelo je da koristi gornje ekstremitete, a tri odrasla bolesnika postala su ambulantna tokom fizikalne terapije. Srednji MASS skorovi poboljšali su se od 3.59 do 1.32 ($p < 0.001$). **Zaključak.** ITB terapija je očigledno popravila kvalitet života i funkcionalnu sposobnost bolesnika sa onesposobljavajućim spasticitetom. Zahvaljujući tome fizikalna terapija bila je mnogo korisnija kod ovih bolesnika. Iako neke abnormalnosti zbog spasticiteta mogu zahtevati parcijalnu hemilaminektomiju da bi se ugradila pumpa, bolesnicima sa upornim spasticitetom treba pružiti šansu za primenu ITB lečenja u najranijem periodu njihove doživotne onesposobljenosti.

Ključne reči:
baklofen; infuzione pumpe; anestezija, spinalna; kateteri, trajni; mišići, spastičnost; mozak, bolesti; kičmena moždina, bolesti; prognoza.

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Introduction

Spasticity is the consequence of several clinical conditions including cerebral palsy (CP), brain injury, spinal cord injury, multiple sclerosis (MS), aneurysm bleeding, and some other neurological disorders [1]. Spasticity can be described as the muscle stiffness and spasm that is accompanied by involuntary jerking and sometimes pain [2]. When the spasticity is generalized and severe, the patient is usually immobilized and has the propensity to very low quality of life and poor care. Grading of the spasticity is achieved by applying the Modified Ashworth Spasticity Scale (MASS) in order to gain a standardized objective determination of the spasticity. It also helps to measure the efficacy of the treatment modalities in the follow-up. Practically, MASS measures resistance during passive soft tissue stretching. It is done in the supine position. Since spasticity is velocity dependent, the joint or the muscle group subject to testing is moved at the speed of gravity. After establishing an accurate diagnosis of spasticity, treatment options are assessed. Therapeutic options for these cases include oral medications, nerve blocks, destructive neurosurgical procedures and intrathecal administration of antispastic agents [3]. Baclofen is used orally to treat spasticity but its systemic side effects limit the dose a patient can take. It is a synthetic analog of gamma aminobutyric acid (GABA) and acts by stimulating the GABA type B receptor subtype in the central nervous system. In 1984 Penn and Kroin [4] introduced intrathecal administration of baclofen to treat spasticity and it was used in patients who had developed resistance to it or could not tolerate orally administered antispasmodic drugs. In the last decade, the use of baclofen delivered intrathecally via an implanted programmable pump became the principal treatment in the management of spasticity [5–7]. Initially, a trial injection of intrathecal baclofen (ITB) is applied and patient's response to the administered dose of the drug is observed. The decision to implant a pump is established on a positive response, that is, at least two-point decrease in Modified MASS and absence of the unwanted adverse effects [8].

We intented to emphasize the difficulties in baclofen pump implantation surgery and share our experience in the management of severe spasticity cases.

Methods

Patients

Patients who attended the Gülhane Military Medical Academy, Haydarpaşa Teaching Hospital and underwent ITP implantation surgery at the Neurosurgery Department between 2012–2015 were recruited from the medical records of the Clinic. The study design was approved by the Ethical Committee of the GATA Haydarpaşa Teaching Hospital and conforms to ethical standards as described in the Declaration of Helsinki. The same team of a neurologist, physiatrist and neurosurgeon obtained the MASS scores for each individual at their follow-up visits (Table 1).

Table 1

Demographical data of the patients enrolled in the study

Patient	Age (years)	Gender	Etiology	Follow up (months)	Final baclofen dose (mcg/dL)	Preop MASS	Postop MASS	Preop VAS	Postop VAS
1	50	M	Aneurysmal subarachnoid hemorrhage	19	62	3	1	8	3
2	38	F	Multiple sclerosis	19	55	3	1	10	5
3	63	F	Cervical spinal cord injury	21	60	3	1	10	5
4	9	M	Cerebral palsy	19	125	3	1	n/a	n/a
5	12	M	Cerebral palsy	18	50	3	1	n/a	n/a
6	47	F	Multiple sclerosis	18	142	4	1	9	4
7	14	M	Cerebral palsy	19	66	4	2	8	3
8	23	M	Cervical spinal cord injury	22	115	3	1	10	6
9	46	M	Transverse myelitis	18	70	3	1	n/a	n/a
10	38	M	Multiple sclerosis	18	60	4	1	9	4
11	7	M	Cerebral palsy	6	140	4	2	n/a	n/a
12	51	M	Cervical spinal cord injury	12	82.5	4	1	10	5
13	10	M	Cerebral palsy	6	65	4	2	8	3
14	44	F	Aneurysmal subarachnoid hemorrhage	6	170	4	2	n/a	n/a
15	34	F	Multiple sclerosis	12	50	4	2	10	4
16	27	M	Multiple sclerosis	13	55	4	1	10	3
17	35	M	Traumatic brain injury	15	70	3	1	n/a	n/a
18	11	M	Cerebral palsy	8	100	4	1	n/a	n/a
19	40	M	Multiple sclerosis	7	70	4	2	9	4
20	9	F	Cerebral palsy	10	55	4	1	n/a	n/a
21	13	M	Cerebral palsy	17	50	4	2	n/a	n/a
22	45	M	Multiple sclerosis	9	70	3	1	10	3
Mean	30.27			14.18	80.95	3.59	1.32	9.3	4

MASS – Modified Ashworth Spasticity Scale; VAS – Visual analog scale; M – male; F – female.

Twenty-two patients met the following criteria for inclusion in the study: ITP placement surgery; non-ambulant patients with severe spasticity (MASS 3–4), impairing function and personal care; at least 3 preoperative assessments free from antispastic drugs; available follow-up visits at the end of the first, third and sixth month postoperatively.

Pump implantation

When an indication of ITP implantation was decided on in case of positive trial response, a pump (SynchroMed II, Medtronic, Minneapolis, USA) was implanted by the first author (HŞ) in the subcutaneous pocket in the lower quadrant of the abdomen on the left side and connected to the intrathecal catheter under general anesthesia (Figures 1 and 2).

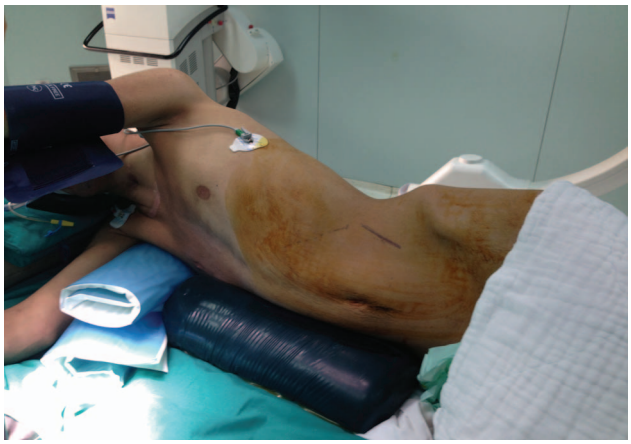

Fig. 1 – All the patients were operated in the lateral decubitus position regardless of the degree of their spasticity or contractures. To obtain the appropriate position and free the hip with possible joint contractures, we fed and supported shoulder and elevated the thorax and lower abdomen with silicon bedding and soft pillows. This allowed us implant the pump in the same position without violating the stiff joints.

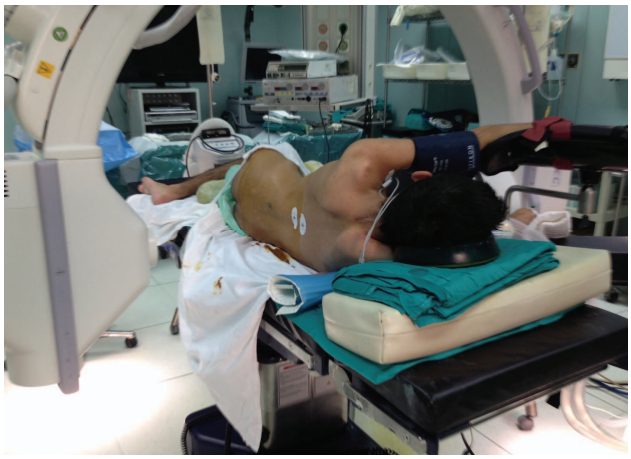

Fig. 2 – We marked dorsal spine with disposable self-adhesive electrocardiographic (ECG) electrodes in order to place the tip of the catheter at the desired level with reduced fluoroscopic shots.

Pumps with 20 mL volume capacity were implanted to the patients in the pediatric group while 40 mL volume capacity pumps were preferred for the adult patients. The level where the tip of the catheter would be left was determined according to the involvement of neck and the upper extremities.

When percutaneous catheter insertion was not successful despite fluoroscopic guidance, this position let us switch to direct catheter placement in the intrathecal space via partial hemi-laminectomy at the same level where percutaneous placement of the catheter was intended. We usually performed partial hemilaminectomy at the lumbar 2–3 interlaminar space. Once the patient was anesthetized and positioned appropriately, we believe that the patient deserved pump implantation in any available way and it was mentioned in the informed consent that was routinely obtained from the patients or their families.

The baclofen start doses are mentioned in Table 1. Starting on the third postoperative day, we began dose adjustments every week till the patient, physiatrist, neurologist and the family were satisfied with the clinical outcome. That usually took 4 to 12 weeks to compromise on an acceptable continuous daily intrathecal constant baclofen dose. We established our outcomes of the ITB therapy on the evaluations that were held in the end of the postoperative first, third and sixth month when available. Once the pump was implanted, depending on the dose-response situation, the patients needed percutaneous refills every 1 to 6 months. The demographic specifications of the patients are also shown in Table 1.

Statistical analysis

Raw data were analyzed using SPSS statistics packet program version 20.0 for Mac. Minimum value, maximum value, mean, and standard deviation (SD) were used to define data. The postoperative MASS scores were compared to that of the preoperative values using the Wilcoxon Signed-Rank test. *P* values less than 0.05 ($p < 0.05$) were considered statistically significant.

Results

Eight of the patients were at pediatric age (mean age 10.6 years, ranged from 7 to 14 years) and they developed spasticity due to CP. They were all non-ambulant and families had great difficulty in caring for the children. Visual analog scale (VAS) scoring could not be applied to 9 patients. Fourteen adult patients (5 females, 9 males) had a mean age of 41.5 years (range: 23–63 years). All of them were not ambulant and were unable to sit in a wheelchair. The cause of spasticity in the series was MS in 7 cases, spinal cord injury in 3, CP in 8, transverse myelitis in 1, aneurysmal subarachnoid hemorrhage in 2, and traumatic brain injury in 1 patient (Table 1). Twenty of the patients were quadriplegic and 2 patients with MS were paraplegic.

All the patients ceased oral medications at least 3 days before the trial day, if applicable, and then underwent a prepump implantation trial of ITB with 50 µg bolus delivered by lumbar puncture and followed by tone assessments over 6 to 8 hours. Records revealed that we had to administer a 50% increased second baclofen dose to only 3 patients during the prepump baclofen benefit trials on the following day. Pediat-

ric group received ITB (25 μg) at the dose that was a half of the dose the adults had received. Reduced mean MASS of at least 2 points was taken as positive response to trial. Again, the patients ceased antispastic oral drugs 3 days before surgery and the same physiatrist and a neurologist/pediatric neurologist evaluated them the day before implantation of the ITP free of oral antispastic medications.

The catheter was placed (between C7-T10 level) through a percutaneous technique into the lumbar subarachnoid space in 18 of the 22 patients. Four of the patients with CP (2 adult and 2 children) had severe spinal rotation and scoliosis, therefore the 16 T gauge Tuohy introducer needle could not be inserted in the intrathecal space and we had to place the catheter after performing a partial hemi-laminectomy in the lateral decubitus position. Positioning the patients was a challenge, but the operation room team got used to manage the condition after several cases. Induction of the anesthesia dissolved spasticity, but muscle contractures remained. Hip motion was usually restricted so in order to give the lateral decubitus position on the right side, we fed the whole body with silicone pads to release the right hip and shoulder and supported the left leg over the right leg with pillows and fixed the body in lateral position. We used anterior and posterior supports for chest and a posterior support at the upper thigh level. Therefore, adductor muscles of the hips with contracture did not encounter violation and extra injury (Figures 1 and 2).

Laminectomy alone was not a reason for a longer hospital stay in any of the patients. The patients did not suffer extra pain because of the laminectomy procedure as they are on analgesic medication for the pump pocket incision site.

Follow-up examinations were arranged at the end of the first, third and sixth month. Mean follow-up time of the overall group was 14.18 months. Owing to the continuous delivery of a constant dose of ITB, all of the patients got a steady relief of spasticity. All the patients enrolled in the study were severely disabled and immobile at the beginning (20 spastic quadriplegic, 2 spastic paraplegic). Mean MASS score of the patients improved from 3.59 to 1.32 (Table 1). P value was lower than 0.001, representing a highly significant difference (Table 2).

Mean VAS score of the available 13 patients was 9.3 in the preoperative assessment and it regressed to 4 at the third month evaluation in the postoperative follow-up. Adult group received an average of 80.8 μg daily baclofen dose, while the pediatric group received 81.4 μg daily baclofen, indicating that there was not a significant difference between the adult and pediatric group regarding the dose they received. The MS patients had remarkable benefit from the baclofen treatment. We recorded at least 2 points of MASS score reduction. Five of seven MS patients were able to walk by the end of three months following appropriate physical training. The patients with CP were able to sit in the wheel chair following the ITB treatment, but they were not able to walk. Since they were not coordinating, they could receive only passive physical training to regain the appropriate range of motion of their joints. Nevertheless, caregivers or families who were attending these patients were satisfied with the ease of care they had after the ITB treatment. Further studies including electrophysiological and mental tests should be performed for these patients to measure any improvement in cognitive functions.

Table 2

Preoperative and postoperative Modified Asworth Spasticity Scores (MASS)

Number of patients	MASS, mean ± SD		z (*p*)
	preoperative	postoperative	
22	3.59 ± 0.50	1.32 ± 0.48	-4,315 (0.001)

Postoperative MASS scores were compared to that of the preoeprative values using the *P*-Wilcoxon Signed-Rank test; values less than 0.05 were considered statistically significant (*p* was found lower than 0.001 in the series).

In the postoperative follow-up period we did not encounter any complications in our series other than in one patient who was referred back to our clinic with fluctuating swellings in the pump area and lumbar incision site. He was a 23-year-old man with cervical spinal cord injury who presented with spastic tetraplegia. ITP implantation surgery was planned, but percutaneous catheter placement was unsuccessful, so we performed partial hemi-laminectomy and transduced the catheter under direct visualization at the lumbar 3rd vertebra level. We revised the pump and found that the pump and the catheter system were intact. CSF was entrapped in the pump pocket and in the lumbar subcutaneous pouch. Lumbar fascia at the laminectomy site was tight but catheter required anchoring, and the tightening sutures were put where it penetrated the fascia. We reimplanted the pump in its pocket and postoperative course was uneventful and he did not experience any other complications (Figure 3).

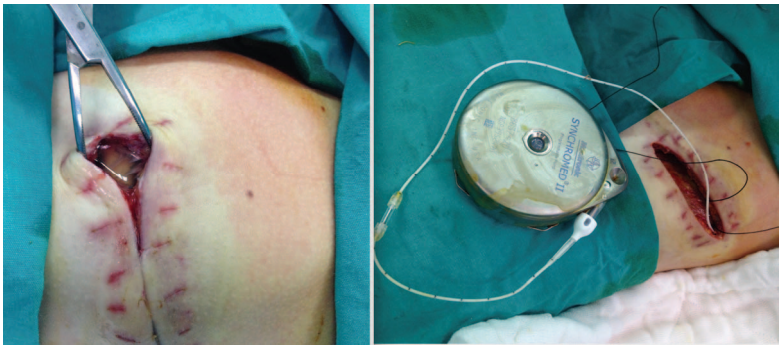

Fig. 3 – One patient experienced cerebrospinal fluid (CSF) collection in the pump pocket and the spinal incision site. We revised the pockets and the pump was reimplanted after ensuring that the system was intact. The fascia that was damaged by the Tuohy needle during our attempts to place the catheter by percutaneous technique was the route of the CSF fistula into the pouch and we repaired it with several stiches and anchored the catheter by a purse-string suture to the fascia.

Discussion

In the case of disabling spasticity, oral medication was the first choice because of easy administration route. Oral baclofen is widely used, however the untoward effects of oral baclofen such as sedation, respiration problems and muscular weakness in higher doses, limit its use. It also modulates pain due to its direct effect on GABA receptors. Though not all patients with spasticity benefit from this treatment, it allows a successful management of the spasticity [7]. Inhibition of spasticity increases mobility and makes the patient more liable to benefit from physical treatment [9, 10]. Overall benefit is increase in quality of life of the patients and caregivers as well [11, 12]. However, as in most of the surgical interventions involving implantation of a catheter and pump as foreign bodies, certain complications including infection, hardware malfunction, displacement of the catheter or the mechanical device, rejection of the system by the host, patient's intolerance to the system, the uncooperative patient profile and alterations in response to medication because of the individual features may be encountered. Inadvertent effects of the medication used via such closed systems are the most challenging of all since the administered drug has pretty narrow therapeutic window. Fluctuations in dose may not be tolerated because both withdrawal and overdose of intrathecally administered baclofen may be potentially life threatening and necessitate intensive care [13]. Although these complications are rare if certain practice guidelines are followed, clinicians should be prepared to recognize and treat them timely. Abrupt withdrawal of ITB can result in high fever, drowsiness and sometimes coma, return of spasticity, muscle rigidity, and in rare cases even death. An acute massive overdose can cause coma, while less severe overdoses can cause drowsiness, lightheadedness, respiratory depression, seizures, hypotonia, and loss of consciousness. The most prominent side effect is hypotonia, and can be addressed in most cases by adjusting the rate of administration [11, 14, 15].

A steady cerebrospinal fluid (CSF) concentration of the drug allows to generate the same effects as those of oral high dose administration of baclofen, except for that untoward side effects including sedation, respiration problems and muscular weakness will be avoided. It takes several weeks to several months to set to the desired effects and dose relation.

In our series, following a series of incremental adjustments, dosage remained stable after three months. That is ideal to find out how the patient would feel like when the drug reached therapeutic concentration, because some patients require doses above or below the designated range and even some patients experience drug toxicity within the therapeutic range.

Actually, during classical trial injections to the patient, incremented dosage was administered and takes 3 days to the longest, and did not need hospitalization unless the patient had a special condition that necessitated so. In the severely disabled group of patients, we did not have to readminister a second or third incremented baclofen dose to observe the benefit of the patients, because they all had significant relief following the initial trial dose. The next step for these patients remained dose adjustments following the implantation of the pump. Overall, continuous intrathecal administration of the baclofen to evaluate its systemic and functional effects could be considered as a helpful method as mentioned by some authors [16], but we believe it would put extra burden, both on the patient and the hospital by hospitalizing and using another trial pump for several days. After coming to a consensus on the implantation of the baclofen pump, all the patients were operated and a catheter was placed in the intrathecal space either via percutaneous route or via partial hemi-laminectomy in the right decubitus position usually at the 2–3 or 3–4 interlaminar spaces. In the severely disabled patients, usually the spinal column is anatomically deformed and intervertebral space does not allow the Tuohy needle to pass through. Since the patient had general anesthesia, we did not give up the implantation procedure in any of the patients and performed the laminectomy to place the catheter in the subarachnoid space. Sometimes the dural compression and arachnoid synechias due to the interrupted CSF turnover do not let CSF to flow through the Tuohy needle although you might be in the thecal sac. After transducing the catheter into the subarachnoid space and placing the tip in the way to reach a desired level, CSF flow is observed. After experiencing a CSF collection in the subcutaneous pouch, we began passing the catheter through the fascia by penetrating it at the intact site to prevent CSF fistula.

In order to measure the goal achievements after implantation, the patients (when available), caregivers, and the family were asked to rate in their terms whether the goals were achieved satisfactorily, or not. Better seating, feeding, improved sleep patterns, mood, eased provision of care, decreased pain were expressions of satisfaction and they were set as a simple statement implying overall benefit from the procedure [17]. Our results were also consistent with the current literature. Current data indicate that the ITB therapy effectively and significantly reduces severe spasticity in non-ambulatory patients caused by various reasons [7, 18]. This striking success of intrathecal baclofen use might be attributed to several factors including appropriate patient selection, education about realistic expectations and careful dose titration in time. To the benefit of the patients, physiatrists were also involved in the decision-making and postoperative assessment period, so early involvement of rehabilitation therapists in the procedure contributes to maximize clinical outcome.

Caregivers report muscle relaxation alone, as a positive benefit of the therapy but further physical treatment is needed. While the patients are under the effect of baclofen, determining the range of motion of each joint and the muscles with intractable contracture, which are candidates for surgical release, is another important issue to deal with. After reaching the physiological limits of the functioning muscles, additional treatment modalities might be considered. After all, when the patients are anesthetized and myorelaxant agents are administered, you can evaluate the limits of physiological motion and contractures at the extremities of these patients. The main goal is gaining the largest span of independent active, and passive movement of the extremities and

the trunk. Since all of the patients in our series were dependent on others in terms of hygiene, feeding, positioning, and ambulation, this also brought ease of care for the caregivers. After the ITB treatment, the MASS scores of the patients improved significantly. As the restriction of the disabling spasticity is decreased gradually, 5 patients began using their hands for grasping and 3 patients began ambulating in the house with an assistive device following intensive physiotherapy. As reported previously, another benefit of the ITB therapy was improvement in nutritional status, particularly in the pediatric patient group [19]. They began putting on weight in the end of the second month firstly because they could swallow easier as mentioned by the parents or caregivers and secondly because their health conditions improved and they had less infectious problems due to decreased pulmonary aspiration. In accordance with the improvement of the MASS scores, we found out pain relief in 13 patients and it was quantified with the VAS assessments (Table 1). Four patients in the pediatric group had improved mood and decreased yelling and crying episodes as noted by their parents. Pediatric neurologist ceased to apply sedative medications to these patients.

Some authors reported impairment in the spinal column alignment and worsening of the scoliosis in some of the patients who received the ITB treatment after a certain period of time [20, 21]. Our overall impression from our study is that the ITB therapy does not interfere with the underlying natural tendency to develop scoliosis for the most severely disabled children. Likewise, we also observed that the number of the orthopedic surgical interventions did not increase because of the ITB therapy, on contrary, both the orthopedics and the families had tendency to reconsider surgical intervention [22, 23]. Because it is rational that if the patient is found relaxed compared to the prepump assessments, one may cancel the preplanned operation for an individual, while he can identify new potential benefit and onsider orthopedic intervention [18]. The most important question of the issue here is if early onset of the ITB therapy will reduce contractures and yield more definite improvements in terms of ambulation and functionality. Besides ease of access to physiotherapy and insurance coverage, consistency of the patients and their families to a regularly based training sessions and home training takes the first place in the improvement of these patients, thereafter [24].

Conclusion

We retrospectively assessed the patients whom we operated on and implanted ITP in a selected group of non-ambulant patients with severely disabling spasticity. Detailed examination of the records revealed that all had satisfactory outcomes in the minimum 6 months of follow-up. Although spinal structural deformities of the severely disabling spastic patients are a challenge in the placement of the catheter, it does not totally restrict surgical intervention. Determining rationale goals preoperatively is the core issue, and is followed by patient and family consistency to treatment. Therefore, while planning this treatment, realistic balance of likely gains and possible losses should be carefully explained to the families and patients if applicable. We observed that families and physiatrist got excited about involving them in physiotherapy with the hope of further improvement. ITB usually produced many other improvements apart from the programmed aims and beyond the expectations of the families or caregivers. The ITB therapy apparently increases quality of life and increases functional outcome. So, as a surgical treatment modality, selected patients should be given this chance at the earliest period of their lifetime disability.

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Received on May 08, 2016.
Revised on January 15, 2017.
Accepted on March 07, 2017.
Online First March, 2017.

ORIGINAL ARTICLE

UDC: 616.12-009.17:616.61
https://doi.org/10.2298/VSP170226038L

Worsening renal function in patients hospitalized with acutely decompensated heart failure

Bubrežna disfunkcija kod bolesnika sa akutnom dekompenzacijom srčane slabosti

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Abstract

Background/Aim. A predictor of a poor prognosis, renal dysfunction often manifests in patients with heart failure, and is associated with an increased mortality in these patients. The aim of the parent study was to determine risk factors associated with worsening renal function (WRF) in patients hospitalized for acutely decompensated heart failure. **Methods.** The study included 330 patients with acutely decompensated heart failure. Patients who developed WRF (n = 215, mean age 72.4 ± 9.8 years) were in the clinical group, and patients without WRF (n = 115, mean age 59.8 ± 11.7 years) were in the control group. Patients in the clinical group were observed according to: the age, gender, lipids, electrolytes, smoking, hypertension, and type of heart failure, with reduced or preserved left ventricle ejection fraction (HFrEF or HFpEF). We used logistic regression to calculate non-adjusted odds ratio (OR) and 95% confidence intervals for occurrence of WRF. **Results.** WRF was determined in 65.2% of patients with heart failure. Non-adjusted OR showed that there was a significant risk for development of WRF with age (OR = 4.3; $p < 0.01$), total cholesterol > 5.2 mmol/L (OR = 1.6; $p < 0.05$), hyponatremia < 135 mmol/L, (OR = 2.8; $p < 0.01$), smoking (OR = 3.9; $p < 0.01$), hypertension (OR = 2.0; $p < 0.05$), and with the presence of HFrEF (OR = 1.3; $p < 0.01$). Presence of HFpEF, hypokalemia, < 3.5 mmol/L, plasma triglycerides, > 1.7 mmol/L, and gender, did not have any significance for the development of renal damage. **Conclusion.** Patients' age, total cholesterol, hyponatremia, smoking, hypertension, and HFrEF were significant risk factors for worsening renal function in heart failure patients. Comparing predictive values, age could be the best prognostic tool for early identification of patients at risk for WRF.

Key words:
heart failure; cardio-renal syndrome; risk factors.

Apstrakt

Uvod/Cilj. Bubrežna disfunkcija se često javlja kod bolesnika sa srčanom slabošću, predstavlja loš prognostički faktor i povezana je sa porastom mortaliteta kod ovih bolesnika. Cilj istraživanja je bio da se utvrde faktori rizika povezani sa razvojem bubrežne disfunkcije kod bolesnika sa akutnom dekompenzacijom srčane slabosti. **Metode.** Istraživanjem je obuhvaćeno 330 bolesnika sa akutnom dekompenzacijom srčane slabosti. Bolesnici koji su razvili bubrežnu disfunkciju (n = 215 ispitanika, starosti 72,4 ± 9,8 godina) činili su kliničku grupu, a bolesnici bez bubrežne disfunkcije (n = 115 ispitanika, starosti 59,8 ±11,7 godina) bili su kontrolna grupa. Kod ispitanika kliničke grupe analizirani su sledeći parametri: godine starosti, pol, lipidni status, koncentracija elektrolita u plazmi, prisustvo pušenja i hipertenzije i tip srčane slabosti. Korišćena je logistička regresija za izračunavanje *odds ratio* (OR) i 95% intervala poverenja za razvoj bubrežne disfunkcije kod ovih bolesnika. **Rezultati.** Bubrežna disfunkcija je ustanovljena kod 65,2% bolesnika sa srčanom slabošću. Pokazano je da su se kao značajni faktori rizika od razvoja bubrežne disfunkcije izdvojili starost ispitanika (OR = 1,6; $p < 0,05$), porast koncentracije ukupnog holesterola, > 5,2 mmol/L (OR = 1,6; $p < 0,05$), hiponatrijemija, < 135 mmol/L, (OR = 2,8; $p < 0,01$), pušenje (OR = 3,9; $p < 0,01$), hipertenzija (OR = 2,0; $p < 0,05$) i postojanje srčane slabosti sa smanjenom ejekcionom frakcijom (OR = 1,3; $p < 0,01$). Srčana slabost sa očuvanom ejekcionom frakcijom, hipokalijemija, < 3,5 mmol/L, visoka koncentracija triglicerida u plazmi, > 1,7 mmol/L i pol ispitanika nisu pokazali statistički značaj za razvoj bubrežne disfunkcije. **Zaključak.** Godine starosti, povišene vrednosti totalnog holesterola, hiponatrijemija, pušenje, hipertenzija i smanjenje ejekcione frakcije značajni su i nezavisni faktori rizika od razvoja bubrežne disfunkcije kod bolesnika sa srčanom slabošću. Poređenjem prediktivnih vrednosti, godine starosti bi mogle da budu najznačajniji faktor rizika za ranu identifikaciju bolesnika koji su pod povećanim rizikom od razvoja bubrežnog oštećenja.

Ključne reči:
srce, insuficijencija; sindrom, kardio-renalni; faktori rizika.

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Introduction

Worsening renal function (WRF) as a predictor of a poor prognosis often manifests in patients with heart failure (HF). WRF is associated with an increased mortality in these patients [1] as well as increased in-hospital costs, in-hospital mortality, length of stay and likelihood of readmission [2]. According to data from large registries, 30% of patients hospitalized for acutely decompensated heart failure (ADHF) exhibit moderate to severe WRF. If patients with mild WRF are included, there is more than 50% of them [3]. WRF is also closely linked with deterioration of renal function over time, after an initial hospitalization, which leads to the development of renal failure, respectively.

In patients with acute HF, hemodynamic balance maintained by kidneys is often disrupted, resulting in decreased organ perfusion and ultimately organ failure and possibly death. Heart failure with reduced left ventricle ejection fraction (HFrEF) is characterized by impaired left ventricular systolic function and poor cardiac output with activation of compensatory mechanisms such as the renin-angiotensin-aldosterone system, the sympathetic nervous system, and other mediators, which interact to maintain the fluid volume [4]. Furthermore, decreased renal perfusion, in addition to nephrotoxic agents, eventually leads to kidney injury in those patients [5]. Heart failure with preserved left ventricle ejection fraction (HFpEF), mostly in patients with thick ventricular walls and a small ventricular cavity, is also characterized by a low cardiac output in acute deterioration of HF. The high end-diastolic pressure is transferred back to the pulmonary capillaries which results in dyspnoea upon exertion, and these pathophysiological abnormalities trigger neurohormonal activation, as happens in HFrEF [6]. The identification of risk factors associated with WRF in patients with HD may provide an opportunity to reduce the risk of complications and improve the outcome in this setting.

Therefore the aim of the parent study was to determine risk factors associated with WRF in patients hospitalized for acutely decompensated heart failure.

Methods

We observed 330 patients with ADHF, of both sexes, hospitalized at the Clinic for Cardiovascular Diseases, Clinical Center Niš, Serbia, between March and November 2014. The study was designed as a cross-sectional, retrospective study, approved by the Ethic Committee of Clinical Center Niš, and conducted in compliance with the Declaration of Helsinki and Good Clinical Practice Guidelines.

The diagnosis of HF was established according to the guidelines of the European Society of Cardiology (ESC) [7]. All patients with left ventricular ejection fraction (LVEF) $\leq$ 45% –HFrEF, or with heart failure with preserved left ventricle ejection fraction (HFpEF), having the New York Heart Association (NYHA) function class II to IV [8], were enrolled in the study. Echocardiography was performed on VIVID 4GE ultrasound system and LVEF was measured according to Simpson biplane method. These measurements were performed as soon as the patients were able to have compliance during the examination.

The reasons for hospitalization were hypertensive crisis, n = 25 (7.5%), chronic ischemic cardiomyopathy, without increased specific markers of myocardial necrosis, n = 58 (17.5%), valvular heart disease, n = 69 (21%), and dilated cardiomyopathy, n = 178 (53.9%), as underlying conditions for the development of ADHF (Table 1). There suffered 95 (28.7%) of patients from acute heart failure and 235 (71.3%) patients from acute decompensation of a preexisting heart disease. Patients who had previous diagnosis of diabetes mellitus (type 1 and 2), chronic renal failure grade 4 and 5, malignant diseases, and chronic inflammatory diseases were not included in the study. The patients with acute coronary syndrome, coronarography or percutaneous coronary intervention during hospitalization and 30 days before were also excluded.

Tabele 1

Reasons for hospitalization

Reasons	Number (%) of patients
Hypertensive crisis	25 (7.51)
Chronic ischemic cardiomyopathy*	58 (17.5)
Valvular heart disease	69 (21)
Dilated cardiomyopathy	178 (53.9)

***Without increased specific markers of myocardial necrosis.**

All patients hospitalized with the HF were divided into two groups, according to the presence of WRF, 7 days after admission. The first subgroup consisted of 215 (65.2%) patients, who developed WRF during hospitalization and they were observed as the clinical group. The second subgroup consisted of 115 (34.8%) patients, without WRF during the hospitalization, and they represented the control group. In the group of patients with WRF, 163 (75.8%) patients were older than 65 years of age and 121 (56.28%) were males. HF with reduced left ventricle ejection fraction was observed in 134 (62.3%) patients while 185 (86.0%) patients had arterial hypertension, or received antihypertensive therapy. Regarding the smoking habits, 112 (52.0%) patients were smokers. In the control group, without WRF, 85 (73.9%) were older than 65 years of age and 72 (62.6%) were males. HFrEF was observed in 38 (33.0%) patients and 89 (77.3%) patients had arterial hypertension, or received antihypertensive therapy.

We assessed kidney function by measuring serum creatinine concentration. An increase in serum creatinine by at least 25%, or more, compared to the baseline values, was defined as worsening renal function [9]. Using the simplified Modification of Diet in Renal Disease (MDRD) formula, a suggested method for assessment in patients with HF, we also estimated glomerular filtration rate (eGFR) [10]. According to this method, we determined stages of chronic kidney disease at admission and after 7 days of hospitalization.

Previous medication included angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers (75.3%), aldosterone antagonists (62.5%), digoxine (69.8%), oral anticoagulants (49%), beta blockers (72.4%) and oral furosemide (87.1%). However, during hospitalization, all pa-

tients with ADHF received furosemide intravenously and vasodilators.

Laboratory measurements

Using standard clinical laboratory methods, biochemical measurements: urea, creatinine, glycemia, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides and electrolytes were obtained from fasting blood samples (5 mL) from each participant at admission, and all analysis were performed by using Erba Mannheim XL600 analyzer (ERBA Diagnostics Mannheim Gmbh, Baden-Wurttemberg, Germany). Impaired lipid level (total cholesterol, LDL, HDL, and triglycerides) were defined according to the National Guidelines for Good Clinical Practice for Diagnostics and Treatment of Dyslipidemia (2012) [11].

Statistical analyses

Characteristics of the study group were expressed as mean ± standard deviation (SD) (continuous variables) with number and % in brackets (categorical variables). We compared data of patients using Student *t*-test for normally distributed data (expressed as mean ± SD) and Mann–Whitney U test for non-parametric variables. Univariate logistic regression was used to calculate odds ratio (OR) and 95% confidence intervals (CI) for occurrence of WRF. The relationship between selected variables was determined by Pearson's correlation coefficient I. All analyses were performed with SPSS version 16.0 (SPSS, Chicago, IL, United States). The significance level was set at $p < 0.05$.

Results

Worsening renal function was determined in 215 (65.2%) patients hospitalized with heart failure after 7 days of hospitalization. Significant differences were found between patients who developed WRF and the control group. The patients with heart failure who developed WRF were older, more hypertensive and less likely to be with the first episodes of acute heart failure and preserved ejection fraction. They had similar lipid profile except higher triglycerides ($p < 0.01$), higher fasting glycemia ($p < 0.05$), serum urea and creatinine concentration ($p < 0.01$). The patients in the clinical group had lower serum potassium ($p < 0.01$), sodium concentration ($p < 0.01$), lower values of systolic ($p < 0.01$), and diastolic blood pressure ($p < 0.01$) and eGFR ($p < 0.01$) at admission, compared to the control group. There were no significant differences according to the patients' gender, LDL and HDL cholesterol concentrations. All data are presented in Table 2.

The analysis of the stages of chronic kidney disease showed significant differences in distribution among subgroups of the HF patients. The patients in the clinical group had severely impaired kidney function compared to the control group at admission ($p < 0.01$). This is presented in Table 2. Moreover, the development of chronic kidney disease grade 3 was registered in 102 (47.4%) of the WRF patients after 7 days of hospitalization (data not shown).

Table 2

Clinical data of 330 heart failure (HF) patients in the control and clinical group at admission

Parameter	Control group (n = 115)	Patients with WRF (n = 215)	*p*
Age (years), mean ± SD	59.8 ± 11.7	72.4 ± 9.8	0.05
Acute heart failure, n (%)	52 (45.2)	43 (20.0)	0.05
Acute decompensation, n (%)	63 (54.8)	172 (80.0)	0.05
Chronic kidney disease, n (%)			
stage 1 (eGFR > 90 mL/min/1.73m^2)	67 (58.2)	48 (22.3)	0.01
stage 2 (eGFR 60–89 mL/min/1.73m^2)	39 (34.0)	85 (39.5)	
stage 3 (eGFR 30–59 mL/min/1.73m^2)	9 (7.8)	92 (42.7)	
Males, n (%)	72 (62.6)	121 (56.2)	NS
Smokers, n (%)	46 (40.0)	112 (52.0)	0.01
Glycemia, (mmol/L), mean ± SD	5.2 ± 0.9	5.9 ± 0.4	0.05
Cholesterol, (mmol/L), mean ± SD	5.5 ± 1.5	5.9 ± 1.5	NS
LDL, (mmol/L), mean ± SD	3.5 ± 1.2	4.0 ± 1.3	NS
HDL, (mmol/L), mean ± SD	1.1 ± 0.3	1.2 ± 0.3	NS
Triglycerides, (mmol/L), mean ± SD	1.2 ± 0.5	2.1 ± 0.6	0.01
Urea, (mmol/L), mean ± SD	6.2 ± 2.4	10.3 ± 6.1	0.01
Serum creatinine (μmol/L), mean ± SD	84.5 ± 12.6	139.4 ± 21.8	0.01
eGFR (mL/min/1.73 m^2), mean ± SD	79.5 ± 11.7	59.5 ± 22.4	0.01
Na, (mmol/L), mean ± SD	136.5 ± 4.9	132.7 ± 2.2	0.01
K, (mmol/L), mean ± SD	4.4 ± 0.7	3.3 ± 0.5	0.01
Hypertension, n (%)	89 (77.3)	185 (86.0)	0.01
SBP, (mmol/L), mean ± SD	138 ± 15	107 ± 10	0.01
DBP, (mmol/L), mean ± SD	80 ± 10	68 ± 8	0.01
HFrEF, n (%)	38 (33.0)	134 (62.3)	0.05
LVEF (%), mean ± SD	50.2 ± 12.3	39.7 ± 12.1	0.01

Data are expressed as mean ± standard deviation (SD) – compared with Student-*t* test or expressed as n (%) compared with *U* test.

WRF – worsening renal function; SBP – systolic blood pressure; DBP – diastolic blood pressure; LVEF– left ventricular ejection fraction; eGFR – estimated glomerular filtration rate; HFrEF – heart failure reduced left ventricle ejection fraction.

Table 3

Analysis of risk factors according to age, gender and left ventricle ejection fractions (LVEF) in 215 patients with worsening renal function

Parameters	n	Urea (mmol/L) mean ± SD	Creatinine (μmol/L) mean ± SD	eGFR (ml/min/1,73m^2) mean ± SD	LVEF (%) mean ± SD
Age (years)	52	7.23 ± 3.8	121.7 ± 38.3	61.9 ± 15.4	47.8 ± 14.2
< 65					
> 65	163	12.1 ± 7.2**	129.9 ± 39.2*	52.8 ± 17.1**	42.9 ± 11.7*
Sex	121	8.4 ± 4.2	117.3 ± 49.7	66.7 ± 18.8	43.1 ± 13.2
male					
female	94	9.9 ± 8.1*	115.8 ± 60.8	55.5 ± 17.9**	48.0 ± 14.4**
HFrEF	134	10.2 ± 4.8	124.6 ± 19.9	57.1 ± 9.3	40.4 ± 12.3
HFpEF	81	8.9 ± 3.2	116.8 ± 19.1*	63.5 ± 18.4**	49.8 ± 11.4**

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

n – number of patients; SD – standard deviation.

eGFR – estimated glomerular filtration rate; LVEF – left ventricular ejection fraction; HFrEF – heart failure with reduced left ventricle ejection fraction; HFpEF – heart failure with preserved left ventricle ejection fraction.

Table 4

Non-adjusted odds ratio (OR) and 95% confidence intervals (CI) for worsening renal function in the study patients

	Control group	Clinical group	OR	95%CI	*p*
Age (years)					
< 65	30 (26.1)	52 (24.1)			
65+	85 (73.9)	163 (75.8)	4.31	3.80–6.00	< 0.01
Sex					
male	72 (62.6)	121 (56.2)	1.09	0.99–1.12	0.11
female	43 (37.4)	94 (43.8)			
Smoking	46 (40.0)	112 (52.0)	3.98	2.55–5.79	< 0.01
TC > 5.2 mmol/L	101 (88.3)	195 (90.6)	1.66	1.46–3.23	< 0.05
TG > 1.7 mmol/L	93 (81.1)	201 (93.5)	0.96	0.33–3.90	0.56
Na < 135 mmol/L	70 (60.9)	177 (82.5)	2.82	2.68–3.22	< 0.01
K < 3.5 mmol/L	54 (47.5)	116 (54.0)	1.27	0.92–1.44	0.886
Hypertension	89 (77.3)	185 (86.0)	2.06	1.15–8.53	0.045
HFrEF	38 (33.0)	134 (62.3)	1.29	1.12–2.81	< 0.01
HFpEF	77 (67.0)	81 (37.6)	1.16	0.68–1.62	0.32

Data are expressed as n (%).

TC – total cholesterol, TG – triglycerides; HFrEF – heart failure with reduced left ventricle ejection fraction; HFpEF – heart failure with preserved left ventricle ejection fraction.

The parameters of global renal function (plasma concentration of urea, creatinine and eGFR) and LVEF according to the age, gender and type of HF (HFrEF or HFpEF) in the subgroup of patients with WRF are shown in Table 3. We found that urea and creatinine concentration were significantly higher and eGFR and LVEF lower in the patients older than 65 years of age. The female patients also had significantly higher urea and lower eGFR compared to the males. The patients with HFrEF had significantly higher creatinine concentration and lower eGFR, thus presenting the older female patients with reduced EF as particularly predisposed to WRF.

Non-adjusted OR for renal dysfunction were presented in Table 4. Non-adjusted ORs showed that there was a significant risk for development of WRF during hospitalization in the patients older than 65 years (OR = 4.3; 95% CI 3.8–6.0), history of smoking (OR = 3.9; 95% CI 2.5–5.7), total plasma cholesterol > 5.2 mmol/L (OR = 1.6; 95% CI 1.4–3.2), hyponatremia < 135 mmol/L, (OR = 2.8; 95% CI 2.6–3.2), hypertension (OR = 2.0; 95%CI 1.1–8.5), and in patients with reduced left ventricle ejection fraction compared to those with preserved LVEF (OR = 1.3; 95% CI 1.1–2.8). Heart failure with preserved left ventricle ejection fraction, elevated plasma triglycerides, > 1.7 mmol/L, hypokalemia, < 3.5 mmol/L, and sex did not prove significant for worsening renal function in the patients with heart failure. Thus, the presence of any of the evaluated risk factors (age over 65 years, smoking, hypertension and hyponatriemia) increased the probability for WRF more than two times.

Discussion

Heart failure is a leading cause of hospitalization in the age group of 65 years and older, represents a significant economic burden [5], and eventually leads to kidney injury. The present study adds to the current data that WRF is an expecting finding among older patients hospitalized for HF. WRF frequency ranges from 35% to 70% in various studies [12], and have a high in-hospital mortality [13]. In a meta-analysis unadjusted mortality rate at one year, follow-up was 51% in the patients with moderate to severe renal impairment, compared to 26% in those without any renal impair-

ment [14]. The prevalence of WRF, as well as the development of kidney failure stages III, (determined as eGFR < 60/mL/min/m^2), in our patients hospitalized with ADHF was 65.2%, and 47.4% prospectively, which is in accordance with current data. Some researchers found "moderate" renal failure (creatinine clearance < 60/mL/min/m^2) in 22.5% of their heart failure patients [15], while in the Valsartan in Heart Failure (Val-HeFT) trial [16] (n = 5010), eGFR was found to be below 60 mL/min/m^2 in 58% of patients. In both studies, however, decreased eGFR was a predictor of a poor prognosis.

Baseline renal insufficiency was reported to increase the number of hospitalizations due to worsening of HF, and these individuals had high likelihood of cardiovascular death [12]. Possible mechanism for development of renal dysfunction is arterial hypotension followed by severe and prolonged kidney hypoperfusion. In 10.2% of our patients with HF, prompt decrease in kidney function at hospital admission was noticed. Age and baseline renal function were determined as risk factors for WRF and exacerbation in chronic HF patients [17].

Several studies showed that the prevalence of kidney failure rises with age [15–18]. Accordingly, in our study significantly more patients older than 65 years experienced WRF, and this was an independent risk factor for its occurrence, (OR 4.3). Traditional risk factors for heart disease, such as hypertension, smoking and total cholesterol, > 5.20 mmol/L, also showed significance for WRF, although with a slightly smaller predictive values.

WRF in our study was equally distributed between gender, despite the fact that cardiovascular diseases are more frequent in males compared to females. This could be explained by physiological changes after menopause, most probably due to the loss of protective estrogen effect on vasculature and great proportion of patients aged over 65 years. Estrogen decreases expression of angiotensin type 1 receptor and angiotensin-converting enzyme and causes the release of angiotensinogen substrate [19].

In our study, HFrEF (≤ 45% LVEF) was found to be a significant risk factor for WRF with moderately higher risk (OR 1.3), unlike HFpEF. However, in other studies various relations between heart and kidney function parameters were determined. Apparently, elevation in both ventricles end-diastolic pressures and venous pressure contribute to renal dysfunction by impairing forward blood flow and by increasing renal venous pressure [20]. Predomination of a single process is probably influenced by a stage of heart disease and neurohormonal status of a patient. Acute renal injury, as a complication in congestive HF patients treated with diuretics, was observed more often in those with HFrEF (40%) than with HFpEF (28%) [17]. Additionally, WRF is certainly favored by pronounced vasoconstriction and sodium retention in acutely decompensated HF patients [21]. Worsening renal function during the first 3 days of hospitalization was also reported in 47% of patients with acutely decompensated HF [22]. Heart failure with reduced left ventricle ejection fraction as the significant risk factor for kidney injury in our HF patients was likely due to its prolonged hypoperfusion effect on kidneys.

Urea, creatinine and eGFR were higher in our patients with WRF that were older than 65 years compared to the younger ones. As a consequence of HF related increase in venous pressure in kidneys, pressures in interstitial space and Bowman's capsule also increase and lead to greater urea reabsorption. Besides, in low-output HF, arterial perfusion is maintained by releasing neurohumoral mediators, among which is arginine vasopressin that mediates urea reabsorption [12, 20]. However, urea may not be reliable index of WRF, mostly compared to eGFR, because its serum concentrations are affected by different elements of metabolism [12]. This is perhaps the reason of insignificant correlation between LVEF and urea, and creatinine values in our study. Prolonged hyponatremia was proven to be an independent cardiovascular risk factor, and also an independent factor of mortality in this group of patients, mostly due to the long-term diuretic therapy.

We suppose that higher urea concentrations in our female patients are due to lower percent of total body water and significantly faster decline of eGFR with age, compared to the males [23].

Conclusion

Worsening renal function is common in patients hospitalized with ADHF. We have demonstrated that age over 65 years, heart failure with reduced left ventricle ejection fraction, smoking, hyponatremia, elevated total cholesterol and hypertension, were significant risk factors for worsening renal function. Our results indicate that age over 65 years could be the best prognostic tool, among evaluated, for identification of worsening renal function in patients with heart damage. The other assessed parameters, heart failure with preserved left ventricle ejection fraction, elevated triglycerides level, hypokalemia, and gender did not have any significance for worsening renal function in patients hospitalized with heart failure.

The results of our study may provide clinicians with very important knowledge about the elderly patients, who are under a greater risk for renal damage and therefore possible prevention of irreversible changes.

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Received on December 15, 2016.
Revised on March 02, 2017.
Accepted on March 03, 2017.
Online First March, 2017.

ORIGINAL ARTICLE

UDC: 577.1:[616.2-008.4:612.821.7
https://doi.org/10.2298/VSP161030041C

Impact of severity of obstructive sleep apnea (OSA) and body composition on redox status in OSA patients

Uticaj težine opstruktivne *sleep* apneje (OSA) i telesnog sastava na redoks status kod pacijenata sa OSA

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Abstract

Background/Aim. There is an increasing number of studies on the existence of systemic oxidative stress in patients with obstructive sleep apnea (OSA) which is an important mechanism linking OSA and endothelial dysfunction with increased risk for cardiovascular diseases. Comorbidities must be also considered, especially obesity, as the most important source of oxidative stress independently of OSA. The aim of this paper was to show if OSA severity increases the level of markers of systemic oxidative stress and reduces antioxidant capacity, independently from body mass index (BMI) and nutritional status. **Methods.** One hundred and twenty-eight patients with OSA were included in the trial. Based on the results of a sleep study-polysomnography, the examinees were divided into two groups according to apnea-hypopnea index (AHI < 15 and AHI ≥ 15). Nutritional status was estimated by using the BMI and body composition. Body composition was determined by dual X-ray absorptiometry (DXA) whole-body scan. Redox status of patients with OSA was determined by measuring the concentration of NO in plasma and antioxidant capacity was evaluated using the plasma level of reduced glutathione (GSH) as a marker of antioxidant capacity. **Results.** Significantly higher mean values of NO were found in the group with AHI ≥ 15 in comparison to AHI < 15 group (1.269 ± 0.789 vs. 0.462 ± 0.373 nmol/mL, respectively; $p = 0.001$), while significantly higher levels of GSH were found in the group with AHI < 15 in comparison to AHI ≥ 15 group (238.08 ± 84.37 vs. 172.77 ± 83.88 mg/mL, respectively; $p = 0.04$). Independent predictors of plasma GSH level (multivariate regression analysis) were: desaturation index (ODI) [B = -0.157; 95% confidence intervals (CI): -0.262–0.053], mean $SatO_2$ (B = -4.76; 95% CI: -9.21–0.306) and min $SatO_2$ (B = 0.118; 95% CI: 0.03–0.206). ODI was singled out as an independent predictor of NO concentration in plasma (B = 0.038; 95% CI: 0.011–0.065). No significant statistical difference was found in mean values of BMI and body composition parameters in patients with AHI < 15 and AHI ≥ 15. None of the markers of systemic oxidative stress was associated with BMI and body composition assessment parameters. **Conclusion.** OSA severity is significantly associated with reduced antioxidant capacity and increased level of systemic oxidative stress. The degree of desaturation during sleep considerably affects systemic oxidative stress in patients with OSA independently from nutritional status and body composition.

Key words:
sleep apnea, obstructive; oxidative stress; nutritional status; body composition.

Apstrakt

Uvod/Cilj. Sve je više istraživanja o postojanju sistemskog oksidativnog stresa kod pacijenata sa opstruktivnom apnejom u snu (OSA), kao mogućoj vezi OSA sa endotelnom disfunkcijom i bolestima kardiovaskularnog sistema. Mora se uzeti u obzir i postojanje komorbiditeta, pre svega gojaznosti, koji mogu doprineti oksidativnom stresu nezavisno od OSA. Cilj ovog rada bio je da pokaže da li težina OSA doprinosi povećanom nivou sistemskog oksidativnog stresa i smanjenju antioksidativnog kapaciteta, nezavisno od indeksa telesne mase (BMI) i telesnog sastava kod pacijenata sa OSA. **Metode.** Istraživanjem je bilo obuhvaćeno 128 pacijenata sa OSA. Na osnovu *sleep* studije – polisomnografije, ispitanici su bili podeljeni u dve grupe prema *apnea-hypopnea* indeksu (AHI): AHI < 15 i AHI ≥ 15. Stanje uhranjenosti je procenjeno pomoću BMI i telesnog sastava. Telesni sasatav je određen pomoću *whole-body* DEXA sken-

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era (Hologic QDR-4000). Redoks status pacijenata sa OSA je određivan pomoću koncentracije NO u plazmi i nivoa redukovanog glutationa (GSH) u plazmi kao markera antioksidativnog kapaciteta. **Rezultati.** U grupi sa AHI ≥ 15 bila je nađena značajno veća srednja vrednost koncentracije NO u plazmi u odnosu na pacijente sa OSA i AHI < 15 (1,269 ± 0.789 vs 0.462 ± 0.373 nmol/mL, p = 0,001), dok je značajno veći nivo GSH u plazmi bio u grupi sa AHI < 15 u odnosu na grupu sa AHI ≥ 15 (238,08 ± 84,37 vs 172,77 ± 83,88 mg/mL, p = 0,04). Nezavisni prediktori nivoa GSH u plazmi (multivarijantna regresiona analiza) bili su: index desaturacije (ODI) [B = -0.157; 95% granice poverenja (CI): -0.262–0.053], srednja vrednost SatO2 (B = - 4.76; 95% CI: -9.21–0.306) i minimalna SatO2 (B = 0.118; 95% CI: 0.03–0.206). Kao nezavisni prediktor koncentracije NO u plazmi izdvojio se ODI (B = 0,038; 95% CI: 0,011–0,065). Nije postojala statistički značajna razlika između srednjih vrednosti BMI i parametra za procenu telesnog sastava u grupi sa AHI < 15 i AHI ≥ 15. Nijedan od parametara za procenu stanja uhranjenosti i telesnog sastava nije značajno uticao na ispitivane parametre sistemskog oksidativnog stresa. **Zaključak.** Sa povećanjem težine OSA smanjuje se antioksidacijski kapacitet i raste nivo sistemskog oksidativnog stresa. Stepen desaturacije tokom spavanja ima značajan uticaj na redoks status pacijenata sa OSA, nezavisno od stanja uhranjenosti i telesnog sastava.

Ključne reči:
apneja u snu, opstruktivna; stres, oksidativni; nutritivni status; telo, sastojci.

Introduction

Obstructive sleep apnea (OSA) is defined by recurrent episodes of breathing interruptions lasting more than 10 seconds (apnea) or reduction in airflow (hypopnea) during sleep associated with sleep fragmentation, awakenings and decreased oxygen saturation. Since the respiratory control is considered to be imperfect, up to five respiratory events during 60 minutes of sleep are tolerated [1]. According to the mechanism of breathing interruptions, different entities of this disorder are: OSA, central sleep apnea (CSA) and mixed sleep apnea (MSA).

In OSA, the airflow is interrupted, but respiratory effort continues. Apnea-hypopnea index (the number of apnea and hypopnea per hour of sleep – AHI) represents a standard for determining the severity of OSA. There are several ways to show the severity of desaturation during sleep as a consequence of OSA. Desaturation index (ODI) is most commonly used and it represents the number of desaturations ($SatO_2$ reduction ≥ 3%) per hour of sleep, the mean oxygen saturation, minimal saturation and percentage of sleep hours with hemoglobin oxygen saturation less than 90% (Sat < 90%). It is assumed that cyclical occurrence of hypoxia-reoxygenation (intermittent hypoxia) that characterizes OSA is the most important mechanism in formation of free radicals, similar to ischemia-reperfusion damages in coronary disease. It is believed that intermittent hypoxia leads to mitochondrial dysfunction, activation of enzymes that use oxygen, such as xanthine oxidase or nicotinamide adenine dinucleatide phosphate (NADPH), activation of leukocytes and endothelial cell dysfunction which leads to synthesis of free radicals and increased inflammation [2]. Oxidative stress can lead to activation of redox-sensitive transcription factors which regulate the synthesis of inflammatory cytokines, chemokines and adhesion molecules [3]. When considering oxidative stress in OSA, comorbidities must also be taken into account, especially obesity, as the most important source of oxidative stress independently from OSA. In a large population study, it was shown that body mass index (BMI) alongside with smoking and diabetes is the most important independent factor associated with systemic oxidative stress, although the contribution of accompanied OSA cannot be excluded [4].

The aim of this paper was to show if the OSA severity increases the level of markers of systemic oxidative stress and reduces antioxidant capacity independently of nutritional status.

Methods

The study included 128 patients with OSA. Patients with concomitant diseases that are characterized by the existence of systemic inflammation were excluded, except for obesity. All examinees underwent the sleep study-polysomnography using Philips STARDUST sleep recorder. AHI, AHI in the supine position (AHI supinatio), AHI in other positions of sleep (AHI non supinatio), desaturation index (ODI), minimum saturation ($minSatO_2$), medium saturation (mean $SatO_2$) and the percentage of time with saturation less than 90 % ($SatO_2$ < 90%) were determined. On the basis of the results of the sleep study based on AHI, examinees were divided into two groups: with mild OSA (AHI ≤ 15) and moderate and severe OSA (AHI > 15). All examinees underwent assessment of nutritional status using the BMI = body mass (BM)/body height (kg/m^2). Body compositions were determined by dual X-ray absorptiometry – (DXA) whole-body scan (Hologic QDR-4000). The absolute value of body fat mass (FM) in kg, body fat percentage (FM%), lean body mass (FFM) in kg and lean body mass index [FFMI = FFM/BM^2 (kg/m^2)] were examined. The data from the National Health and Nutrition Examination Survey (NHANES) for FM% reference values using DXA measuring were used [5].

Determination of nitric oxide (NO): NO decomposes rapidly to form stable metabolite nitrite/nitrate products. Nitrite (NO_2^-) was determined as an index of nitric oxide production by using Griess reagent (1). 0.1 mL 3N PCA, 0.4 mL 20 mM EDTA and 0.2 mL plasma were kept on ice for 15 min, then centrifuged for 15 min at 6000 rpm. After the supernatant was poured off, 220 µl of K_2CO_3 was added. Nitrites were measured at 550 nm. Bidistilled water was used as a blank probe [6].

Detemination of reduced glutathione (GSH): Plasma level of (GSH) was determined according to Beutler [7] and is based on GSH oxidation via 5,5 dithiobis-6,2-nitrobenzoic acid (DTNB). Bidistilled water was used as a blank probe. Measurement was done at 420 nm.

Method of descriptive statistics was used for description of general characteristics of the examinees in observed groups, as well as results obtained on the basis of the completed test. Independent *t*-test was used for comparison of arithmetic means of characteristics of two populations. Correlation was used to test the relationship between the parameters, and its presentation and interpretation of the significance was performed by using the linear correlation coefficient. The influence of individual variables as independent predictors for observed parameters was examined by using the multivariate regression analysis.

Results

Patients were divided into two groups according to AHI (cut off = 15). In the group with AHI < 15 there were 56 (43.8%) patients (44 males) and in the group with AHI ≥ 15, there were 72 (52.2%) patients (48 males). Demographic characteristics of examinees, mean values of parameters for assessing nutritional status, body composition and the sleep study in examined groups (*t*-test) are presented in Table 1.

A risk predictors for AHI ≥ 15 in comparison to AHI < 15 were analyzed by using the univariate logistic regression analysis (Table 2).

The influence of individual variables as predictors of GSH value was analyzed by using the univariate linear regression analysis (Table 2).

Table 1

Patients' characteristics

Observed Parameters	AHI < 15 (n = 56) mean ± SD (min–max)	AHI ≥ 15 (n = 72) mean ± SD (min–max)	*p*
Age (years)	42.57 ± 13.63	52.44 ± 11.38	0.033
Smokers (%)	32 (57.1)	40 (55.5)	0.565
AHI	10.2 (5.1–14.8)	24.3 (15.1–82.0)	< 0.001
ODI	4.63 ± 7.06 (0–23.5)	51.66 ± 23.26 (13.5–84.8)	< 0.001
Mean SatO2	93.54 ± 2.90 (88–97)	85.63 ± 12.52 (43–96)	0.035
Min SatO2	86.08 ± 4.80 (80–94)	67.75 ± 13.26 (39–85)	< 0.001
SatO2 < 90%	4.90 ± 10.04 (0–32.6)	41.86 ± 38.43 (1–99)	0.001
GSH (mmol/mL)	238.08 ± 84.37 (80.79–352.38)	172.77 ± 83.88 (27.15–285.16)	0.04*
NO (nmol/mL)	0.462 ± 0.373(0.42–1.25)	1.269 ± 0.789 (0.125–3.46)	0.001*
BMI (kg/m^2)	32.29 ± 5.58 (23.5–42.2)	36.55 ± 6.74 (26.8–52.6)	0.156
FM%	31.97 ± 4.13 (26.8–40.3)	34.25 ± 9.36 (24.3–49.1)	0.234
FM (kg)	31.68 ± 7.78	34.89 ± 9.64	0.483
FFM (kg)	64.01 ± 12.14 (40.75–82.72)	59.0 ± 10.16 (40.26–72.33)	0.929
FFMI	20.80 ± 3.46 (14.1–27.38)	20.8 (17.89–23.81)	0.172

AHI – apnea-hypopnea index; ODI – desaturation index; Mean SatO2 – medium saturation; Min SatO2 – minimum saturation; SatO2 < 90% – time with saturation less than 90%; GSH – reduced glutathione; NO – nitric oxide; BMI – body mass index; FM% – body fat percentage; FM – body fat mass; FFM – lean body mass; FFMI – lean body mass index; SD – standard deviation.

Table 2

Influence of individual variables on reduced glutathione (GSH) value in obstructive sleep apnea (OSA) patients

The observed risk factors	Univariate analysis	
	#B (95% CI)	*p*
The age	0.089 (0.021–0.199)	0.109
AHI	0.418 (0.088–0.748)	0.017*
ODI	-1.555 (-3.063–0.048)	0.046*
MeanSatO2	0.194 (0.028–0.36)	0.037*
MinSatO2	3.988 (-0.02–7.995)	0.047*
SatO2 < 90%	-0.192 (-0.284–0.10)	0.01*
Smoking	1.192 (-1.844–4.229)	0.198
BMI	0.141 (-0.415–0.697)	0.737
FM%	0.033 (-0.019–0.084)	0.220
FMkg	-0.184 (-0.386–0.018)	0.303
FFM kg	0.068 (-0.098–0.233)	0.437
FFMI	0.022 (-0.138–0.122)	0.703

#Unstandardized coefficient B; *statistically significant;

AHI – apnea-hypopnea index; ODI – desaturation index; Mean SatO2 – medium saturation; Min SatO2 – minimum saturation; SatO2 < 90% – time with saturation less than 90%; GSH – reduced glutathione; NO – nitric oxide; BMI – body mass index; FM% – body fat percentage; FM – body fat mass; FFM – lean body mass; FFMI – lean body mass index; CI – confidence interval.

The multivariate regression analysis determined independent predictors of GSH as follows: ODI, mean $SatO_2$, min $SatO_2$ (Table 3).

Table 3

Independent predictors of reduced glutathione (GSH) value in obstructive sleep apnea (OSA) patients

The observed risk factors	Multivariate analysis, $R^2 = 0.32$	
	B (95% CI)	*p*
ODI	-0.157 (-0.262 -0.053)	0.004*
MeanSatO2	-4.76 (-9.21 -0.306)	0.001*
MinSatO2	0.118 (0.03–0.206)	0.033*
SatO2 < 90%	-0.131(-0.80–0.537)	0.231
AHI	0.024 (-0.036–0.085)	0.687

[#]Unstandardized coefficient B; *statistically significant

AHI – apnea-hypopnea index; ODI – desaturation index; Mean SatO2 – medium saturation; Min SatO2 – minimum saturation; SatO2 < 90% – time with saturation less than 90%; CI – confidence interval.

The influence of the individual variables as predictors of NO value was analyzed by using the univariate linear regression analysis (Table 4).

Table 4

Influence of individual variables on nitric oxide (NO) value in obstructive sleep apnea (OSA) patients

The observed risk factors	Univariate analysis	
	[#]B (95%CI)	*p*
The age	0.016 (-0.005–0.036)	0.129
AHI	0.010 (0.001–0.019)	0.029*
AHI supinatio	0.016 (0.007–0.025)	0.003*
AHI nonsupinatio	0.016 (0.023–0.028)	0.017*
ODI	0.011 (0.002–0.02)	0.019*
MeanSatO2	0.009 (-0.02–0.039)	0.514
MinSatO2	-0.020 (-0.040–0.000)	0.052
SatO2 < 90%	0.001 (-0.008–0.01)	0.811
Smoking	-0.320 (-0.873–0.233)	0.247
BMI	0.014 (-0.029–0.058)	0.509
FM%	0.018 (-0.023–0.058)	0.372
FMkg	-0.004 (-0.041–0.032)	0.800
FFM kg	-0.025 (-0.052–0.002)	0.067
FFMI	-0.026 (-0.149–0.097)	0.664

[#]Unstandardized coefficient B; *statistically significant;

AHI – apnea-hypopnea index; ODI – desaturation index; Mean SatO2 – medium saturation; Min SatO2 – minimum saturation; SatO2 < 90% – time with saturation less than 90%; GSH – reduced glutathione; BMI – body mass index; FM% – body fat percentage; FM – body fat mass; FFM – lean body mass; FFMI – lean body mass index.

Multivariate regression analysis determined ODI as independent predictor of NO concentration in plasma (B = 0.038; 95% CI: 0.011–0.065) (Table 5).

Table 5

Multivariate linear regression analysis for nitric oxide (NO)

The observed risk factors	Multivariate analysis, $R^2 = 0.45$	
	B (95%CI)	*p*
ODI	0.038 (0.011–0.065)	0.011*
AHI	-1.221 (-2.558–0.117)	0.069
AHI supination	0.507 (-0.222–1.237)	0.150
AHI nonsupinatio	0.170 (-0.629–0.290)	0.425

[#]Unstandardized coefficient B; *statistically significant;

AHI – apnea-hypopnea index; ODI – desaturation.

Discussion

In our study, significantly lower plasma level of GSH and significantly higher mean values of NO concentration were found in plasma in the group with AHI ≥ 15 in comparison to subjects with AHI < 15. As independent predictors of a GSH level in plasma in OSA patients were found to be ODI, mean SatO2, min SatO2, while an independent predictor of NO concentration in plasma was ODI. We found no statistically significant difference in mean values of BMI and body composition parameters in patients with AHI < 15 and AHI ≥ 15. None of the markers of systemic oxidative stress in our research was significantly associated with BMI and body composition assessment parameters.

Obstructive sleep apnea is a major health problem due to the high prevalence, association with obesity (60%–90%), and there is an increasing amount of evidence suggesting that OSA is an important risk factor for cardiovascular diseases. GSH is an important antioxidant which, by using free radicals, prevents cell damage. Once oxidized, it can be reduced back by glutathione reductase, using NADPH as an electron donor. The ratio of reduced/oxidized glutathione (GSH/GSSG) is often used as a parameter for cell toxicity. Makris et al. [8] demonstrated that the level of GSH decreased during the night for about 15% in patients with OSA, and increased averagely for about 63% in patients without OSA. In a study done by Ntalapascha et al. [9] it was shown that GSH concentrations in plasma during night (morning-night), differed significantly between the patients with severe OSA and the control group (AHI < 5). This prospective research among the population with OSA, without significant comorbidities, provides evidence that OSA can independently be the cause of an increase in the level of systemic oxidative stress. In our study, as independent predictors of GSH in plasma were determined to be ODI, mean $SatO_2$, min $SatO_2$, while an independent predictor of NO concentration in plasma was ODI. This may indicate that the severity of desaturation during sleep, especially intermittent hypoxia (ODI) has a substantial impact on systemic oxidative stress in patients with OSA. In a study done by Simiakakis et al. [10], in the group with AHI > 15, antioxidant capacity was notably lower than in the control group (AHI < 5). Minimum desaturation was the most important predictor of levels of the biological antioxidant potential (BAP). Results of this study showed that OSA may affect the oxidative stress by reducing antioxidant capacity due to nocturnal hypoxia.

However, there are also studies with opposite results that did not show increased oxidative stress in OSA. In a study by Svatikova et al. [11], in patients with OSA without other chronic diseases, there was no increase in level of markers of systemic oxidative stress (thiobarbituric acid reactive substances – TBARS, oxidized low-density lipoproteins – LDL, isoprostanes) in comparison to the control group without OSA.

A source of oxidative stress in overweight is similar to that in patients with OSA, so the independent role of OSA in the occurrence of systemic oxidative stress is not yet completely clear. In a large population study, it was shown that

BMI, alongside with smoking and diabetes, is the most important independent factor associated with systemic oxidative stress, although the contribution of accompanied OSA cannot be excluded [4]. In our study, no statistically significant difference in mean values of BMI and body composition parameters in the patients with AHI < 15 and AHI ≥ 15 were found. None of the markers of systemic oxidative stress in our research were significantly associated with BMI and body composition assessment parameters. In the paper by Fujita et al. [12] no substantial association was found between markers of oxidative stress and parameters for OSA severity assessment. However, it has been shown that the waist/hip ratio (WHR) is an independent predictor of glutathione peroxidase (GPX) concentration and a total antioxidant status (TAS) (r = -0.317). The results of this study indicate that oxidative stress in OSA is more expressively associated with central obesity than with intermittent hypoxia and AHI [12].

Further studies are required to confirm whether the severity of OSA is an independent predictor of systemic oxidative stress which can significantly contribute to cardiovascular morbidity and mortality.

Conclusion

The results of our study are in favor of that severity of OSA is significantly associated with reduced antioxidant capacity and more expressed systemic oxidative stress. The degree of desaturation during sleep significantly affects systemic oxidative stress in patients with OSA aside from nutritional status and body composition.

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Received on October 30, 2016.
Revised on March 02, 2017.
Accepted on March 06, 2017.
Online First March, 2017.

ORIGINAL ARTICLE

UDC: 615.46::616.314-77-092.9
https://doi.org/10.2298/VSP170112045K

Denture base resins biocompatibility testing *in vivo*

Ispitivanje biokompatibilnosti akrilata za izradu zubnih proteza *in vivo*

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Abstract

Background/Aim. The wearing of acrylic dentures is the cause of the inflammatory reaction of the oral mucosa. The aim of this study was to investigate the response of rat tissues to subcutaneous and intramuscular implantation of different acrylic samples, by histopathological analysis of the tissue. **Methods.** The study included two samples of hard and three samples of soft acrylic resins (heat and cold polymerized), that were subcutaneously and intramuscularly implanted in rats tissues. Implantation tests were designed to test the biological response of the surrounding tissue to the tested materials after their application for the period of two weeks and the period of four months. **Results**. After two weeks, regardless of the type of implantation, histopathological analysis showed an acute inflammatory response. There was an intense hyperplasia of inflammatory cells, multiplication of connective tissue as well as formation of many new blood vessels. The highest level of inflammatory changes was observed after the application of cold-polymerized resins. A lower intensity of inflammation in the case of heat polymerised resin was the result of its more complete polymerization. After the second observation period, fibrotic capsules were formed around the implanted samples indicating a chronic course of the inflammatory process. Less visible signs of inflammation and chronicity of the processes indicate that with time, i.e. with the length of the observation period, reduces inflammation. **Conclusion.** The **s**ubcutaneous and intramuscular implantation of acrylic resins material samples led to inflammatory response whose intensity was decreased over time. Heat polymerized resin was a biologically more acceptable in comparison to the cold polymerized acrylates.

Key words:
dentures; acrylates; biocompatible materials; rats.

Apstrakt

Uvod/Cilj. Nošenje akrilatnih proteza uzrokuje inflamatorne reakcije oralne sluzokože. Cilj ovog istraživanja bio je da se ispita odgovor tkiva pacova nakon potkožne i intramuskularne implantacije različitih akrilatnih uzoraka pomoću patohistološke analize tkiva. **Metode.** U studiji su korišćena dva uzorka tvrdih i tri uzorka mekih akrilatnih smola (toplo i hladno polimerizovanih) koji su supkutano i intramuskularno implantirani u tkivo pacova. Implantacioni testovi su osmišljeni tako da se ispita biološki odgovor okolnog tkiva na testirane materijale nakon njihove primene u periodu od dve nedelje i periodu od četiri meseca. **Rezultati**. Posle dve nedelje, bez obzira na vrstu implantacije, patohistološka analiza pokazala je akutni inflamatorni odgovor. Došlo je do intenzivne hiperplazije inflamatornih ćelija, umnožavanja vezivnog tkiva kao i formiranja brojnih novih krvnih sudova. Najviši nivo upalnih promena primećen je nakon aplikacije hladno polimerizovanih akrilatnih smola. Niži intenzitet inflamacije kod uzoraka toplo polimerizovanih smola rezultat je njihove potpunije polimerizacije. Nakon drugog perioda posmatranja uočeno je formiranje fibroznih kapsula oko implantiranih uzoraka što ukazuje na hroničnan tok upalnog procesa. Manje vidljivi znaci zapaljenja ukazuju na transformaciju zapaljenske reakcije u hronični oblik u kojoj se vremenom, odnosno tokom dužeg perioda posmatranja samo zapaljenje smanjuje. **Zaključak**. Supkutana i intramuskularna implantacija akrilnih uzoraka dovela je do inflamatornog odgovora čiji je intenzitet smanjen tokom vremena. Toplo polimerizovani akrilati su biološki znatno prihvatljiviji u odnosu na hladno polimerizovane akrilate.

Ključne reči:
zubna proteza; akrilati; biokompatibilni materijali; pacovi.

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Introduction

Wearing of denture plates is very frequently the cause of inflammatory reactions of submucose of the oral cavity [1, 2]. Hypersensitivity to acrylates was observed in almost 17% of patients wearing dentures [3].

Adverse effects related to acrylates are, in most cases, of local character and may be presented in form of cheilitis and stomatitis, stinging and burning in the mouth, painful sensations of different intensity and candidiasis [4–8]. Allergic reaction to acrylic denture may occur in more severe form such as erythema multiforme [9]. Potential toxicity of temporary acrylic dentures was well-documented. Contact stomatitis in children caused by wearing orthodontic appliances was described in clinical practice as well [10].

The above mentioned changes are more frequent in patients with already infected, inflammed submucose of the oral cavity damaged with different drugs or vomitting [11, 12]. Some regions of the oral cavity are particularly sensitive to irritating effects of acrylic dentures [13]. Apart from being placed to endure additional strain, zones with keratinized epithelium represent places less sensitive to effect of harmful components of acrylates as well [14].

Intense gingival inflammation under acrylic veneers of bridges, may, among other things, be explained by porousness and superficial roughness as the result of a great abrasion of acrylic material. Regarding the complexity of the potential clinical biocompatibility investigation of acrylic materials used to manufacture dentures, it is easier to analyse tissue reaction to acrylic materials after implantation of the samples in tissues of experimental animals. Such studies comparing effects of different types of commercially available acrylic materials *in vivo* conditions has not been performed so far.

The aim of the study was to perform pathohistological analysis of the tissues after subcutaneous and intramuscular implantation of samples of different acrylic materials.

Methods

Tested material

The tested material included two hard and three soft acrylates used in prosthodontic dentistry for construction and readaptation of mobile dental restorations. Cold and hot polymerized acrylates were used in the study (Table 1).

Parallelepiped shaped material samples with rounded edges 1 × 2 × 3 mm were made. Upon polymerization the samples were polished using standard procedure in order to avoid mechanical irritation during the implantation in tissue of experimental animals.

According to the type of the tested materials the samples were divided into five experimental groups (G1-G5), each of which were further subdivided into two groups depending on the place of the implantation (subcutaneous and intramuscular). Each experimental group consisted of twelve samples (n = 12), six samples for subcutaneous and six samples for intramuscular implantation.

A pink wax (Cavex, Holland BV) sample with identical shape and dimensions was made as a negative control for each implanted sample of the tested material (n = 60). Samples were a combination of paraffin, microwax and beeswax and its neutral effect on tissues was previously experimentally proven [17].

All of the tested samples were desinfected with 70% ethanol and rinsed with saline (0.9% NaCl). The samples were stored in sterile Petri dishes at room temperature untill implantation. Immediately before implantation, they were removed to Petri dish with sterile saline (no more than 60 minutes).

Experimental animals

Laboratory Wistar male rats, 10 to 12 weeks old and 180–200 g of average weight were used in the experiment. Twelve animals were used for each of the tested materials (n = 60).

Table 1

Tested acrylic materials

Tested material	Experimental group (G)	Manufacturer	Acrylic type	Content	
				powder	liquid
Bosworth Trusoft	G1	HG Bosworth Company USA	soft cold polymerized acrylate	poly (ethyl methacrylate)	ethyl alcohol, butyl benzyl phthalate
Lang Flexacryl	G2	Lang Dental MFG.Co. USA	soft cold polymerized acrylate	poly (ethyl methacrylate)	n-buthyl methacrylate
Lang Immediate	G3	Lang Dental MFG.Co. USA	soft cold polymerized acrylate	poly (ethyl methacrylate)	methyl methacrylate
Triplex Cold	G4	Ivoclar Vivadent, Lichtenstein	hard cold polymerized acrylate	poly (methyl methacrylate)	methyl methacrylate, ethylene glycol dimethacrylate
Triplex Hot	G5	Ivoclar Vivadent, Lichtenstein	heat polymerized acrylate	poly (methyl methacrylate)	methyl methacrylate, ethylene glycol dimethacrylate

The animals were healthy and acclimatized to laboratory environment and standard laboratory nutrition. They were followed for the behaviour changes, disease onset and weight loss to eliminate potential irregularity that will affect plausibility of the obtained data.

Experimental investigations on animals were approved by the Ethics Committee of the Faculty of Medicine in Niš (number 01-2066-1).

Experimental design

Implantation tests were designed for examining biological response of the surrounding tissue to the tested materials upon their application (ISO 10994-6: 2007) [18].

All animals were operated on under general anesthesia. Premedication included the application of atropin sulphate (Verofarm, Russia) and diazepam (Galenika, Serbia) in a dose of 0.2 mg/100 g of body weight. General anesthesia of 30–60 minutes was administered intraperitoneally with 0.3 mL of 10% Ketamidor® (Richer Pharma A.G., Austria).

Anesthetised animals were placed in prone position on a special wooden framework. The operating field was prepared by removing hairs on interscapulary portion of the back and both thighs.

The implantation region was rinsed with povidone iodine. The implantation procedure was performed using a sterile needle 4/18. The samples were subcutaneously implanted in the interscapulary portion of the back. The sample of the tested material was implanted on the left side of medial dorsal line and the sample of sterile pink wax was implanted on the right side. The sample of the tested material was implanted in *m. gastrocnemius* of the left leg of the experimental animal, while the sample of the pink wax was implanted in the muscle on the opposite side. The wounds were healed with povidone iodine and left to heal spontaneously.

No antibiotic protection of experimental animals was performed. Postoperative recovery was monitored every day, and there were no signs of infection.

The two-week observation period and four-month observation period were designed. After each of the observation periods three animals from each experimental subgroup were sacrificed.

Euthanasia of experimental animals was performed by exanguination of the left ventricle and extirpation of complete blood. Changes in the subcutaneous tissue of the experimental animal after the four-week implantation period were both macroscopically and microscopically observed.

Preparation of samples for microscopic analysis

Tube-like portions of subcutaneous and muscular tissue where resins tested had been implanted were taken as samples for analysis. The samples of the implanted materials were carefully separated from the tissue by twizzers and fixated in 100% formalin. The material was further dehydrated in growing concentrations of ethanol (from 50% to absolute). Upon xylol illumination the material was put in paraffin molds. Tissue blocks molded in paraplast were cut on microtom (LKB Broma, Sweden), (1.5 μm) and stained using classical method – hematoxylin & eosin (HE) and special method for staining of collagen fibres – trichrome staining according to Masson.

The stained preparations were analysed histopathologically on the image analysis system Lucia 3.2 G (Laboratory Imaging, the Czech Republic) on microscope NU-2 (Carl Zeiss, Germany).

The histomorphological analysis of tissues was designed for all samples of subcutaneous and muscular tissue that were in contact with the tested material and negative control for both observation periods.

The evaluation of results by microscopic analysis was performed on the basis of the presence of inflammatory reactions and tissue fibrosis, number and distribution of inflammatory cells and the existence of degenerative changes as well as potential necrosis and destructive changes in capillary walls (Table 2).

Results

After the two-week implantantion period it was noticed that the implantation of Lang Immediate sample material caused macroscopically apparent changes on subcutaneous tissue in the form of mild erythema and local hemorrhage (Figure 1). There were no local macroscopic changes on the implantation site of control samples and other tested materials.

Table 2

Evaluation of a degree of inflammatory reaction [19]

Degree of inflammatory reaction	Score	Changes in the surrounding tissue
Slight reaction	0	Formation of fibrous capsule with sparce inflammatory cells.The blood vesels are of small calibre with visible endothelial cells.
Mild reaction	1	Presence of fibrous capsule with lower number of lymphocytes and plasmocytes. Low proliferation of conective tissue was followed by spherical blood vessels without congestion.
Moderate reaction	2	Formation of fibrous capsule with presence of macrophages, polymorphonuclears, lymphocytes and plasmocytes. Congestion of blood vessels was not noted.
Intensive reaction	3	Presence of a large accummulation of polymorphonuclears lymphocytes, plasmocytes, macrophages, giant cells of foreign body type and capillaries with prominent congestion.

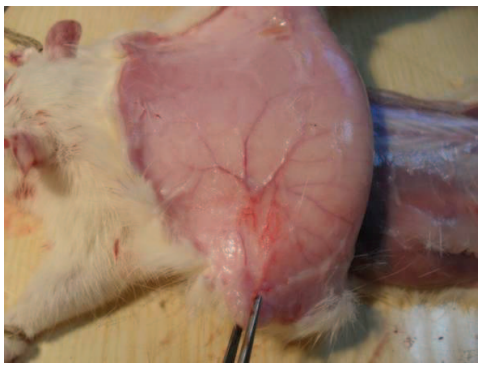

Fig. 1 – Changes in subcutaneous tissue observed at the implantation site of Lang Immediate sample.

Pathological analysis of subcutaneous tissue showed acute inflammatory reaction to the presence of the tested materials. It included intesive hyperplasia of inflammatory cells (polymorphonuclears, lymphocytes, plasmocytes and macrophages), duplication of connective tissue as well as formation of a great number of new blood vessels. Fusion of macrophages led to formation of giant cells as the response to the presence of a foreign body.

The highest degree of inflammatory changes were observed in cold polymerized acrylates (Figure 2). The presence of Triplex Hot sample caused low intensity inflammatory changes in relation to other tested materials which may be attributed to its more complete polymerization (Figure 3).

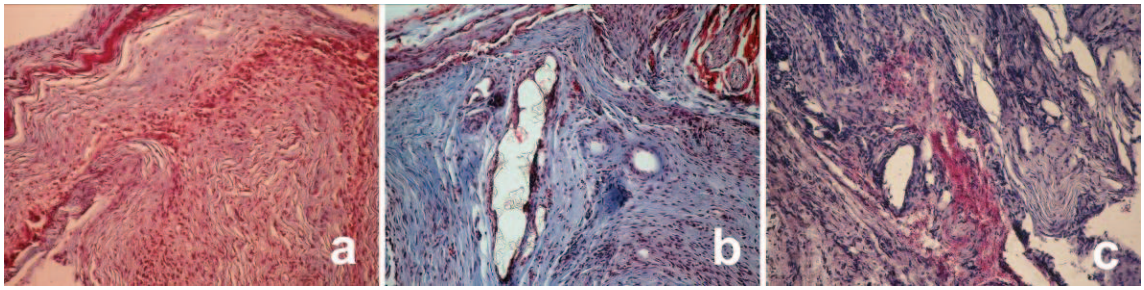

Fig. 2 – Application of samples in subcutaneous tissue of experimental animal after two weeks caused the occurrence of the mild degree fibrosis, hyperplasia of inflammatory cells and formation of new blood vessels: a) Bosforth Trusoft; b) Lang Flexacryl; c) Triplex Cold (Trichrome staining according to Masson, ×100).

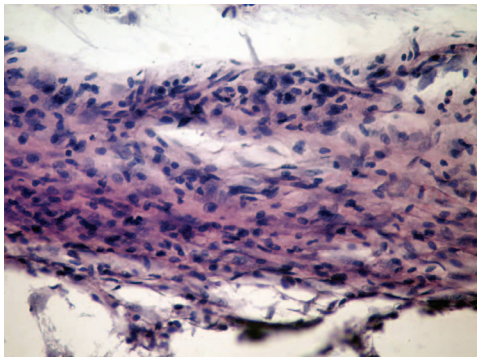

Fig. 3 – After two weeks, subcutaneous application of Triplex Hot sample caused proliferation of connective tissue cells and fibrosis (HE; ×200). HE – hematoxylin eosin.

There was no inflammatory reaction on the site of subcutaneous implantation of control sample, which eliminates mechanical trauma during the application as the cause of occurrence of the above mentioned inflammatory reaction.

After a four-week observation period,-there were no macroscopic changes at the implantation site of acrylic materials in all tested experimental groups.

Fibrous capsules were formed around the implanted samples as the result of the presence of material in the subcutaneous tissue. The hyperplasia degree of inflammatory cells was lower in comparison to the first observation period. Tissue fibrosis with lower number of connective tissue cells indicated chronic course of inflammatory process (Figure 4). Less prominent inflammatory signs and chronic course showed reduction of inflammation over the observed period of time.

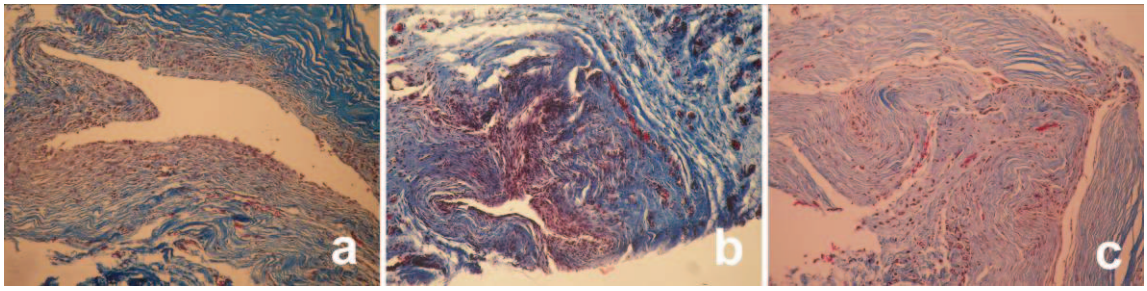

Fig. 4 – After four months of the subcutaneous implantation of material samples focal tissue fibrosis occurred with slight inflammatory infiltrate: a) Lang Immediate; b) Triplex Cold; c) Triplex Hot (Trichrome staining according to Masson, ×100).

Implantation of acrylic samples in *m. gastrocnemius* of experimental animals during the two-week observation period caused no macroscopic changes of the tissue. Histopathological analysis of the surrounding muscle tissue after removal of material samples showed mild to strong inflammatory reaction, hyperplasia of connective inflammatory cells, intramuscular proliferation of connective tissue and a great number of newly formed blood vessels in the tested materials of all experimental groups (Figures 5 and 6).

Formation of fibrous capsule around the application site represented the result of a four-month implantation of the tested material samples on macroscopic level. The tissue fibrosis was from mild (Bosforth Trusoft, Lang Flexacryl, Triplex Cold, Triplex Hot) to moderate degree (Lang Immediate). Pathohistological findings of all tested materials confirmed domination of connective fibres in relation to inflammatory cells, suggesting chronic inflammation, that is, a scar formation in muscle tissue (Figure 7).

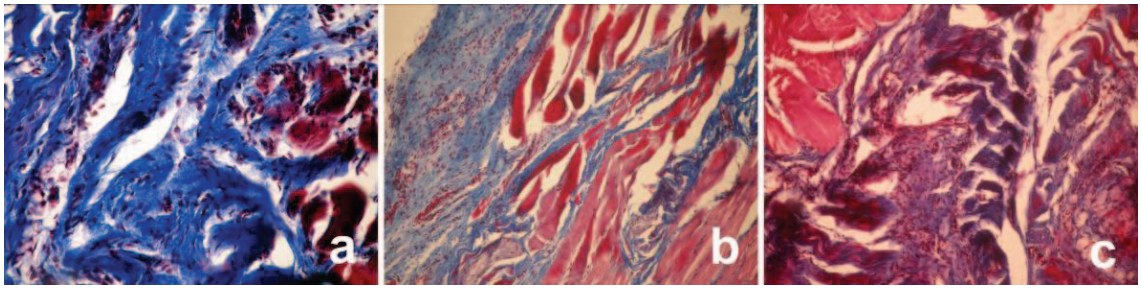

Fig. 5 – After two weeks of the implantation period of material samples Lang Flexacryl (a) and Lang Immediate (b) hyperplasia of giant cells of foreign body type and intensive proliferation of connective tissue were observed. Fibrous reaction with numerous newly formed blood vessels occurred around hollow spaces with Triplex Cold sample (c) (Trichrome staining according to Masson, ×200).

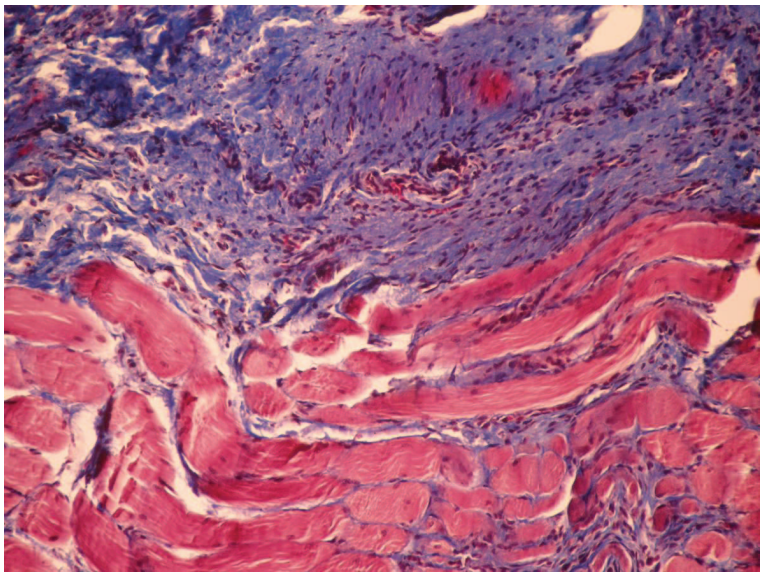

Fig. 6 – A two-week implantation period of Triplex Hot sample caused proliferation of connective tissue cells and intensive fibrosis in muscle tissue (Trichrome staining according to Masson, ×100).

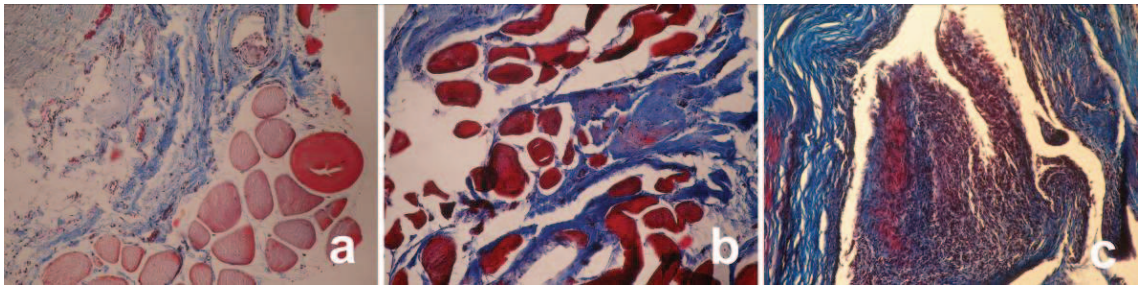

Fig. 7 – The mild degree fibrosis was present in muscle tissue after a four-month implantation of Lang Flexacryl sample (a) and Triplex Hot (b) sample, while the moderate degree fibrosis was present in Lang Immediate sample (c) (Trichrome staining according to Masson, ×200).

Inflammation caused by the presence of implantes acrylic samples reduced over time, that is, along with the duration of the observation period. Implantation of control samples of pink wax caused no significant changes in subcutaneous tissue and muscle tissue in the whole observation period (Table 3).

Table 3

The average values of the prominence degree of inflammatory reaction of tissue after the subcutaneous and intramuscular implantation of acrylic samples

Experimental group (G)*	Subcutaneous implantation		Intramuscular implantation	
	after 2 weeks	after 4 months	after 2 weeks	after 4 months
G1	3	1	2	1
G2	3	1	3	1
G3	3	1	2	2
G4	3	1	2	1
G5	2	1	1	1
Control group	0	0	0	0

***see Table 1**

Discussion

The test of tissue reaction to the implanted material (ISO 10994-6: 2007) has no direct implication on clinical application [18]. However, immediate contact of implanted material and tissue offers a more precise picture of a body reaction to its presence. Subcutaneous implantation turned out to be an efficient method for the examination of biological features of dental materials [19, 20]. The reaction of subcutaneous tissue may be considered analogous to that of submucous tissue regarding their unique histological form. On the other hand, implantation of dental materials in muscle is considered to be a less sensitive method for examination of biocompatibility of dental materials, but the obtained data certainly contributes to the analysis of tissue reaction to acrylates.

Samples of acrylic materials and control samples of pink wax were implanted in the subcutaneous tissue and muscle of rats during the two-week period and four-month period. Changes in the tissues of experimental animals after removal of material samples may be considered analogous to the tissue reaction in direct contact with the denture plates. The disadvantage of the applied test is reflected in the failure to identify the influence of salivary flow and its buffering capacity on the tissue reaction in contact with acrylic material.

Histopathological analysis of subcutaneous tissue showed acute inflammatory reaction after removal of material samples. Intensive hyperplasia of inflammatory cells or granulomatous reaction, duplication of connective tissue as well as formation of numerous new blood vessels were the results of the two-week long presence of acrylic material. Fusion of macrophages led to the development of giant cells as the response to the presence of a foreign body. A more intensive reaction of subcutaneous tissue to the implantation of cold polymerized acrylates represented further evidence of its biological inferiority as compared to heat polymerized acrylate. The obtained data may be explained by greater porousness and superficial adherence of materials as well as greater amount of non-polymerized potentially toxic substances in samples of soft acrylates and Triplex Cold in relation to Triplex Hot. Findings of Kallus [1, 2] also confirmed greater granulomatous reaction of subcutaneous tissue of rats to the presence of cold as compared to hot polymerized acrylate. The results of this study did not indicate significant changes in inflammatory reaction of tissue to the presence of different types of soft acrylic materials.

After the second observation period fibrous capsules formed around implanted samples as the result of the presence of material. Less prominent inflammatory signs and chronic course of the process showed inflammation reduction over the period of time, which is in accordance with the findings of Kallus [2] and Zmener [19].

The presence of Triplex Hot sample caused low intensity inflammatory changes in relation to other tested materials, which may be attributed to its more complete polymerization [21]. Analysing different materials for denture base Ebadian et al. [22] indicated higher biocompatibility of polymerized poly (methylmethacrylate) (PMMA) in comparison to Co-Cr alloys after implantation in buccal vestibulum of dogs.

Pathohistological analysis of muscle tissue surrounding the tested acrylic materials that were removed after the first observation period showed moderate to strong granulomatous inflammatory reaction, hyperplasia of connective inflammatory cells, intramuscular proliferation of connective tissue and a great number of newly formed blood vessels. The most intensive inflammatory response was noticed in soft acrylic material Lang Flexacryl and solid cold polymerized Triplex Cold. After implantation of the Lang Flexacryl sample hyperplasia of giant cells of a foreign body type in muscle tissue was observed. Implantation of Bosforth Trusoft and Lang Immediate samples led to moderate proliferation of young connective tissue in the muscle and duplication of inflammatory cells.

The presence of the hot polymerized Triplex Hot samples in muscle tissue led to the proliferation of connective tissue cells and intensive fibrosis, and reaction had chronic course from the very beginning. In accordance with this study, Stinson [23] showed that implantation of PMMA sample in gluteal muscles of rats led to formation of fibrous capsule over the period of time. Dillingham et al. [24] found mild toxicity of PMMA after it had been implanted in paravertebral muscle of a rabbit. Biocompatibility of PMMA based materials was proved after their implantation in the bone as well [17].

Formation of fibrous capsule surrounding the application site represented the result of a four-month implantation of the cold polymerized material samples on microscopic level. The tissue fibrosis ranged from the mild (Bosforth Trusoft, Lang Flexacryl, Triplex Cold, Triplex Hot) to the moderate degree (Lang Immediate). The histopathological findings of all tested materials confirmed domination of connective fibres in comparison to inflammatory cells, indicating chronic type of inflammation. Inflammation induced by the presence of implanted acrylic samples reduced over the observation period.

Histopathological analysis of the prominence degree of inflammatory reaction of tissue that was in immediate contact with the tested material sample showed more prominent

inflammatory reaction in the subcutaneous implantation as compared to the intramuscular one in all tested experimental groups of acrylic materials. Control samples caused no inflammation of the surrounding tissue and the occurrence of mild fibrosis in few of them was probably the result of mechanical tissue damage during the implantation procedure.

Cold polymerized acrylates induced the most intensive granulomatous reaction after both implantation procedures, which is in accordance with the results of examination of their cytotoxic effect [25–27]. The results of this study are in positive correlation with the findings of *in vitro* studies that proved stronger cytotoxic effect of soft and hard cold polymerized acrylates as compared to hot polymerized acrylates [28–31].

Conclusion

The subcutaneous and intramuscular implantation of the samples of the tested acrylic materials led to acute inflammatory reaction which become chronic over time. Heat polymerized acrylate showed the least proinflammatory effect. Therefore, the authors recommend heat polymerited acrylates as the material of choice for construction and readaptation of dentures.

Acknowledgment

This study was supported by the Project No 41017 founded by the Ministry for Education, Science and Technological Development of the Republic of Serbia.

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Received on January 12, 2017.
Revised on February 28, 2017.
Accepted on March 10, 2017.
Online First March, 2017.

SHORT COMMUNICATIONS

UDC: 614:[616.9-036.22:616.34
https://doi.org/10.2298/VSP161118032R

Intestinal parasitosis in asylum seekers from the Middle East and South Asia

Parazitoze kod azilanata sa Bliskog istoka i iz južne Azije

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Abstract

Background/Aim. It is estimated that about 230 million refugees and asylum seekers circulates worldwide. Parasitosis are diagnosed in recent years with increasing frequency both in Europe and other developed countries. International migration of population, as an inalienable part and a result of the process of globalization, has an increasing impact on health of the population of countries through which migrants pass or settle. The aim of this study was to determine the incidence of intestinal parasitic diseases in asylum seekers on the territory of Belgrade. **Methods.** The study group included 97 asylum seekers from the Centre for Asylum seekers in Obrenovac, in the period December 2013–January 2014. Stool samples were taken less than seven days after arriving to the Center and sent to the Laboratory for Parasitological Diagnosis of the Public Health Institute of Belgrade. Detection of parasites in stool samples was performed by a direct native slide made of fresh sample and direct slide made after stool concentration applying "Mini Parasep" technique. Statistical analysis included application of χ^2-test of matching and χ^2-test of independence. **Results.** The study showed that the parasites were detected in 9.3% of cases, in the group of 15–24 years of age. Protozoa were found in 6.2% and helminthes in 3.1% of the samples. **Conclusion.** Most intestinal parasitosis were found in asylum seekers from Bangladesh. All parasitosis were found in males and the most frequently detected parasite was *Giardia lamblia.*

Key words:
parasitic diseases; refugees; diagnosis; feces; serbia.

Apstrakt

Uvod/Cilj. Procenjeno je da širom sveta cirkuliše oko 230 miliona izbeglih lica i azilanata. Parazitoze se poslednjih godina dijagnostikuju sa sve većom učestalošću kako u Evropi, tako i u drugim razvijenim zemljama sveta. Međunarodna migracija stanovništva kao neotuđivi deo procesa globalizacije i kao njena posledica, sve više ima uticaja na zdravlje stanovništva zemalja kroz koje migranti prolaze i u kojima se nastanjuju. Cilj ovog rada je bio da se utvrdi učestalost parazitoza kod tražioca azila na teritoriji Beograda. **Metode.** Ispitivanjem je obuhvaćeno 97 azilanata koji su boravili u Centru za smeštaj azilanata Obrenovac, u periodu decembar 2013–januar 2014. godine. Uzorci stolice ispitanika, ne duže od sedam dana nakon dolaska u Centar, su dostavljani u laboratoriju za parazitološku dijagnostiku Gradskog zavoda za javno zdravlje (GZZJZ) Beograd. Za pregled stolice na prisustvo crevnih protozoa i helminata korišćen je direktan nativni preparat svežeg uzorka i direktan preparat nakon primene "Mini Parasep" koncentracione tehnike. Za statističku obradu podataka korišćeni su χ^2-test podudarnosti i χ^2-test nezavisnosti. **Rezultati.** Pozitivan nalaz parazita u stolici utvrđen je kod 9,3% ispitanika, uzrastne grupe 15–24 godina. Protozoe su izolovane kod 6,2%, a helminti kod 3,1% uzoraka. **Zaključak.** Istraživanjem je ustanovljeno da je najčešće izolovana *Giardia lamblia* u stolici azilanata muškog pola iz Bangladeša.

Ključne reči:
parazitne bolesti; izbeglice; dijagnoza; stolica; srbija.

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Introduction

Parasitosis are diagnosed in recent years with increasing frequency both in Europe and other developed countries [1]. International migration of population, as an inalienable part and a result of the process of globalization, has an increasing impact on the health of the population of countries through which migrants pass or settle [2]. It is estimated that about 230 million refugees and asylum seekers circulates worldwide [2, 3]. In August 2015, the number of asylum applications in the European Union (EU) reached a record with over 148,880 applications [4]. Global demographic forecast indicate that the impact of factors that encourage international migration will become more intense in all regions of the world [2], contributing to raising local health issues to the international level [5]. Such predictions are related to diseases caused by intestinal parasites that were once considered rare and were linked only to tropical and subtropical areas as well as for areas with low living, hygienic and sanitary standards. Given that the use of human origin fertilizers is still common in certain less developed parts of the world, this can lead to the ingestion of parasite eggs over insufficiently washed vegetables and fruits [6]. Also, the process of inadequate treatment of waste water, being discharged directly into rivers and lakes, is a clear danger to human health [6]. Features of parasitic infections in immigrants can vary, as well as the time of their infestation [1]. To a large extent, they depend on exposure to parasites in the countries of origin, direction of movement and the characteristics of the areas which they finally inhabitate [1].

The data on the incidence of parasitic infections in people who have migrated to developed countries are highly variable and dependent on the various circumstances that lead to migration of the population [7], and development of the health systems of the countries with temporary or permanent immigration [8]. The overall prevalence of potentially pathogenic parasites among migrants is in the range of 8%–86% [8].

In Serbia, the data on the incidence of parasitic infections in immigrants have not yet been published. On the other hand in the United States (US) these data are available and the highest incidence of intestinal parasitosis were observed in individuals originating from Asian countries [9].

In developed countries, there is no agreement on how to control parasitic diseases brought by migrating population. Frequent diagnosis of malaria and intestinal parasites in Somali refugees registered in the US led to the implementation of the recommendations that all refugees over 2 years of age should be presumptively treated with single dose albendazole since 1997 [5]. Given the poor organized health care for refugees and a high risk of transmission of infection, the first contact with health services in the new environment should be fully focused on implementation of screening and treatment of high-risk patients [9]. In September 2015, the European Asylum Support Office (EASO) launched a project to introduce protocols for monitoring infectious diseases in immigrants [4]. In the EU countries, the recommendations include general and selective screening and careful observation of the health status of immigrants.

The aim of this study was to determine the incidence of intestinal parasitic diseases in asylum seekers at the Centre for Asylum Seekers in Obrenovac.

Methods

The study included 97 asylum seekers who were in the Centre for Asylum Seekers in Obrenovac, in the period December 2013 – January 2014. Stool samples (taken not more than seven days after arriving to the Center) were collected in special containers for feces (Dunavplast, Indjija, Serbia) and sent to the Laboratory for Parasitological Diagnosis of Public Health Institute of Belgrade. Each sample contained around 10–20 g of stool collected in the morning in the facility where asylum seekers were accommodated. For detection of intestinal protozoa and helminthes two slides were used: a direct native slide made of a fresh sample [10] and direct slide made after stool concentration by "Mini Parasep" concentrator as modified Ridley-Allan method for fecal parasite concentration (Mini Parasep® Faecal Parasite Concentrator, DIASYS, Wokingham, Berkshire, England) [11]. The concentrator consists of a freestanding mixing chamber with a 10% formalin solution and ethyl-acetate (Triton X-100 solution), two-stage filtration matrix for the elimination of large particles of about 425 μm, the chamber for dispersion of grease which removes fatty particles and remnants of feces and lower conical part where a sediment is formed by centrifugation [11]. For detection of intestinal helminthes, we also used the Kato technique [10].

Statistical analysis

Commercial software package SPSS 13.0 for Windows was used for statistical analysis of the obtained results and for descriptive statistical characteristics of examined variables. The presence of statistically significant difference between the incidence of parasitic infections in the general population of the city of Belgrade and the studied group of asylum seekers in Obrenovac was examined by χ^2-test of matching. To test differences in the prevalence of parasitic infections by special categories of the patients (gender, age, country of origin) χ^2-test of independence was used. Statistically significant difference was considered for a p-value ≤ 0.05.

Ethical aspects

Examination was carried out in accordance with ethical standards of the Helsinki Declaration from 1975 revised in 1983. Detection of parasitic infections in asylum-seekers was conducted in accordance with the legislation of the Republic of Serbia, according to the Ordinance on medical examinations of asylum seekers when entering the Asylum Centre [12].

Results

The study group comprised 97 asylum seekers from countries in the Middle East and South Asia, median age of 24.5 years, predominantly males (Table 1).

Table 1

Frequency of positive parasitological findings in the study group by age, sex and country of origin

Characteristics	Total	Positive findings
Age (years), n (%)		
0–14	6 (6.2)	0 (0)
15–24	46 (47.4)	7 (15.2)
25–34	34 (35.0)	2 (5.9)
35–44	5 (5.2)	0 (0)
45+	6 (6.2)	0 (0)
Gender, n (%)		
male	86 (88.7)	9 (10.5)
female	11 (11.3)	0 (0)
State of origin, n (%)		
Syria	18 (18.6)	0 (0)
Pakistan	50 (51.5)	4 (8.0)
Bangladesh	28 (28.9)	5 (17.9)
Afghanistan	1 (1.0)	0 (0)
Total, n (%)	97 (100)	9 (9.3)

Parasites in faeces were detected in 9/97 (9.3%) of cases. Although the sample of asylum seekers is too small, this value is significantly higher than reported by the Public Health Institute of Belgrade where the incidence of intestinal parasitism in general population on the territory of Belgrade in 2010 was 1.9% ($p = 0.000$) [13]. The presence of parasites in stool was most commonly found in male asylum seekers, 9/86 (10.5%), in the age group 15–24 years, 7/46 (15.2%) and in those who originated from Bangladesh, 5/28 (17.9%) (Table 1).

Of all positive samples (n = 9), protozoa were found in 6/97 (6.2%) and helminthes in 3/97 (3.1%). The most commonly found were *Giardia lamblia* 4/9 (44.4%), *Blastocytis hominis* 2/9 (22.2%) and *Trichuristrichiura* 2/9 (22.2%), while *Taenia spp.* was discovered in only one person 1/9 (11.1%) (Figure 1).

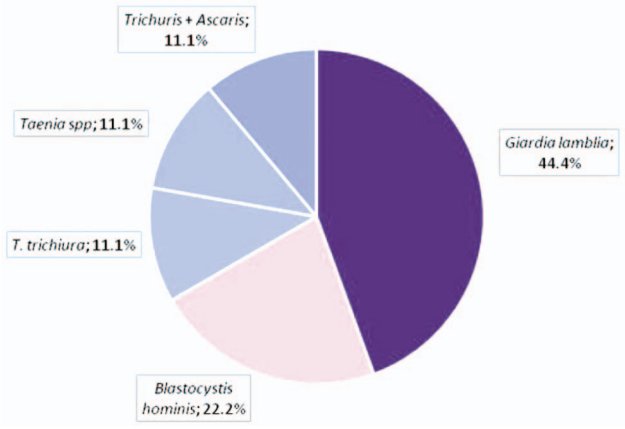

Fig. 1 – Species of parasites found in stool samples of asylum seekers.

In the group of people with positive test for parasites in stool, most people with *Giardia lamblia* were from Pakistan 3/4 (75%), ($p = 0.338$); People from Bangladesh had positive findings in stool for *Blastocystis hominis* (2/2; $p = 0.025$) and *Trichuris trichiura* (2/2; $p = 0.025$), while in asylum-seekers from Syria no parasites were found in stool samples. There were no positsive findings of parasites among children and women.

Discussion

In developed countries, a more detailed assessment of health of immigrants is performed and that can serve to identify diseases which could later become public health problem in these countries. In the United States, medical screening for adult refugees and asylum seekers is required, and it includes assessment of mental health, radiological examination of the thorax [sputum test for tuberculosis (TB) is done if radiological finding is atypical] and testing for syphilis and human immunodeficiency virus (HIV) [5]. Spanish authors point to the need of establishing prevalence and demographical characteristics of the patients with eight diseases that have potential risk of transmission: latent and active TB, syphilis, HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV), Chagas disease, *Giardia intestinalis* and *Entamoeba histolytica* / *Entamoeba dispar* [14]. In Serbia, medical examination of asylum seekers, according to the Regulation from the Law on Asylum [15], under Article 3 include: history (infectious and non-communicable diseases, immunization status); physical examination; other diagnostic examinations (laboratory, Rtg) and under Article 4: laboratory testing of blood (eritrocite sedimentation rate, blood cells count); X-ray for pulmonary tuberculosis; laboratory testing of stool for: typhoid, paratyphoid and other *Salmonella*, shigellosis and intestinal protozoa [12].

In literature, there is still a dilemma whether to apply empirical therapy in groups of immigrants upon arrival to the land, or to perform the screening of parasitic infections [5]. In the United States, the accepted stand is that introducing presumptive treatment of parasitosis is necessary [5], while the EU countries point to the necessity of screening for parasites (whose life cycle is not tied to the host country), especially in immigrants who come from tropical and subtropical parts of the world, with the aim to prevent the spread of infections [16].

Although screening for parasitic infections in immigrants is legally regulated in Serbia, there is no published data on the prevalence of parasitosis in these risk groups. This study showed that the incidence of parasitosis in immigrants from countries of the Middle East and South Asia was significantly higher than the incidence of parasitosis in the general population of Belgrade territory in 2010 (9.3% vs. 1.9%, $p = 0.000$) [13]. We have no data if these persons originate from rural or urban areas. In comparison, the Mediterranean countries (Greece), reported the presence of intestinal parasites in 27.2% of immigrants from all continents and in 7.9% of those originating from the Asian countries [16]. The incidence of parasitosis in people who immigrated to Italy was recorded to be between 31%–61.9% 1,16, while in Sweden it was 36% [17]. US screening studies of 10.358 refugees showed that the incidence of intestinal parasitosis is the highest in immigrants originating from Asian countries (33.6%) [9]. The variability of data, observed in the results presented in literature, is explained by the fact that immigrants belonged to different geographical origins, had different age

groups, and living conditions (including the quality of drinking water, sanitary disposal facilities, availability of adequate footwear), eating habits, education levels and different exposure to parasites in countries which they passed through. In addition, one of the reasons for lower incidence of parasitism in our study group refers to a relatively small number of subjects, and the possibility that asylum seekers, passing through different countries on the way to Serbia, have been tested and treated for parasites.

In this study, no parasitic diseases were detected among people who migrated from Syria, which can be explained by the fact that the reason for migration in these people was fear of persecution because of different political opinions during the war in this country which is often associated with a higher educational level of refugees and their better socio-economic status. People from Bangladesh and Pakistan, who often migrate for socio-economic reasons, were more likely to be found positive for parasites in stool.

Local conditions and habits of the population have the most important role in the development and survival of the parasites [16]. In the case of certain types of helminthoses, eggs or larvae continue embryonic development in the ground or in the host before they become infective to humans [16]. Two-thirds of all asylum seekers found positive for parasitosis in this study had protozoa in stool, while one-third had helminthes. This is in accordance with the known fact that protozoa are easier to be transmitted by direct and indirect contact while helminthes are rarely transmitted by direct contact. *Giardia lamblia* was the most often found and it was present in 4.1% of immigrants originating from countries of the Middle East and South Asia. This is in accordance with data reported by Spanish authors, from the study that included 2,464 immigrants, where the incidence of *Giardia lamblia* was determined to be 5.4% [14]. In the US screening study of 10,358 immigrants from Asian countries, the most often found were *Trichuris trichiura* (7.1%), *Giardia lamblia* (5.7%) and *Ascaris lumbricoides* (2.1%) [9]. A recent Spanish study of 242 subjects has found a high incidence of *Ascaris lumbricoides* (35.5%) and *Giardia lamblia* (28.5%) among immigrants from sub-Saharan Africa [17]. Swedish study of 1,377 refugees and asylum seekers revealed that *Giardia lamblia* was found in 17% of cases and *Ankilostoma duodenale* in 19% [18]. The same study showed that intestinal parasites were more frequently found in asylum seekers from Southeast Asia (48%), Africa (43%) and South America (42%) compared to those from Eastern Europe (22%) and the Middle East (32%) [18].

Masucci et al. [1] showed that intestinal parasites were more often present in men, although some species (especially *A. lumbricoides* and *Taenia spp.*) were detected more frequently in women. In this study group of immigrants, there was ten times less women than men, and none of the women were positive for parasites. Also, parasites were not discovered in children (a total of six children of up to 4 years of age) which can be associated with the absence of parasitism in women in this study group. According to literature, the prevalence of parasitic infections is usually higher in children compared to adults, and is explained by the usual children's behavior (eating dirt or neglecting hand washing after using the toilet) which directly contributes to a higher degree of exposure to infection [16].

It is very important for the future work within the public health strategy to focus on improving the screening, monitoring and follow-up of parasitosis in asylum seekers. Also, the cost-effectiveness of screening compared to introduction of presumptive therapy should be evaluated.

Conclusion

Most intestinal parasitosis were found in asylum seekers from Bangladesh. All parasitosis were found in males and the most frequently detected parasite was *Giardia lamblia.*

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Received on November 18, 2016.
Revised on December 27, 2016.
Accepted on February 21, 2017.
Online First March, 2017.

SHORT COMMUNICATION

UDC: 616-001: 616.36-001.45
https://doi.org/10.2298/VSP161215042R

Gunshot liver injuries grade I–III and related liver enzyme values

Vrednosti hepatičnih enzima kod povreda jetre vatrenim oružijem, I–III stepena

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Abstract

Background/Aim. The liver is one of the most commonly injured solid organ in patients with abdominal gunshot wounds. The aim of this study was to investigate correlation between aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels as well as correlation between liver enzymes and Injury Severity Score (ISS) among patients sustained a gunshot liver injury. **Methods.** The study included 30 patients with a gunshot liver injury. Patients were divided into three groups, according to the American Association for the Surgery of Trauma injury grade. We included only patients with first (I), second (II) and third degree (III) injury. AST and ALT levels were also initially measured, and then consecutively each day, up to the fifth post-traumatic day, in order to determine which of them is better and more stable predictor of severity of gunshot liver injury. **Results.** ALT had significant positive correlation with a low-degree gunshot liver injury, on the day zero, post-traumatic day one and day two. Nevertheless, AST/ALT relation throughout post-traumatic five day period regarding an injury grade correlates best in II grade injury. At the end, strong positive correlation between ALT and ISS was observed ($p < 0.05$). **Conclusion.** Presented data clearly shows that ALT is better gunshot liver injury predictor than AST, with strong predictive value regarding injury severity, in first days after liver trauma. Therefore, it could be easily available, cheap and reliable prognostic tool for complexity of liver trauma. ALT prediction value is more significant for I and II injury, grade. Correlation between AST and ALT exists only for specific injury grade (II), but not in general.

Key words:
wounds, gunshot; liver; injury severity score; transaminases.

Apstrakt

Uvod/Cilj. Jetra je jedan od najčešće povređivanih organa kod pacijenata sa povredama zadobijenim projektilima male početne brzine. Cilj rada bio je da se utvrdi korelacija između vrednosti jetrenih enzima, aspartat aminotransferaze (AST) i alanin aminotransferaze (ALT), kao i korelacija između i skora na Internacionalnoj skali za merenje ozbiljnosti povreda (ISS) među pacijentima sa strelnim povredama jetre. **Metode.** Ispitivanjem je bilo obuhvaćeno 30 bolesnika sa strelnim povredama jetre. Oni su bili podeljeni u tri grupe u skladu sa sistemom vrednovanja Američke asocijacije za traumatsku hirurgiju. Studijom su obuhvaćeni ispitanici sa prvim (I), drugim (II) i trećim stepenom strelnih povreda jetre. Nivoi AST i ALT mereni su inicijalno, kao i svakog narednog dana, do petog dana posle traume, u cilju određivanja enzima koji bi bio bolji prediktor stepena ozbiljnosti strelnih povreda jetre. **Rezultati.** Pozitivna korelacija sa niskogradusnim strelnim povredama jetre utvrđena je za ALT nultog dana, kao i prvog i drugog posttraumatskog dana. Pored toga, AST/ALT odnos najbolje je korelisao sa II stepenom oštećenja jetre tokom svih pet dana posle traume. Nađena je pozitivna korelacija između vrednosti ALT i ISS ($p < 0.05$). **Zaključak.** Našom studijom je dokazano da je ALT bolji indikator strelnih povreda jetre u odnosu na AST, sa jakom prediktivnom vrednošću u odnosu na stepen povrede, prvih dana posle traume. Taj enzim bi mogao biti pouzdan, lako dostupan i jeftin indikator kompleksnosti strelnih povreda jetre. Pored toga, prediktivna vrednost ALT je značajnija za I i II stepen povrede. Korelacija između enzima AST i ALT postoji samo za II stepen strelnih povreda jetre, ali ne i uopšteno.

Ključne reči:
rana vatrenim oružjem; jetra; povrede, indeksi težine; aminotransferaze.

Introduction

Liver is one of the most commonly injured solid organ in patients with abdominal gunshot wounds [1] and its injury is reported in approximately 5% of all trauma [2]. Leading cause of death in severe liver injuries is uncontrollable bleeding, while multiple organ failure and residual sepsis are the primary causes of late death and morbidity [3].

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The Injury Severity Score (ISS) correlate well with survival and provides a numerical description of the overall severity of injury for patients with multiple trauma [4]. It could be also releated to the severity of liver injuries which is universally classified according to the American Association for the Surgery of Trauma (AAST). The majority of patients admitted for liver injuries have grade I, II or III [5]. Liver injury releases transaminases, mitochondrial and cytoplasmic enzymes that are found in hepatocytes, neurons, pancreatic and muscle cells and the two most common transaminases are aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [6]. AST and ALT elevation are known to correlate to liver injury and occur immediately after the trauma [7–9]. Nevertheless, it appears that high-grade (AAST grades III–VI) liver injury results in higher AST and ALT levels than low-grade liver injury (AAST grades I–II) [10].

The aim of this study was to investigate the correlation between AST and ALT levels as well as correlation between liver enzymes and ISS score among patients sustained a gunshot liver injury.

Methods

We evaluated 30 patients with gunshot liver injuries and accompanying injuries of other organs if sustaind in some cases, but any of them was not life-threatening. The patients were divided into three groups, according to AAST injury grade. We included only patients with first (I), second (II) and third degree (III) gunshot liver injury. There were 10 patients in each group. Every patient underwent abdominal computed tomography (CT) during initial evaluation with ISS scoring; AST and ALT levels were also measured, initially and then consecutively each day, up to the fifth post-traumatic day in order to determine which of them was better and more stable predictor of severity of gunshot liver injury. The ELISA tests on Cobas c 311/501 analyzer (COBAS, Roche Diagnostics GmbH, D-68305 Manheim, Germany) for measurement of AST and ALT levels in human serum was used. The threshold of blood AST and ALT upper margin normal reference range level was set at 50 IU/L [6]. The patients with severe, life-threatening injuries were excluded as well as those with severe bleeding, previous liver diseases or any other chronical disease and those who had initially increased levels of AST and ALT upon admission. Parameters observed were: age, gender, ISS, blood AST and ALT levels and CT diagnosed liver injury grade, according to to the ASST scale [6].

Statistical analysis

Statistical analysis was performed by the Statistical Package for the Social Sciences (SPSS) version 11 for Windows software package (SPSS Inc., Chicago, USA). Since data do not follow normal distribution we used the nonparametric Spearman's correlation. Methods of statistical description included the Student *t*-test in order to determine statistical significance. The difference of the obtained values was considered to be significant at $p < 0.05$.

Ethics

Each subject signed the acceptance of the study protocol, in which the Ethical Principles for Medical Research Involving Human Subjects (The Helsinki Declaration) were clearly stated. They all signed the cinformed onsent form.

Results

The patients were divided into three groups, according to the gunshot liver injury grade. Each grade comprises 10 patients, with levels of AST and ALT measured from day zero up to the fifth post-traumatic day. Average age of patients was 41.3 ± 15.2 (ranging from 19 to 72) years and 21 out of 30 patients were males. There were 11 patients who sustained concomitant right colon injury, 7 patients had right kidney injury, while 3 patients had accompanied duodenal penetration. In Table 1 acorelation between serum enyzmes values injury grades is given and it is evident that ALT had significant, positive correlation with low-degree gunshot liver injury, on the day zero, post-traumatic day one and day two.

Table 2 represents AST/ALT ratio throughout post-traumatic five day period in regard to injury grade. It correlated best with II grade injury. Table 3 provides information about correlation between AST and ALT levels among patients as well as correlation between serum liver enzymes values and ISS where strong positive correlation between ALT and ISS was observed ($p < 0.05$).

Table 1

Correlation between liver injury grade and liver enzyme values during five days after injury

Days after injury	AST		Days after injury	ALT	
	Injury grade I–II	Injury grade II–III		Injury grade I–II	Injury grade II–III
0	-0.341	0.093	0	0.692*	0.157
1	0.389*	0.509*	1	0.574*	-0.407
2	0.480*	0.438*	2	0.492*	-0.421
3	-0.157	0.531*	3	0.063	-0.392
4	-0.137	0.470*	4	0.261	-0.530
5	0.577	0.265	5	0.218	-0.530

*** Statistically significant correlation among groups.**
AST – aspartate aminotransferase; ALT – alanine aminotransferase.

Table 2

AST/ALT correlation throughout post-traumatic five day period regarding injury grade

AST/ALT ratio throughout five days after injury	Grade injury I	Grade injury II	Grade injury III
AST 0 / ALT 0	-0.081	-0.040	-0.001
AST 1 / ALT 1	0.362	-0.125	0.291
AST 2 / ALT 2	-0.304	0.550*	-0.051
AST 3 / ALT 3	-0.404	0.385*	0.038
AST 4 / ALT 4	-0.248	0.510*	0.063
AST 5 / ALT 5	-0.412	0.578*	0.409*

***Statisticaly significant correlation between groups.**
AST – aspartat aminotransferase; ALT – alanine aminotransferase.

Table 3

Spearman's rank correlation coefficient between AST and ALT and ISS during five days after injury for 30 patients, regardless of injury grade

Spearman's rank correlation (ρ)		ALT 0	ALT 1	ALT 2	ALT 3	ALT 4	ALT 5	ISS
AST 0	p	- 0.073	-0.218	0.090	0.181	0.133	-0.006	0.119
	t-test	0.703	0.246	0.637	0.339	0.483	0.973	0.532
AST 1	p	0.146	0.275	0.187	0.135	0.112	-0.059	0.252
	t-test	0.442	0.141	0.322	0.477	0.557	0.758	0.179
AST 2	p	0.194	0.377*	0.272	0.301	0.397*	0.253	0.021
	t-test	0.303	0.040	0.147	0.105	0.030	0.178	0.914
AST 3	p	0.193	0.389*	0.283	0.318	0.447*	0.329	-0.076
	t-test	0.306	0.034	0.130	0.087	0.013	0.076	0.691
AST 4	p	0.192	0.366*	0.120	0.188	0.315	0.191	0.121
	t-test	0.308	0.047	0.528	0.321	0.090	0.312	0.525
AST 5	p	0.117	0.393*	0.146	0.231	0.357	0.313	-0.025
	t-test	0.537	0.032	0.441	0.220	0.053	0.092	0.896
ISS	p	0.084	0.335	0.401*	0.383*	0.400*	0.397*	
	t-test	0.658	0.071	0.028	0.037	0.029	0.030	

*** Statistically significant correlation with corresponding group**.
AST – aspartat aminotransferase; ALT – alanine aminotransferase; ISS – Injury Severity Score.

Discussion

This study evaluated patients with I, II and III degree gunshot liver injury and according to recent data, a majority of patients admitted for liver trauma worldwide refer to these grades [5]. Correlation between AST and ALT levels in patients with liver trauma and grade of gunshot liver injury could be releated to ISS which predicts survival rate. Bruhn et al. [6] found equally reliable significance of AST and ALT in detecting gunshot liver injury. However, in general, our study showed that there was no significant positive correlation between AST and ALT, and that significant correlation existed only between ALT and ISS which could signify strong prediction value of ALT in trauma severity. According to a study conducted by Narci et al. [11], ISS was more valuable than other trauma scoring systems for prognostic evaluation of trauma patients; thus, based on our study, ALT could be easily available, cheap and reliable prognostic tool for complexity of gunshot liver trauma. Nevertheless, when we observed separate enzyme values regarding a gunshot liver injury grade, we noticed a significant positive correlation of AST/ALT ratio with II grade gunshot liver injury ($p < 0.05$). A study made by Zagory et al. [12] showed the same positive trend of correlation between liver enzymes and gunshot liver injury grade, although it referred to pediatric patients. It is also evident that in each injury grade there exists negative correlation between them on the day zero of injury ($\rho = -0.08$, $\rho = -0.04$, $\rho = -0.001$). Some authors [13, 14] tried to determine whether AST and ALT could predict severity of gunshot liver injury. Only Koyama et al. [9] established optimal cut-off values for AST (> 100 U/L) and ALT (> 80 U/L) as a useful screening tool for CT scan in otherwise stabile patients. However, blood samples were taken only immediately upon arrival, unlike in our study where we checked enzyme levels every day, up to the fifth post-traumatic day, and revealed that ALT developed strong positive correlation between I and II injury grade on the day zero, as well as the first and second postinjury day ($\rho = 0.69$; $\rho = 0.57$; $\rho = 0.49$, respectively). This means that ALT could be better predictor for I and II grade gunshot liver injury than AST. On the other hand, AST showed strong positive correlation on the first, second and third post-injury day among patients with II–III grade injury, which means that AST could be better predictor of severity of gunshot injury grade after the first day of the injury. We did not determine cut-off values for liver enzymes. Instead, we tried to predict injury grade in regard with AST or ALT blood level. Besides that, study presented here included patients with gunshot injury, instead of blunt liver trauma observed in forementioned studies. We believe that this makes presented research different and important. Bruhn et al. [6] claimed that initial evaluation of AST and ALT could be a useful diagnostic tool to predict the need for CT.

Unlike that study, we proved that ALT could be important in estimation of severity of gunshot liver injury, in first days upon trauma which could predict patients outcome, especially in I and II grade injuries. On the other hand, AST correlates with II and III grade injuries, but only after 24 h of trauma initiation, which could be too late for a decision on further diagnosis (CT) or treatment options.

Data gained from the study performed are consistent with the existing literature in the context that the presence of significant gunshot liver injury can indeed be expected to result in raised liver enzyme levels [6]. Unlike the other recently published studies, we tried to estimate the prediction values of liver enzymes concerning the severity of gunshot liver injury and to determine whether any of these enzymes significantly correlated with specific injury grade in first hours of injury. Hovewer, limitation of our study is the absence of IV, V and VI liver injury grade, inability to confirm positive predictive trend of ALT even in those with severe injury grades as well as lack of liver enzyme correlation with CT findings which could be of significant importance for further treatment modalities. The reason is high mortality rate of patients with those grades in prehospital period. Nevertheless, there are no contemporary studies concerning gunshot liver injury and relation between liver enzymes and ISS nor predictive value of AST and ALT regarding a gunshot liver injury grade. Thus, we think that our study deserves attention and could be of significant importance for future research of this subject.

Conclusion

Presented data clearly shows that ALT is better predictor of gunshot liver injury than AST, with strong predictive value regarding injury severity (ISS) in first days after liver trauma. Therefore, it could be easily available, cheap and reliable prognostic tool for complexity of liver trauma. ALT prediction value is more significant for I and II injury grade. Correlation between AST and ALT exists only for specific injury grade (II), but not in general.

Acknowlegdement

During the research, Dr Radojevic was a fellow of Fogarty International Center of the National Institutes of Health's "Research Ethics Education in the Balkans and Black Sea Countries" (Award Number R25TW008171), provided by Icahn School of Medicine at Mount Sinai New York USA and School of Medicine University of Belgrade, Serbia. As so, ethical principles conducted during the research were influenced by the education acquired. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Received on December 15, 2016.
Revised on March 02, 2017.
Accepted on March 03, 2017.
Online First March, 2017.

GENERAL REVIEW

UDC: 616.379-008.64-02
https://doi.org/10.2298/VSP151216028N

Diabetes mellitus and obesity as a result of a disrupted homeostatic microbiome. New data on etiopathogenesis of diabetes mellitus

Dijabetes i gojaznost kao rezultat narušavanja homeostatskog mikrobioma. Novi podaci o etiopatogenezi dijabetesa

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Key words:
microbiota; diabetes mellitus; obesity; pancreas; digestive system.

Ključne reči:
mikrobiota; dijabetes melitus; gojaznost; pankreas; gastrointestinalni sistem.

Introduction

Diabetes mellitus is a disease in expansion. According to International Diabetes Federation in 2014 an estimated 387 million people in the world have diabetes mellitus (DM), with expected increase of 200 million in 20 years [1]. Diabetes mellitus is characterized by metabolic disorder and hyperglycemia and results from insulin deficiency or decreased effects of insulin on the target tissues. The etiology of DM is reasonably well known as well as the mechanism of its pathogenesis.

Diabetes mellitus type 1 is characterized by absolute lack of insulin and physiological destruction of β cells of pancreas. Autoimmune damage of pancreatic islets is a long-term process and clinical manifestation of the disease occurs when more than 80% of the β cells are irreversibly damaged.

Diabetes mellitus type 2 is a metabolic disease caused by defective insulin secretion and insulin resistance [2]. It is believed that many factors influence the onset of the disease: genetic factors, reduced physical activity, overweight, malnutrition in fetal and prenatal period, certain drugs (steroids, diuretics, anti-hypersensitive). Adipose tissue produces leptin, tumor necrosis factor (TNF) alpha, resistin, adiponectin and interleukin (IL)-6 thereby affecting insulin resistance and possible dysfunction of pancreatic β cells [3–5]. New possible factors in etiopathogenesis of DM include microbiological agents, such as disrupted saprophytic flora (the homeostatic microbiome – HM) and infections of the pancreas. Some studies [6, 7] showed that certain types of bacteria and fungi could cause increased or decreased insulin secretion in the body thereby causing insulin resistance and the development of diabetes mellitus and obesity. This leads to the question how microorganisms affect insulin secretion and the occurrence of diabetes mellitus. Microorganisms in the pancreas could lead to destruction of β cells due to activation of immune system as a response to infections inducing in this way DM type 1. In interaction with pancreatic cells, microorganisms could lead to increased (*Candida*) or decreased (bacteria) insulin secretion thereby increasing chances for the onset of DM type 2. Saprophytes in the intestinal tract and other organs lined with mucosa secretory products are able to pass via the bloodstream to the pancreas thus indirectly affecting insulin secretion. This report suggests that the total body load of microbes (the microbiome) is an important physiological factor, and that maintenance of this HM is important for human health. A speculation that can be derived from this is that disruption in the HM may contribute to DM and obesity. There are several lines of evidence that support this hypothesis.

Infections of the pancreas

One of the important factors that might affect insulin secretion is an infection of pancreas. It is well known that certain pathological conditions such as acute pancreatitis, necrotic pancreatitis and cysts are caused by following microorganisms: Gram (+) bacteria, 74%, Gram (–) bacteria, 21% (*Enterobacter*, up to 58%), *Candida albicans*, 5–24% [6, 8]. These microorganisms are mostly saprophytic and they are part of the normal gastrointestinal flora [6–9]. However, the extent of pancreas infection and effects and types of the infection are unclear. For example, severe cases of acute pan-

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creatitis might lead to numerous complications such as DM, and a damaged pancreas could be infected by bacteria from the small intestine. Symptoms of inflammation include fever, increased number of leukocytes, and, in severe cases, organ failure may also adversely effect the pancreas.

Possible mechanisms of pancreatic infection

Microorganisms from the intestine may enter the pancreas in three different ways (Figure 1): directly from the duodenum through the pancreatic duct (*ductus pancreaticus*) [10, 11], penetration into body cavity due to injuries and via the blood. Certain pathological conditions that can facilitate direct penetration of microorganisms from the intestine to the pancreas include reduced secretion of pancreatic juice (in patients with acute and chronic pancreatitis), weakening of the sphincter of Oddi (sphincter muscles get weaker with age, a major risk factor for the onset of DM type 2), anatomical changes in intestinal tract, tumors, and excessive and frequent food intake that can cause distension of the stomach and intestine, disrupting the sphincter of Oddi so that it remains open or to expand the lumen of the sphincter.

In support of this hypothesis, there is the observation that the microorganisms found in the pancreas originate mainly from the small intestine and that they have colonized the pancreas though the sphincter of Oddi [6, 7]. Among tested samples, 31% were contaminated by bacteria (*Pseudomonas aeruginosa*, *Enterobacter* spp. and *Staphylococcus* spp.), 24% were contaminated with *Candida albicans* and 45% were sterile [6, 7].

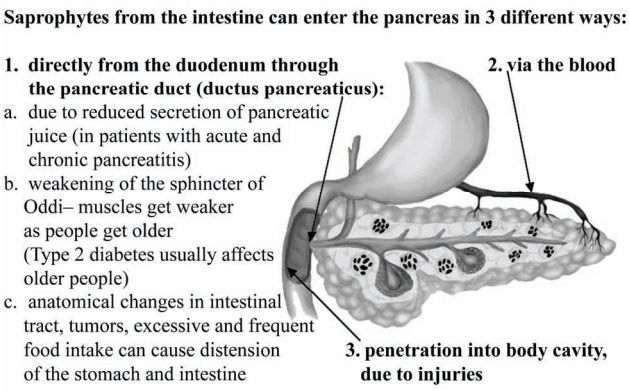

Fig. 1 – Possible ways of pancreatic infection.

Effects of infections *in vivo* on insulin secretion

Infections in the body can influence insulin secretion and glucose metabolism. Infection causes marked changes in whole body glucose metabolism as a result of acceleration in endogenous glucose production due to increased gluconeogenesis [12]. It was shown that acute infection in humans causes insulin resistance and glucose intolerance [13]. Endotoxin or lipopolysaccharide (LPS) is a potent stimulator of inducible nitric oxide synthase (iNOS). LPS injection lead to hyperglycemia, insulin resistance and increased iNOS protein expression and activity [14]. One study with rats infected with *E. coli* demonstrated that infected animals were hyperthermic and showed the increased rates of glucose metabolism as well as mild hyperlacticacidemia. Plasma catecholamine concentrations were increased by 50%–70% [15]. Results of experiment with male rats treated with bacterial endotoxin (*Salmonella enteritidis*) suggest that variations in an individual's early life bacterial environment may contribute to differences in glucose homeostasis, insulin action and disease susceptibility later in life [16]. The likelihood of elevated C-reactive protein (CRP) concentrations increase with increasing of HbA1c levels. Exposure to multiple pathogens could cause a chronic low-grade inflammation, resulting in insulin resistance [17].

The homeostatic microbiome

The HM consists of all microorganisms that normally inhabit the human body (gastrointestinal system, urogenital system, skin and all cavities lined with mucosa) and it is necessary to maintain normal homeostasis and health. It follows that the HM (i.e., saprophytic microorganisms) is an important contributor to physiology. Since there is a mutual interaction on genetic and biochemical levels between microorganisms that inhabit different places in human body, so there is their communication with other physiological systems that participate in the homeostasis of the human body – the law of connected vessels (Figure 2).

Fig. 2 – Connection of the homeostatic microbiome with other physiological systems involved in maintenance of homeostasis in organism (endocrine, nervous, muscular, integumentary, skeletal, female and male reproductive, urinary, digestive, respiratory, cardiovascular and lymphatic system).

After birth, many microorganisms rapidly colonize the intestinal tract and remain there throughout life. After the death they contribute to the decay of the organism. Homeostatic microorganisms in the body may have two effects: primary, being involved in the digestion of food (food as nutrient medium for their growth and development), and secondary, the production of metabolites that are absorbed by the blood thereby affecting the me-

tabolism and physiological state of the organism. Factors that cause disorder of saprophytic flora are improper diet, too cold or too hot food and beverages (growth of *Candida albicans* may be very invasive at 25°C), immunological insufficiency and long-term treatment with antibiotics, corticosteroids and immunosuppressive drugs [7]. Disruption of the HM may lead to intensive growth of some microorganisms. When these organisms enter other organs they can cause severe diseases and even death of the host.

The HM microorganisms can flourish and increase their numbers primarily in the gastrointestinal tract (GIT), and in the urogenital system, and could have indirect effects on insulin secretion. Until now, most attention is focused on one part of the HM – the gut microbial community. A healthy adult human harbors some 100 trillion bacteria in gut alone [18]. The human body contains a total of 10 times more microbes than the number of somatic cells and at least 100 times more genes in relation to the complete human genome [19].

Microbiome should be considered as an organ since it weighs 1–2 kg. Metagenomic analyzes of human mucosal and fecal samples established that phyla *Bacteroidetes* and *Firmicutes* constitute 90% of the microbiota and the rest (10%) are *Actinobacteria, Proteobacteria* and *Fusobacteria* [19, 20]. *Pseudomonas aeruginosa* and *Enterobacter* belong to the group of *Proteobacteria*, while *Staphylococcus* belong to the largest group, *Firmicutes* (Figure 3).

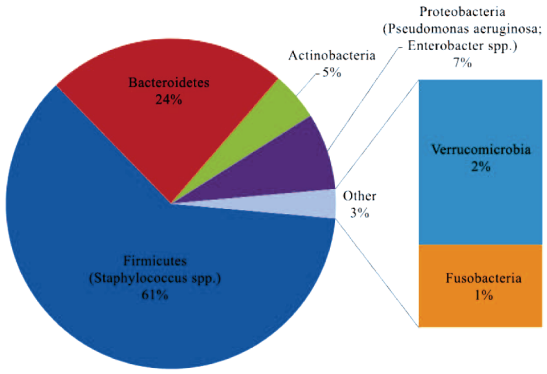

Fig. 3 – Percentage of specific phyla obtained by metagenomic analyses of human mucosal and fecal samples. *Pseudomonas aeruginosa* and *Enterobacter* belong to the group *Proteobacteria* while *Staphylococcus* belong to the largest group, *Firmicutes,* represented by 90%.

Microbiome researches are relating primarily to bacteria, not fungi. However, opportunistic infections caused by *Candida* are obvious example of disrupt homeostatic microbiome. The yeast *Candida albicans* is a commensal and a constituent of the normal microflora in 80% of human population where predominately colonize mucosal surface of GIT, but also urogenital tract and to a lesser extent the skin [21, 22]. It can cause infections that range from superficial to systemic and potentially life-threatening diseases, such as in patients with weakened immune system, i.e., patients with human immunodeficiency virus (HIV) or cancer [23] and in patients after long-term antibiotic therapy [24], as the growth of intestinal saprophytes (bacteria) is normally kept under control.

The growth of *Candida* is normally limited by immune system and other microorganism occupying the same location of the body. Immune response to *Candida* infections in human is not well known. I suppose it is not likely that the yeast growth is directly correlated with activation of gut associated lymphoid tissue (GALT). It is more likely that saprophytic bacteria normally present in intestinal tract have substantial influence on *Candida* growth control and antibiotic therapies could disrupt this relation leading to persistent *Candida* infections. Each microorganism in the human body occupies a particular ecological niche. They overlap and act as a system of connected vessels. Reduction in the number of one type of microorganism leads to an increase in the number and spread of other types. Microorganisms in a certain amount are an essential factor of every human HM. Due to increased number, some microorganisms may become pathogenic and threaten the health of the host. If the presence of all microorganisms in the HM is presented as 100%, it is not determined what percentage occupies each microorganism. Determining their ideal relationship, it would be easier to predict and prevent certain diseases in humans. The HM plays an important role in health preserving of the host. Disorder in the HM leads to insulin resistance, obesity and diabetes mellitus, but it is also assumed that disorder in the HM can provoke other diseases.

The HM may affect heart disease. Amount of formic acid in urine is inversely proportional to blood pressure which is a risk factor for heart disease. Important source of formic acid is microbiome in intestine [25]. It is known that microorganisms by mimicry can provoke an autoimmune attack and the onset of diabetes mellitus or multiple sclerosis [26]. There is a possible connection between microbiome and autism [27]. It is known that typhus, caused by infection of GIT, is characterized by headaches, constipation, and at a later stage, by diarrhea and disturbance of consciousness (neurological symptoms) [28]. Some data suggest the impact of microbiota on the incidence of cancer [29]. Formation of the HM is shown in Figure 4 (Ring of Life) and takes place in several stages: pregnancy and childbirth, breastfeeding, contact with family and wider environment, nutrition and sexual contacts.

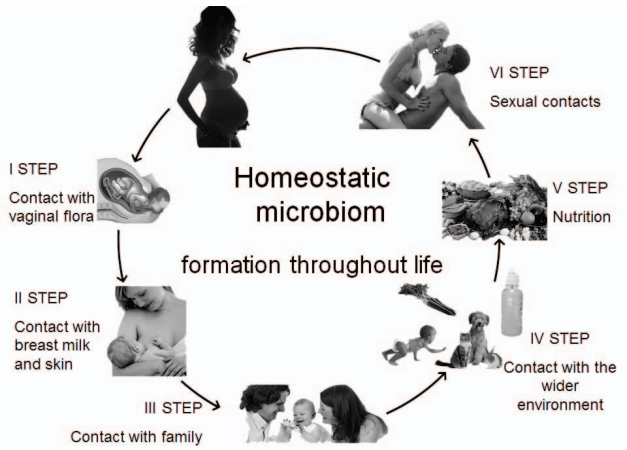

Fig. 4 – Ring of life. Steps important in formation of homeostatic microbiome throughout life.

Pregnancy

Microbial abundance and diversity might differ in pregnancy. Metagenomic analysis of DNA that was isolated from the vagina showed that the diversity of microbiota was reduced in pregnancy with dominance of *Lactobacillus* species and the orders *Lactobacillales* (and *Lactobacillaceae* family), *Clostridiales*, *Bacteroidales*, and *Actinomycetales* [30]. Thus, during pregnancy, the first group of the HM organisms is present which will come into contact with the baby during birth.

Childbirth

At a delivery, the child comes into contact with the HM of its mother (microflora of the urogenital and GIT). Several lines of evidence suggest that this can influence the likelihood in later life of overweight and obesity. For example, the composition and development of infant gut microbiota are influenced by weight and weight gain of mothers during pregnancy. Fecal microbial composition showed significantly higher concentrations of *Bacteroides* and *Staphyloccocus* and lower concentrations of *Bifidobacterium* group in infants of overweight mothers [31]. Kalliomaki et al. [32] reported that numbers of the genus *Bifidobacterium* was higher in children with normal weight than in children developing overweight. In addition, number of fecal *S. aureus* organisms was lower in children with normal weight than in obese children. They suggest that *S. aureus* may act as a trigger of low-grade inflammation, contributing to the development of obesity [33]. *In vitro* studies showed that *Staphyloccocus* spp. drastically reduce insulin secretion of human pancreatic islets leading to insulin resistance and obesity [6, 7].

Breastfeeding

Infants receive their first microbial "package" at the time of the birth. These bacteria reflect the microbiota of maternal vagina and GIT. Further intestinal colonization takes place during breastfeeding by bacteria in breast milk and breast skin. This finding begs the question as to what role this community plays in colonization of the infant GIT and maintaining mammary health [34]. Bacteria are better for extracting nutrition from mothers' milk since induce glycoside hydrolases which converts carbohydrate glycans, which are abundant in milk, into usable sugars. The HM has the capacity to break down complex, indigestible carbohydrates and creates small fatty-acid molecules as waste products, particularly formic acid, acetic acid and butyric acid that can pass through the gut wall into the bloodstream [19, 20]. Breastfeeding protects infants from diarrheal and respiratory diseases and it is associated with a reduced risk of developing obesity [35, 36].

Culture-dependent methods confirmed the presence of *Staphylococcus* and *Streptococcus* (most abundant species) in human milk [37]. Techniques based on amplification of bacterial 16S rRNA in human milk detected several genera of bacteria, including *Lactobacillus* and *Bifidobacterium* (relative abundance 2%–3%). *Staphylococcus* was represented by 22%–59%. Previous studies showed that the microbiota present in the lower GIT [38], vagina [39], oral cavity [40], and more importantly, the differential composition of these communities in healthy versus diseased states, are related to the health of the human host. Human milk contains oligosaccharides with probiotic properties that promotes the growth of bifidobacteria after birth [33, 41, 42].

Contact with family

Contact with family is essential for the formation of a functioning HM. Analyzes of the genome of *Methanobrevibacter smitthii* and *Bacteroides thetaiotaomicron* strains of close relatives who live together showed it is identical in 96% [42]. Thus, early gut colonizers, such as those acquired from parents and siblings, may remain in the intestine throughout the life of the individual.

Recent studies [43] have shown during five-year monitoring of 37 patients, by using a method for bacterial 16S rRNA amplicon sequencing, that individual microbiota was remarkably stable, with 60% strains (approximately 100 species) remaining over this period. This suggests that most strains are intestinal residents for decades. Members of *Bacteroides* and *Actinobacteria* are significantly more stable components than the population average.

Contact with the wider environment (ecological niche)

A child with its microbiome represents an entity which occupies one ecological niche. This niche will inevitably overlap with other spatial niches, animal, plant and ecological niches of other children, which could also lead to the formation of specific microbiome.

Sexual contacts

There are indications that human microbiota have a protective role in the prevention of sexually transmitted diseases (bacterial vaginosis) [44]. In my opinion, sexual contact is also important in formation and maintenance of the HM. Sexual contact inevitably leads to the exchange between the microbiome of partners. This contact is an essential and useful for individual to maintain social contact with other person, but it can also be harmful to the HM leading to its disruption if microbiome of two individuals is not compatible. Incompatibility of two microbiome may lead to sterile marriages. Promiscuous persons are subjected to frequent distortions of their HM. Probably microbiome of reproductive system affects sperm motility and thus indirectly affects the sex of the child. This subject is not sufficiently explored.

Nutrition

Nutrition is one of the key factors in the early development and long-term maintenance of homeostatic microbiome. The question is how diet affects obesity and whether it is the main cause of obesity and diabetes mellitus. Nutritionists recommend for healthy diet the following: 20% of

protein intake, to 50%–60% of carbohydrate intake and lipid intake less than 30% [45]. It has long been known that carbohydrates are necessary for the health preservation. One of the most ancient data is written in the Bible [46]. During the seven years of hunger, Jacob sent his sons in Miser for wheat: "I hear there is wheat in Miser. Go there and buy it so we stay alive and don`t die of hunger". Back then, they knew they would not survive without wheat, and they had cattle, sheep, goats, and camels. In contrast to this recommendation [45], a nation from the north, the Inuit, does not have carbohydrates in their thousand-year-long traditional diet. They mostly eat fishes, marine mammals and caribous. Inuit are not prone to diabetes mellitus and cardiovascular diseases. What sets them apart from other nations? The answer is the composition of the HM.

A research on twins shown that genetic factors do not have a decisive influence on obesity [47]. He found out that twins of which one is nourished and the other is malnourished had different microbiome. Recent studies (rodent model and in human) have shown that changes in gut microbiota may play an important role in the development of obesity [48]. Genetically obese mice had 50% reduction in *Bacteroidetes* and a proportional increase in *Firmicutes* compared to lean mice. Comparing distal gut microbiota of obese and lean human subject, Ley et al. [49] demonstrated that obese people had lower *Bacteroidetes* and more *Firmicutes* than lean subjects. After a fat restricted or a carbohydrate restricted low calorie diet, the ratio of *Bacteroidetes* to *Firmicutes* approaches a lean type.

In a series of experiments, Bäckhed et al. [45] showed that conventionally reared mice had 40% higher body fat content than germ-free mice, even they consumed less food. After conventionalization (transplantation of distal gut microbiota from normal mice to germ-free mice) body fat content increase 60% after 2 weeks without increase in food consumption. Cani et al. [50, 51] proposed that metabolic endotoxemia may provoke metabolic diseases such as diabetes mellitus and obesity in response to a high-fat diet. They hypothesized that bacterial lipopolysaccharide (LPS) from Gram-negative intestinal bacteria can be a triggering factor.

There are attempts of surgical interventions (Roux-en-Y gastric bypass) to reduce obesity and insulin resistance [52]. Approximately 80% of the patients with diabetes type 2 experience complete remission, defined as normoglycemia which can be explained by a reduction in body weight. It has also an impact on the food flow. Taken food bypasses the duodenum and directly goes to jejunum. Since there are no nutrients in the duodenum, microflora can not be held. Therefore, the possibility of penetration of the conditional pathogens through sphincter of Oddi into the pancreas is significantly reduced. Their impact and penetration into the pancreas is previously discussed [6, 8]. Metagenomic analysis of samples from the jejunum reveals that most represented bacteria is of genus *Streptococcus*, whereas dominant bacteria in the distal ileum, colon and rectum are *Bacteroidetes* and *Firmicutes*. This is anatomically very dangerous operation; it bypasses most of the stomach and the upper part of the small intestine and reduces the metabolic and exogenous effects of the pancreas and liver.

Obese and lean people have a different composition of the microflora, so it can play a role in the development of obesity. High-fat and low-fiber diet changes microbiome, leading to metabolic endotoxemia, increased depositing of lipids and decreased sensitivity to insulin resistance [50, 51].

Reduced number of *Bifidobacterium* and increased number of *Firmicutes* spp (274 genera, including *Staphylococcus*, *Enterococcus* and *Streptococcus*) lead to increased permeability of the intestinal epithelium and elevated levels of fatty acid and endotoxin in the blood and at the same time reduce the concentration of ghrelin, angiopoietins (angiopoietin-like protein 4, that inhibits the absorption of fatty acids from circulating triglycerides in adipose and muscle tissue, a potent inhibitor of lipoprotein lipase), and in turn, lead to an increase lipogenesis in the liver and adipose tissue, number of B-cells and insulin secretion in pancreas decrease and insulin resistance occurs in muscle (reduced insulin sensitivity) [53].

To restore normal relationship within the microbiome that inhabits GIT it is recommended to take prebiotics in the diet. Probiotics may change the composition of the microflora only briefly because without intake of probiotics comes to normalization and the establishment of the previous microflora in GIT.

A prebiotic is a non-digestible food ingredient, particularly oligosaccharides, that selectively stimulates the growth of beneficial commensal colonic microbiota (e.g., *Bifidobacterium* and *Lactobacillus* species) and thus improves host health [54]. Among the natural non-digestible oligosaccharides that fulfill the criteria of a colonic food are fructo-oligosaccharides that are fermented by a number of colonic bacteria to modulate the growth of beneficial bacteria [55]. The number of *Bifidobacteria* increases in the presence of inulin-type fructans. This increase occurs within a few days, but rapidly disappears upon withdrawal of prebiotics, after one week [56]. The extent of increase in number of *Bifidobacteria* depends on their initial number.

Dietary fructans, present in various fruits and vegetables or as food additives, are used as an energy substrate by bacteria, including *Bifidobacterium* spp, that express β-fructofuranosidase, which promotes their growth in the gut. The number of *Bifidobacterium* spp in mice with diet-induced or genetically determined obesity increase with intake of inulin-type fructans [57]. The number of *Bifidobacteria* was inversely correlated with the development of fat mass, glucose intolerance and lipopolysaccharide (LPS) level [58].

Summary-impact of contemporary nutrition on the homeostatic microbione

Food with long-term preservation is present on today's market. This means that such food produced on farms does not contain the necessary and essential microbial agents. Herbal food is produced on large surfaces. In such large plantations it is necessary to treat the surface with insecticides, herbicides and particularly fungicides that directly destroy the natural microorganisms normally inhabiting the surface of fruits and vegetables.

It is the same case with animal food. Animals are mostly grown on large farms and treated with antibiotics.

The food that they eat is also obtained by modern farm growing. Animals (cows, pigs and poultry) generally lead sedentary lifestyle, which means that they have no contact with the microbiome of the soil and the environment. Such food is usually represented in highly developed countries where the highest percentage of diabetes mellitus and obese people is presented. Organically produced food was grown in the villages and individual farmers (e.g., in Serbia), the food was produced in small quantities and was not treated with chemical preparations and originally contained a certain percentage of indigenous bacteria and fungi, and that is the advantage of such a diet. One of the factors that accompanies the onset of diabetes mellitus and obesity is the migration of the population. A person grew up in one ecological system and had contact with one microbiome through diet and direct contact, and once moving, for example, from Europe to America comes to an entirely different surroundings. This is direct evidence that the homeostatic saprophyte flora acquired by a healthy diet for years must be maintained throughout life and without a big attack by intake of unknown and new types of microorganisms. To understand better the impact of local microbes on food, it is enough to mention the original production of cheeses that are very different from place to place and have different tastes and odors, originating mostly from local microbiological agents. All listed above indicate that food should be considered not only as a source of essential nutrients but also as a necessary source of microbiological agents. Many harmful microbiological agents if present normal amounts in the HM represent the real source of essential metabolites and enzymes that contribute to maintaining the host health, but if their numbers begin to grow without control, they can cause severe illness and death of the host. Example of the symbiosis of the organism and the microbiome is peristalsis of GIT. It is well known how dangerous it is to interrupt normal movement of food and bowel motion as well as the consequences of persistent diarrhea for the body. *E. coli* causes diarrhea, but the question is whether its presence in allowed quantity is necessary for the regular emptying of the bowel.

The question is which is the favorable ratio of microbes in the body, particularly in the intestine. Their percentage in healthy people and in members of the same population should be determined and their relationship and tolerance to change of amount should be expressed in mathematical formulas. By establishing a mathematical model for the HM, it is easy to determine disorders and predisposition for certain diseases, in this case diabetes mellitus. Why food rich in sugar is often accompanied by overgrowth of the fungus *Candida*. According to our results *Candida* increases insulin secretion of pancreatic islets, thus helping the elimination of high glucose doses in the blood and prevents glucotoxicity. It is obvious example of the necessity to take food and associated microbiome together – *Candida* is the most present in the sugar-rich food. Intake of probiotics is desirable, but in the disrupted HM they cannot compensate all microbial agents that are necessary for the preservation of human health. It is known that certain members of the HM regulate and affect the insulin secretion.

Our research results demonstrated that microorganisms can influence on pancreatic islets insulin secretion. Namely, they perform their impact directly (when present in pancreas) [59] and indirectly, by secreting their metabolites which have influence on pancreas islets trough the blood vessels - as a consequence of the increase in their number in human body, disorder of the HM emerged [60] (Figure 5).

Bacterial agents (*Enterobacter* spp, *Pseudomonas aeruginosa*, *Staphyloccocus* spp.) reduce insulin secretion leading to postprandial hyperglycemia.

Fungal agents (e.t., *Candida albicans*) increase insulin secretion causing postprandial hypoglycemia and insulin resistance. It is known that increased insulin secretion is frequent in obese persons.

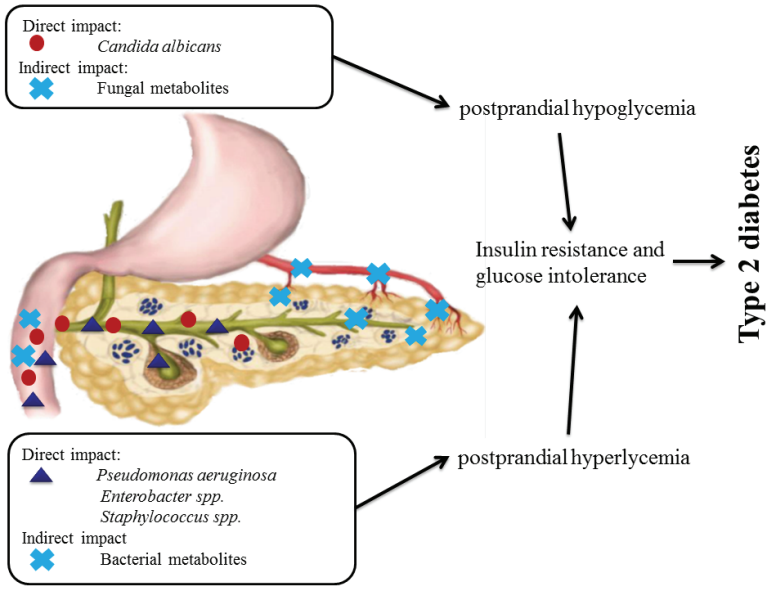

Fig. 5 – Direct and indirect influence of Candida and bacteria on the development of diabetes mellitus type 2.

Both cases lead to glucose intolerance and insulin resistance and in some cases the development of the DM type 2 and obesity.

In a healthy body, microorganisms are part of the homeostatic microbiome and play a key role in maintaining health, digestion and metabolism. Formation of the HM (Ring of Life) takes place in several stages: pregnancy and childbirth, breastfeeding, contact with family and wider environment, nutrition and sexual contacts. Many internal and environmental factors can lead to disorders of homeostatic microbiome, which leads to certain diseases, including disorder of glucose homeostasis.

Conclusion

We can conclude that infection of pancreas and change (disrupt) of the HM play an important role in etiopathogenesis of diabetes mellitus and obesity.

The HM is a great enigma and a challenge for further scientific research. The goal is to initiate extensive researches – interactive collaboration of scientific community in various fields, researches linking the man as an individual in a sustainable ecosystem, but they should be strictly controlled because the knowledge and the results can be manipulative, used not only for treatment but also for causing the disease. Complete understanding of the human microbiome could easily compromise moral, ethical and religious principles of understanding life and it is a serious subject for another debate and discussion.

Acknowledgment

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant No. 41002).

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Received on December 16, 2015.
Revised on January 17, 2017.
Accepted on February 14, 2017.
Online First March, 2017.

CASE REPORTS

UDC: 616-006-07:618.19-079.4
https://doi.org/10.2298/VSP161026010H

Contribution of 18-fluorodeoxyglucose positron emission tomography (FDG PET) imaging in the detection of underlying carcinoma in a woman with nonspecific mastitis

Doprinos snimanja pozitronskom emisionom tomografijom pomoću 18-fluorodeoksiglukoze u otkrivanju skrivenog karcinoma kod žene sa nespecifičnim mastitisom

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Abstract

Introduction. Differentiation between a malignancy and inflammatory process is still a diagnostic challenge. Mammography (MG) and ultrasonography (US) have low sensitivity and specificity in dense breasts in order to detect malignancy. On the other hand, malignant mass lesions can also be masked on magnetic resonance imaging (MRI) by diffuse inflammatory process. 18-fluorodeoxyglucose positron emission tomography (FDG PET) imaging can be a promising alternative imaging method in the evaluation of suspicious breast masses, especially in patients with accompanying inflammatory breast diseases. **Case report.** We report an atypical case of a patient suspected for malignancy in right breast on physical examination and radiologic findings in favor of mastitis. Neither MG nor US revealed any mass lesion consistent with malignancy. Moreover, MRI findings were primarily considered as infectious or granulomatous mastitis. However, FDG PET determined the accurate borders of tumor and dissemination of breast cancer with superiority to other conventional radiological methods. **Conclusion.** This case report emphasizes the contribution of FDG PET imaging to other conventional radiological methods with regard to primary tumor diagnosis, determination of the biopsy site, and also staging the disease especially in patients with accompanying inflammatory breast disease.

Key words:
breast diseases; breast neoplasms; mastitis; diagnosis, differential; positron-emission tomography.

Apstrakt

Uvod. Razlikovanje malignih i inflamatornih procesa još uvek je dijagnostički izazov. Mamografija (MG) i ultrasonografija (US) imaju nisku senzitivnost i specifičnost u gustinom tkiva dojke da bi se mogao prepoznati malignitet. S druge strane, na snimanju magnetnom rezonancom (MRI) lezije maligne mase mogu takođe biti maskirane difuznim inflamatornim procesom. Snimanje pozitronskom emisionom tomografijom pomoću 18-fluorodeoksiglukoze (PDG PET) može biti alternativna metoda koja obećava u proceni sumnjivih masi dojke, posebno kod bolesnica sa pratećim inflamatornim oboljenjima dojke. **Prikaz bolesnika.** Prikazujemo neobičan slučaj bolesnice sa sumnjivim malignitetom desne dojke na fizikalnom pregledu i radiološkim nalazima koji su išli u prilog mastitisa. Pomoću MS i US nije otkrivena lezija koja bi odgovarala mastitisu. Osim toga, MRI nalazi primarno su bili razmatrani kao infektivni ili granulomatozni mastitis. Međutim, pomoću FDG PET određene su tačne granice tumora i diseminacije karcinoma dojke, sa superiornošću u odnosu na druge, konvencionalne radiološke metode. **Zaključak.** Ovaj prikaz bolesnika naglašava doprinos FDG PET snimanja ostalim konvencionalnim radiološkim metodama u dijagnostikovanju primarnog tumora, određivanju mesta biopsije i, takođe, gradiranju bolesti, posebno kod bolesnica koje imaju prateće inflamatorno oboljenje dojke.

Ključne reči:
dojka, bolesti; dojka, neoplazme; mastitis; dijagnoza, diferencijalna; tomografija, kompjuterizovana, emisiona.

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Introduction

Differentiation between a malignancy and inflammatory process is still a diagnostic challenge. There is no radiologic criterion to allow definitive diagnosis both for inflammatory carcinoma and benign inflammatory disorders including infectious and noninfectious diseases (e.g. granulomatous mastitis, mastitis/abscess formation and fat necrosis) [1, 2]. Breast edema, enlargement, skin thickening, nipple discharge/retraction, abscess and axillary lymphadenopathy are common and nonspecific findings of all above mentioned benign and malignant inflammatory entities [1]. Because benign and malignant inflammatory conditions may have similar signal characteristics and contrast enhancement patterns, as in our case, malignant mass lesions can be masked by diffuse inflammatory process on magnetic resonance imaging (MRI) [1]. On the other hand, 18-fluorodeoxyglucose positron emission tomography (FDG PET) can depict areas of abnormal uptake with a higher sensitivity and shows the site where biopsy should be taken [3]. FDG PET imaging can be a promising alternative imaging method in the evaluation of inflammatory breast diseases and may obviate extensive use of MRI. Here, we present a woman with a mass suspected for malignancy in theright breast and radiologic findings in favor of mastitis. FDG PET determined the correct location and dissemination of breast cancer with superiority to other conventional radiological methods.

Case report

A 35-year-old woman presented with the complaints of the right breast enlargement and nipple retraction. In addition to them, orange peel appearance (*peau d'orange*) was seen on physical examination but no focal mass was detected (Figure 1). Conventional imaging methods were planned with suspicion of inflammatory breast cancer (IBC) or invasive breast cancer. Ultrasonography (US) revealed diffuse skin thickening and edema without discrete lesion on the right side consistent with benign mastitis. Several lympadenopathy were also noted on the right axillar region. Mammography (MG) was performed shortly after US and confirmed nipple retraction, diffuse skin thickening and marked trabecular thickening (Figure 2).

The breast parenchyma was hyperdense and microcalcification or spicules contoured mass lesion consistent with malignity were not present on MG. One week later, MRI was performed in order to depict any malignant focus by using a dedicated breast coil (Figure 3). MRI showed diffuse areas of low signal intensity on T1-weighted (Figure 3A) and widespread hyperintense areas on T2-weighted images suggesting edema (Figures 3B, 3C). These edematous areas demonstrated marked contrast enhancement (Figure 3D). However, no focal abnormal signal changes suggesting malignancy was observed, and time-signal intensity curves did not suggest malignancy. Therefore, MRI findings were primarily considered as infectious or granulomatous mastitis. Excisional biopsies made twice from the most suspicious areas were reported as chronic nonspecific mastitis. Due to continued doubts about breast cancer, patient's clinical whole-body FDG PET scan was planned one month after MRI. FDG PET images revealed heterogeneously increased FDG uptake extents in upper outer quadrant of the right breast (SUV max = 5.5), focal increased FDG uptake in 6 right axillar (SUV max = 9.2) and 1 right supraclavicular (SUV max = 3.2) lymph nodes. Moreover, FDG PET showed focal increased FDG uptake in upper part of sacrum (SUV max = 7.4) consistent with bone metastasis (Figure 4). Excisional biopsy was repeated from upper outer quadrant of the right breast according to FDG PET results, and revealed as infiltrative ductal carcinoma. Tumor size was 2.5 cm and the estrogen and progesterone receptors status were both negative. Her2 neu score was positive. Bone metastasis in sacrum was also confirmed by MRI.

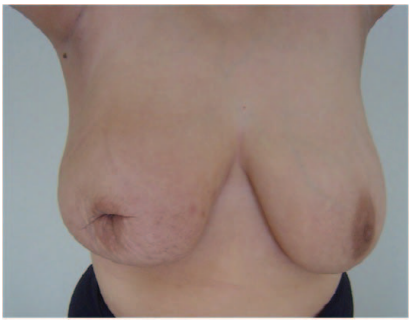

Fig. 1 – Nipple retraction, orange peel appearance and enlargement is seen in the right breast.

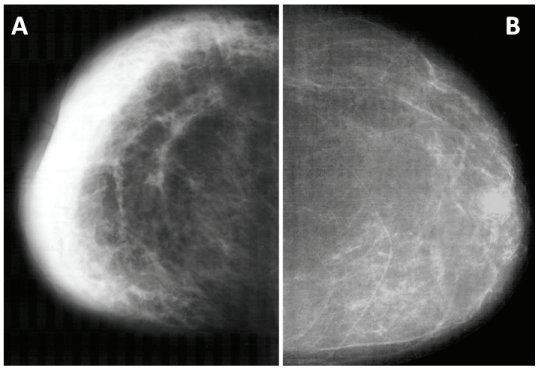

Fig. 2 – Craniocaudal views of (A) right and (B) left breast on mammography show nipple retraction, diffuse skin thickening and marked trabecular thickening in the right breast and normal parenchyma in the left breast.

Discussion

Mastitis is a benign inflammatory disease of the breast which may mimic breast cancer clinically and radiologically. It includes a group of acute and chronic inflammatory conditions. Nearly half of the patients have clinical and radiological findings suggesting a malignant tumor. Punch biopsy and histopathologic examination are often necessary to determine a definitive diagnosis [4].

The pathophysiology of breast cancer presenting as mastitis is unclear. It is possible that it is caused by tumor cells damaging epithelial cells and basement membrane surrounding the ductal lumen. This causes death of ductal tissue and behaves as a source of chronic infection. Another possible explanation may be related to recurrent infection of the necrotic areas within the tumor. Thus, these results in atypical manifestation of breast cancer as inflammatory mastitis [5].

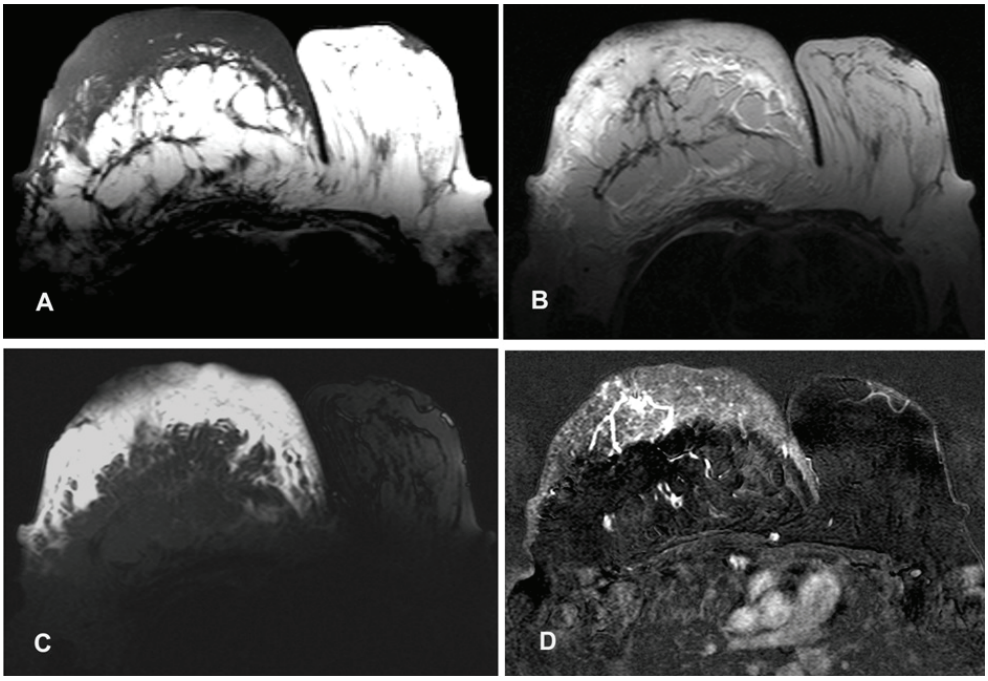

Fig. 3 – A) Axial turbo spin echo (TSE) T1-weighted (TR 550 msec; TE 11 msec; thickness 4 mm); B) TSE T2-weighted (TR 4000 msec; TE 120 msec; thickness 4 mm) and C) fat saturated TSE T2-weighted images were obtained. An axial 3-dimensional (3D) T1-weighted fast field echo (FFE) sequence (TR 6.5 msec; TE 3.2; flip angle 25; thickness 4 mm) was acquired before and after administration of intraveous paramagnetic contrast media. 3D T1-weighted postcontrast sequence was repeated five more times and post-processing of images included subtraction of postcontrast series from precontrast one (D) and calculation of time-signal intensity curves. A) Diffuse areas of low signal intensity on T1-weighted and B, C widespread hyperintense areas on T2-weighted images suggesting edema were seen. D) These edematous areas demonstrated marked contrast enhancement. However, no focal abnormal signal changes suggesting malignancy was observed.

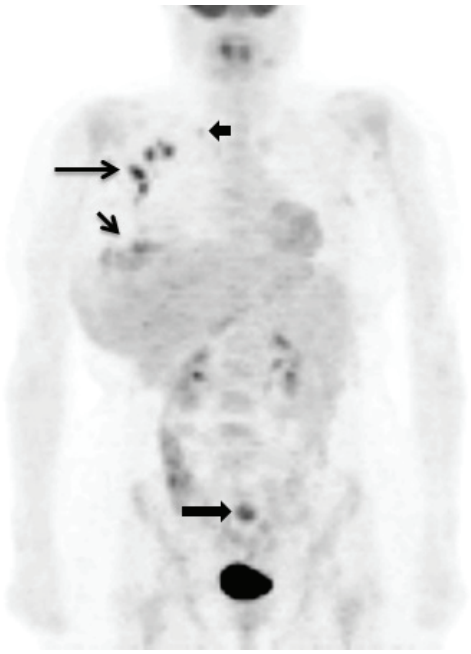

Fig. 4 – Maximum intensity projection (MIP) FDG PET image shows heterogeneously increased FDG uptake in upper outer quadrant of the right breast (SUV max = 5.5) (short thin arrow), focal increased FDG uptake in 6 right axillar lymph nodes (SUV max = 9.2) (long thin arrow) and 1 right supraclavicular lymph node (SUV max = 3.2) (short thick arrow), increased FDG uptake in upper part of sacrum consistent with bone metastasis (SUV max = 7.4) (long thick arrow).

It still remains a challenge to differentiate IBC from benign mastitis as well as from breast cancer since they are often misdiagnosed as in our case. Breast erythema, edema, tenderness, pain, warm breast, *peau d'orange* and swelling are the most common signs suggesting both benign and malign inflammatory conditions. IBC is a rare entity and constitutes 2.5% of all cases with breast cancer. It is the most aggressive variant of breast cancer and has a very poor prognosis. Skin thickening without a mass is the most common radiographic finding on MG and US for IBC as in our case. MRI plays a crucial role in the differential diagnosis of IBC. Tumor emboli blocking the dermal lymphatics are the pathognomonic features on histology which leads to diagnosis [6]. IBC was excluded by several excisional biopsies in our case.

MG and US provide benefits in many cases for the detection of breast masses and the diagnosis of malignancy. Malign breast lesions tend to appear on MG as microcalcification (76%), soft tissue densities (11%) or both (13%) [4]. However, both of them have low sensitivity and specificity in dense breasts as in our case, and also in patients having a prosthesis or prior surgery. Additionally, it is not uncommon to experience difficulty in differentiation of inflammatory breast diseases and breast cancer using US and MG since both could have false negative findings [7].

Since MRI is highly sensitive in detecting breast cancer, it is often used as a diagnostic tool to evaluate equivocal mammographic findings. Although MRI can not replace to MG or US for a complete diagnostic evaluation due to its high cost and limited specificity [8], it may play an important role in differentiation of breast tumor form IBC. Skin enhancement without a mass-like formation, diffuse cutaneous /subcutaneous/ prepectoral edema and skin thickening are the most important findings for IBC in MRI in most cases [6]. While tumors differ from inflammatory conditions with more localized findings, non-mass-like lesions can cause difficulty in diagnosis with MRI. Patients with breast cancers more frequently present with a lobulated or irregular margin than those with benign mastitis or IBC. A vessel adjacent to the lesion is another indicator suggesting malignancy on MRI [9].

FDG PET is widely used in oncology for diagnosis, staging, re-staging and treatment evaluation. The diagnostic accuracy of FDG PET imaging in breast tumors has been shown superior to MRI, MG especially in dense breasts and after augmentation mammoplasty which have complicated conventional radiological findings to interpret [3, 10]. The major advantages of FDG PET include showing multicentricity in breast cancer and lymph node, lung, bone metastases using only one imaging procedure. FDG uptake in tumors is well correlated with the amount of viable cancer cells in the tumor [10]. It is reported that FDG accumulation was correlated with the pathologic grade of the tumor. Well-differentiated subtypes such as tubular or lobular carcinoma and carcinoma *in situ* show low FDG uptake. Tumor size is also important as FDG PET has difficulty in detecting small-sized tumors, especially lesions smaller than 1 cm may show low FDG uptake [11].

Unfortunately, since FDG is not a tumor specific agent, infectious or inflammatory mastitis may also cause false positive FDG uptake for malignancy [5, 12]. Mastitis is a well-known pitfall for FDG PET, and several cases of increased FDG uptake in acute and chronic infectious mastitis were previously reported [13]. The use of a dual-time-point imaging would add to diagnostic accuracy, especially for lesions with lower standardidzed uptake values (SUVs) and in differentiating inflammation from malignant lesions [12]. However, it is quite possible to differentiate inflammation from breast cancer by visual interpretation, careful clinical history and radiologic correlation.

FDG PET can be used as complementary to conventional radiological imaging techniques especially in equivocal cases or in the presence of negative radiologic findings in patients with a high clinical probability of malignancy. Additionally, FDG PET is a valuable imaging technique to show the extent of the disease regarding nodal status or distant metastases for initial staging in patients with IBC since the probability of metastases is high at presentation [13].

Conclusion

FDG PET imaging may show encouraging contribution to conventional radiological methods with regard to primary tumor diagnosis, determination of the biopsy site, and also disease staging especially in patients with accompanying inflammatory breast disease.

Consent

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

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Received on October 26, 2016.
Revised on December 09, 2016.
Accepted on December 27, 2016.
Online First January, 2017.

CASE REPORT

UDC: 616.21-089
https://doi.org/10.2298/VSP160921011D

Endoscopic antrostomy in the treatment of odontogenic maxillary sinusitis – two cases report

Endoskopska antrostomija u lečenju dentogenog maksilarnog sinuzitisa

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Abstract

Introduction. Maxillary sinusistis of odontogenic origin is a well-known condition that occurs due to close relationship of the maxillary posterior teeth to the maxillary sinus. We presented two patients with symptoms and signs of chronic inflammation of the maxillary sinus of odontogenic origin. **Case report.** In both patients, after clinical examination, microbiological testing, skin prick tests to inhalant allergens, and endoscopy of the nasal cavity, we performed the cone beam computed tomography (CBCT) of paranasal sinuses, which showed thickening of the mucosal lining of the maxillary sinus. The mucosal oedema resulted in obstruction of the osteomeatal complex in both patients. The presence of a foreign body in the right alveolar recess in the first case and in the left osteomeatal complex in the second case were noticed. The both foreign bodies had densities similar to bone. The alveolar recesses in both cases were below the level of the nasal cavity floor. The patients were treated by endoscopic approach, a combination of lower and middle meatal antrostomy. The thickened mucous membrane was removed in the region of the osteomeatal complex, and then the foreign bodies were removed in both cases. Histopathological analysis proved that both foreign bodies were tooth roots. **Conclusion.** This case report show how be able to successfully surgically remove foreign bodies from the maxillary sinuses using endoscopic approach, a combination of both, lower and middle meatal antrostomy.

Key words:
maxillary sinusitis; diagnosis; cone-beam computed tomography; foreign bodies; otorhinolaryngologic surgical procedures; endoscopy.

Apstrakt

Uvod. Maksilarni sinuzitis dentogenog porekla je dobro poznato stanje koje nastaje zbog blizine korenova gornjih zuba i maksilarnog sinusa. Prikazali smo dva bolesnika sa simptomima i znacima hroničnog zapaljenja maksilarnog sinusa dentogenog porekla. **Prikaz bolesnika.** Kod oba bolesnika, nakon kliničkog pregleda, mikrobioloških ispitivanja, kožnih proba sa inhalacionim alergenima, kao i endoskopije nosne šupljine, urađena je kompjuterizovana tomografija konusnog zraka – [*cone beam computed tomography* (CBCT)], koja je pokazala zadebljanje sluznice maksilarnog sinusa. Otok sluznice doveo je do opstrukcije ostiomeatalnog kompleksa. Uočeno je prisustvo stranog tela u desnom alveolarnom recesusu u prvom, i u predelu ostiomeatalnog kompleksa, u drugom slučaju. Oba strana tela davala su senku sličnu koštanoj supstanci. Dno alveolarnog recesusa sinusa je u oba slučaja bilo ispod ravni poda nosne šupljine. Bolesnici su operisani endoskopskim pristupom, kombinacijom srednje i donje antrostomije. Odstranjena je zadebljala sluznica u predelu ostiomeatalnog kompleksa, a nakon toga su uklonjena strana tela. Histopatološka analiza je u oba slučaja pokazala da su strana tela bili korenovi zuba. **Zaključak.** Ovim prikazom se ukazuje na mogućnost uspešnog hirurškog uklanjanja stranih tela iz maksilarnog sinusa endoskopskim pristupom, kombinacijom srednje i donje antrostomije.

Ključne reči:
maksilarni sinuzitis; dijagnoza; kompjuterizovana tomografija konusnog zraka; strana tela; hirurgija, otorinolaringološka, procedure; endoskopija.

Introduction

The maxillary sinus is a horizontally placed three-sided pyramid, with the base facing nasal cavity. Maxillary sinus with its alveolar recess is in close contact with the posterior teeth of the upper jaw. The bottom of the maxillary sinus, in approximately 70% of the cases, is below the level of the hard palate, in 20% of cases in the level of the hard palate, and only in 10% of cases is above the hard palate [1]. The most common iatrogenic causes of odontogenic maxillary sinusitis

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are: placing the instrument too deep into the roots of teeth during the endodontic therapy, interradicular perforations of curved root canals and suppression of canal filling material into the sinus, sinus perforation during tooth extraction, suppression of the root or the whole tooth into the sinus during the extraction, protruded dental implants, etc. Spontaneous causes of odontogenic maxillary sinusitis can be: acute periapical abscesses, infected follicular cysts of impacted teeth and pericoronitis of the third upper molar [2]. The use of cone beam computed tomography (CBCT) allows lowering of radiation to the patient, which is only about 10% of the conventional multi-slice computed tomography (MSCT), and gets precise information about the position and relationships between the maxillary sinus and tooth [3]. CBCT showed considerable advantage in the detection of the periapical lesions in comparison to two-dimensional radiographic procedures [4].

We presented two cases of successful surgical removal of foreign bodies from the maxillary sinuses using endoscopic approach as well as a significance of using CBCT diagnostics in preparing patients for surgery, which proved to be sufficiently reliable and safe for the patients.

Case report

Case 1

A 25-year-old female patient visited the hospital because of history of 18-months difficult breathing through the nose, followed by a reduced sense of smell and deficient postnasal secretion. The patient had headache in the frontal area. Because of difficult nasal breathing, the patient often used decongestant nasal drops. About two years ago, the patient had an intervention of tooth extraction in the upper jaw on the right side. She denied that she had difficulties after tooth extraction. By anterior rhinoscopy, we noticed the presence of swollen mucosa of the right inferior turbinate and the right nasal cavity, which was confirmed by endoscopic examination of the nasal cavity. CBCT of the paranasal sinuses showed thickening of the mucosa of the right maxillary sinus as well as obstruction of the right osiomeatal complex (Figure 1). In the alveolar recess of the same sinus, a hyperdensic lesion was seen, which, by its properties, corresponded to the tooth root. That lesion seemed to be a foreign body. Defects of bone structures were not observed. The intensity of inflammation, graded according to Lund-Mackay scoring system, was 3 (1 unilateral incomplete shadows of the maxillary sinus and 2 complete unilateral obstruction of ostheomeatal complex). Skin prick tests showed sensitization to inhaled allergens and microbiological analysis of swabs of the nasal mucous membranes showed normal nasal flora. In consultation with the oral surgeon, we ruled out the presence of oroantral fistula in the alveolar ridge gingival area.

The patient was operated, in general anaesthesia, by endoscopic approach using functional endoscopic sinus surgery (FESS), combining the middle and inferior antrostomy. After infiltration of the mucosa of the uncinate process with solution of epinephrine (dilution 1 : 100 000), we made an uncinectomy and bulectomy. Then, we dilated the natural maxillary sinus ostium. Due to the position of the foreign body, an opening was made in the medial wall of the maxillary sinus at the level of the inferior nasal meatus, directly in front of the nasolacrimal duct. Finally, we removed the foreign body through the inferior antrostoma (Figures 2 and 3). Foreign body was sent to pathohistological analysis, which showed that it was a tissue of tooth root. The nasal pack was placed in the right nasal cavity and removed after four days. Postoperatively, the patient received peroral antibiotic therapy, and started nasal washing with 0.9% solution of sodium chloride. At follow-up examinations, the patient was free of symptoms and nasal finding was normal.

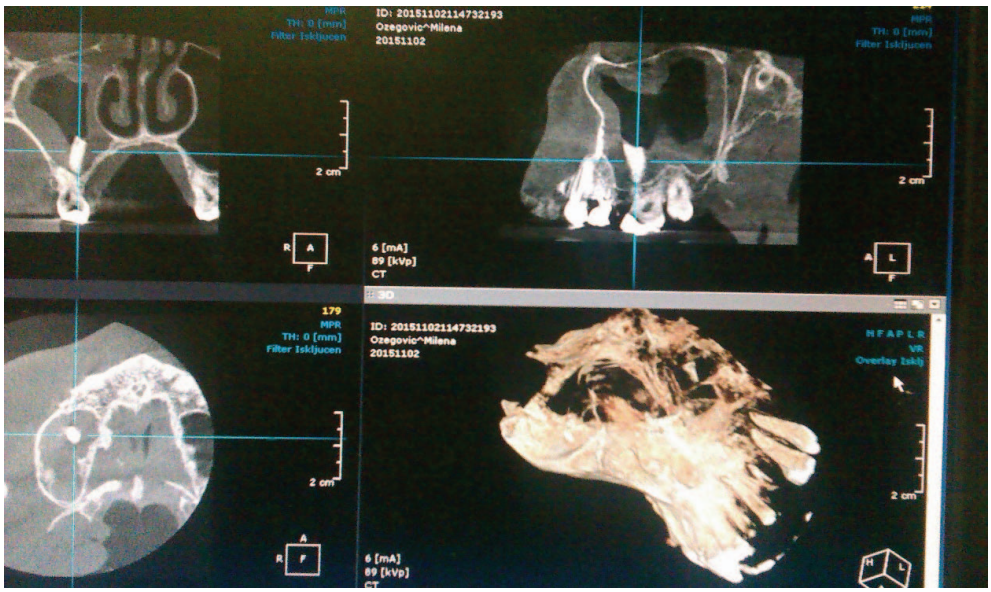

Fig. 1 – Cone-beam computed tomography (CBCT) of the paranasal sinuses shows the opacification of the right ostiomeatal complex, thickening of the right maxillary sinus mucosa and a hyperdensic lesion inside the alveolar recess of the right maxillary sinus which corresponds to the root of the tooth (the first presented patient).

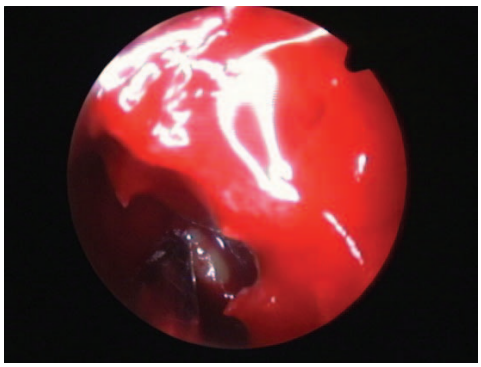

Fig. 2 – Intraoperative endoscopic view of the right maxilary sinus: it can be seen a part of the tooth through the inferior antrostoma (the first patient).

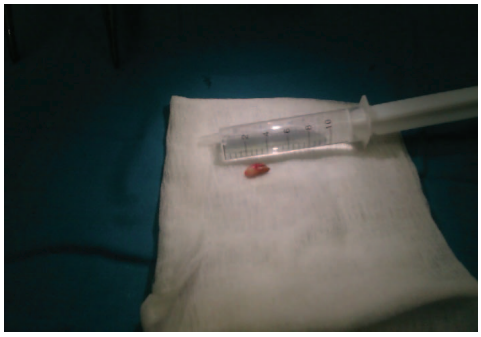

Fig. 3 – Removed part from the tooth root of the first patient.

Case 2

A 36-year-old patient came to the otorhinolaryngologist with the sense of facial pressure on the left side and discrete swelling of the left side of the face. The patient explained that he had the extraction of both upper molars long ago, with opening of both maxillary sinus and procedures of conservative and surgical closing of oroantral openings. Examination was completed with endoscopic examination of nasal cavities. The allergy tests were negative. Bacteriological examination of the throat and nose showed the presence of physiological flora. Fungi were not isolated by mycological analysis. CBCT of paranasal sinuses showed the presence of a foreign body of tooth density inside the left maxillary sinus as well as total opacification of the same sinus. In the area of the alveolar ridge, a dehiscence of sinus floor was seen and it was completely covered with mucous membrane, without signs of oroantral fistula (Figure 4).

The patient was treated endoscopically, under general anaesthesia, combining the middle and inferior antrostomy. The foreign body and hypertrophic mucosa were removed from the sinus and sent for pathohistological analysis which showed that it was the tissue of tooth (Figure 5) surrounded by hypertrophic and chronically inflamed mucosa of the maxillary sinus.

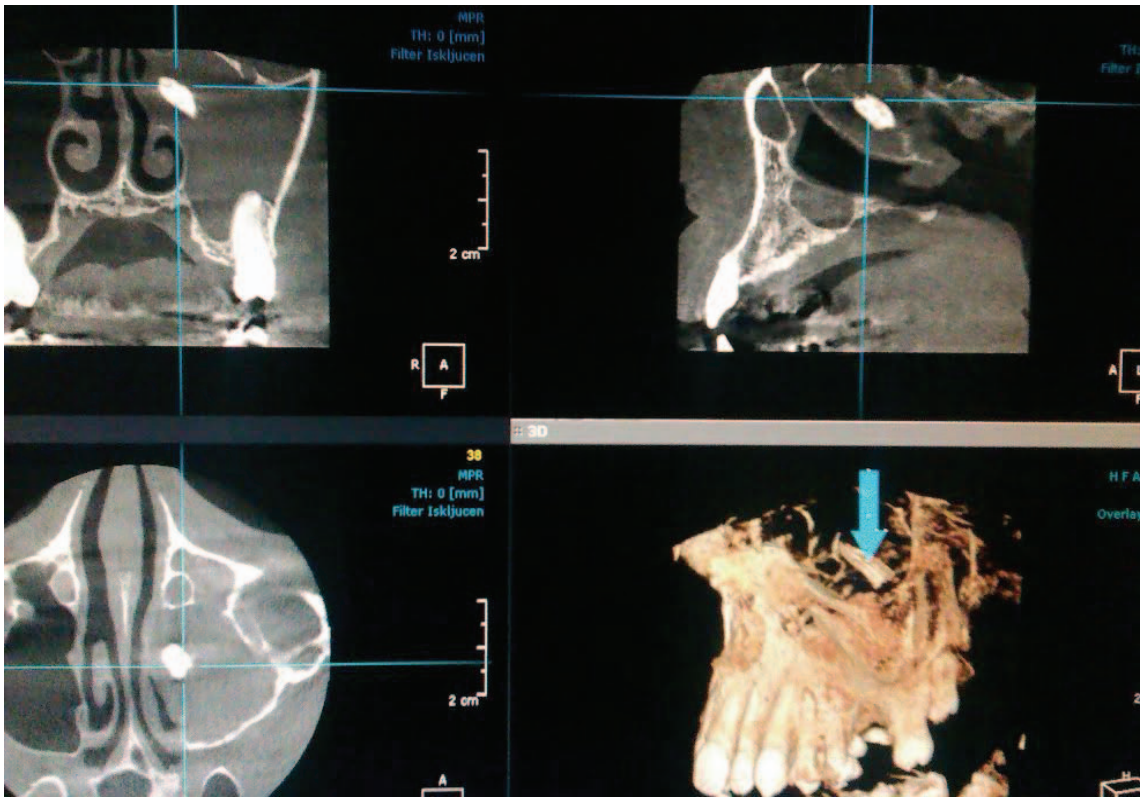

Fig. 4 – Cone-beam computed tomography (CBCT) of the paranasal sinuses shows the complete opacification of the left maxillary sinus. A foreign body, a part of tooth with intracanal filling, is seen in the area of ostheomeatal complex (the second patient).

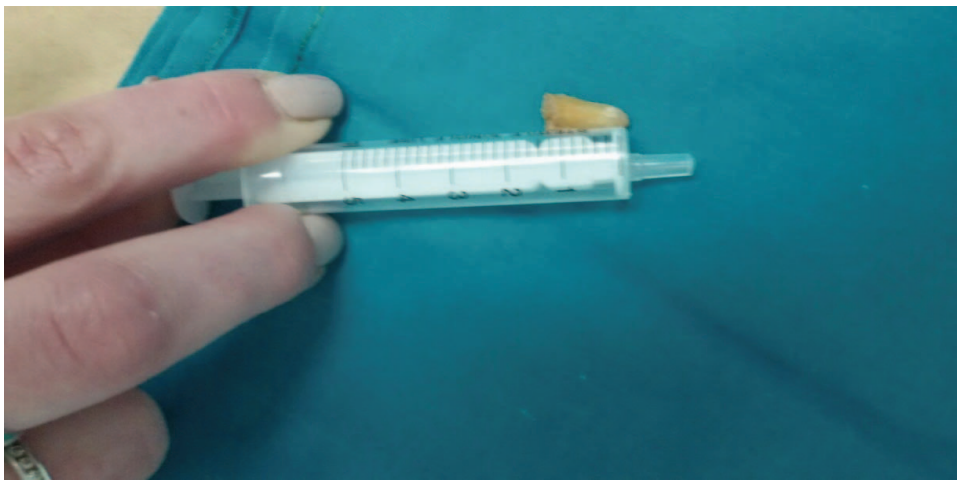

Fig. 5 – Removed part from the tooth root from left maxilary sinus (the second patient).

Discussion

Chronic rhinosinusitis is a chronic inflammation of the nasal and paranasal sinuses mucosa, characterized by the presence of symptoms for a minimum of 12 weeks [5]. Upon the recommendation of the multidisciplinary working group for rhinosinusitis [Rhinosinusitis Task Force (RTF) American Academy of Otolaryngology-Head & Neck Surgery], the diagnosis is made based on the presence of large, i.e., major symptoms, or by the presence of one large and two small (so-called minor) symptoms [6]. The major symptoms of chronic rhinosinusitis are: nasal obstruction, nasal/postnasal discharge, pain/pressure in the area of the face, a feeling of fullness in the area of the face, and impaired sense of smell (hiposmia/anosmia). The minor symptoms are: headache, bad breathing, weakness/fatigue, pain in the teeth, cough, pain/pressure/fullness in the ears and fever. It should be noted that facial pain/pressure and elevated temperatures are not by themselves sufficient criteria for a clinical diagnosis of chronic rhinosinusitis if not present at least one major factor. Elevated body temperature is a major factor for acute rhinosinusitis, but a minor factor for chronic rhinosinusitis. Occasionally, maxillary rhinosinusitis [chronic maxillary rhinosinusitis (CMRS)] is a clinical entity that occurs in 25.2% of cases of chronic rhinosinusitis [7], and in about 10%–20% of cases it is of odontogenic origin [8], although some studies suggest that the incidence of dental factors in pathogenesis of CMRS can be up to 40% of cases [9]. Odontogenic CMRS is mostly of iatrogenic origin (65.7%), and apical dental pathology is the cause of inflammation in 25% of cases [10]. Iatrogenic odontogenic maxillary sinusitis is in 47.5% of cases complicated with oroantral fistula [11]. Inflammatory process in CMRS is leading to the reversible mucociliary dyskinesia, making extremely difficult maxillary sinus drainage and creating favourable conditions for bacterial colonization and infection [12]. CBCT of paranasal sinuses is of great importance for the diagnosis of CMRS [13, 14]. Unilateral CMRS is in 55% of cases the consequence of the first molar disease and in 34% of the second molar pathology [14].

Inflammation of the maxillary sinus mucosa of odontogenic origin usually occurs after injury of the maxillary sinus during tooth extraction. This especially occurs when chronic apical periodontitis destroy sinus bone tissue floor, when the apex of the tooth is covered only with mucous membrane of the sinus which can be perforated during the extraction. Oroantral communication created this way very often heals spontaneously, without the appearance of sinusitis. Chronic sinusitis is localized mainly in the area of the alveolar recess; if there is an oroantral fistula which cannot heal spontaneously it requires surgical treatment.

In the etiology of odontogenic maxillary sinusitis, the following factors should be considered: whole tooth or part of the tooth as well as other foreign bodies, instrument for root canal treatment and periapical filling which is not absorbed. Infectious contents of the tooth canal that enters the maxillary sinus and irritation caused by a foreign body, rapidly cause inflammation. Removal of foreign body from the maxillary sinus only prevents the development of complications [15].

FESS can be a treatment of choice for odontogenic maxillary sinusitis [16] in all the cases of sinusitis non-complicated with oroantral fistula. We propose the combination of two approaches: by middle nasal meatus (by osteomeatal complex) and by inferior nasal meatus. A study performed by Hinohira et al. [17] showed normal maxillary sinuses in all patients with chronic sinusitis after middle and inferior meatal antrostomy treatment. Periodontitis, periapical lesions and endodontically treated teeth indicate the possible presence of bacterial infection. As these processes can last for a long time, dental infections are easily overlooked during routine ear, nose and throat examination [18].

Conclusion

CBCT imaging technique of the nasal cavity and paranasal sinuses in addition to lower doses of radiation than that the standard computed tomography scan needs, provides a three-dimensional visualising of the anatomic relationships in the nasal-sinus area and better planning of surgical approach.

Compared to classical radical operation of the maxillary sinus by Caldwell-Luc, the combination of inferior and mid-

dle antrostomy preserves the integrity of the anterior wall of the maxillary sinus as well as its floor and shortens recovery time. In our cases, endoscopic approach with the combination of lower and middle antrostomy was selected because of the position of foreign bodies, easier removal of a chronic inflamed mucosa of the maxillary sinus and to ensure good ventilation and postoperative sinus drainage, while preserving the mucociliary transport. Therefore, the FESS could be a treatment of choice in all the cases of odontogenic maxillary sinusitis that are not associated with the presence of oroantral fistula needing its closure.

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Received on September 21, 2016.
Revised on December 17, 2016.
Accepted on December 27, 2016.
Online First January, 2017.

CASE REPORT

UDC: 612.017:[616.13/.14-002+616.16-002+616.28+617.7
https://doi.org/10.2298/VSP161212001G

Cogan's syndrome – A case series

Cogan-ov sindrom – serija slučajeva

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Abstract

Introduction. Cogan's syndrome is a rare variable vessel vasculitis. It can be typical and atypical. Basis of the treatment comprises glucocorticoids, and in patients with systemic manifestations, immunosuppressive drugs. **Case report.** We wanted to present the experience of the Clinic for Rheumatology and Clinical Immunology of the Military Medical Academy, Belgrade, in diagnosing and treating patients suffering from Cogan's syndrome. The analysis included 7 patients. Patients' demographic characteristics, disease manifestations, course of the disease, applied treatment and treatment outcome were analysed. Five of the patients were women and 2 were men, with the average age of 39 ± 13 (25–65) years. The typical form of the disease manifested in 1 patient. In 6 patients, the first manifestation was the audiovestibular dysfunction. In 1 patient, systemic manifestations were the first to appear. In the cases where the disease manifested atypically, 3 patients developed conjunctivitis, 2 episcleritis, and 1 uveitis. They all had systemic manifestations. One female patient was diagnosed with aortitis and aortic insufficiency. They all tested positive for inflammatory biohumoral syndrome. Four patients had positive antinuclear antibodies, 3 anticytoplasmic antibodies, and 1 positive rheumatoid factor. They were all treated with glucocorticoid and immunosuppressive drugs. Methotrexate was administered to all the patients in doses up to 20 mg per week. Pulses of cyclophosphamide were administered to 2 female patients. All patients went successfully into remission. The female patient with the typical form of the disease experienced permanent bilateral hearing loss. **Conclusion.** Patients with a rapidly developed audiovestibular dysfunction should be viewed as suffering from Cogan's syndrome from the viewpoint of differential diagnosis. A timely treatment with glucocorticoids can prevent hearing loss and the development of systemic manifestations of the disease. Precedence should be given to methotrexate when selecting an immunosuppressive drug.

Key words:
cogan syndrome; vasculitis; vestibular diseases; keratitis; adrenal cortex hormones; immunosuppressive agents.

Apstrakt

Uvod. Cogan-ov sindrom je zajedno sa Behcet-ovim sindromom prema najnovijoj klasifikaciji svrstan u grupu posebnih vaskulitisnih sindroma. Može biti tipičan i atipičan. Osnov lečenja čine glukokortikoidi, a kod bolesnika sa sistemskim manifestacijama i imunosupresivi. **Prikaz slučaja.** Prikazali smo iskustvo Klinike za reumatologiju i kliničku imunologiju Vojnomedicinske akademije, Beograd, u dijagnostici i lečenju bolesnika sa Cogan-ovim sindromom. Analizom je obuhvaćeno sedam bolesnika dijagnostikovanih i lečenih u periodu od 2004. do 2016. godine. Analizirane su demografske karakteristike obolelih, manifestacije i tok bolesti do dijagnoze, primenjena terapija i ishod lečenja. Od ukupno sedam bolesnika pet su bile žene i dva muškarca, prosečne životne dobi od 39 ± 13 (25–65) godina. Tipičnu formu bolesti imao je jedan, a atipičnu 6 bolesnika. Kod šest bolesnika prva manifestacija bolesti bile su vrtoglavica i naglo slabljenje sluha. Samo kod jednog bolesnika prvo su se ispoljile sistemske manifestacije. U slučajevima atipičnog Cogan-ovog sindroma tri bolesnika su imala konjuktivitis, dva episkleritis i jedan uveitis. Svi bolesnici imali su sistemske manifestacije. Kod jedne bolesnice dijagnostikovan je aortitis sa posledičnom insuficijencijom aortne valvule. Svi bolesnici imali su pozitivan zapaljenski biohumoralni sindrom. Tri bolesnika imala su pozitivna antinuklearna antitela a dva perinuklearna anticitoplazmatska antitela. Svi su lečeni glukokortikoidima i imunosupresivnim lekovima. Metotreksat je primenjen kod šest bolesnika u dozi do 20 mg nedeljno. Pulsne doze ciklofosfamida primenjene su kod dve bolesnice. Kod svih bolesnika postignuta je remisija bolesti. Kod bolesnice sa tipičnom formom bolesti gubitak sluha bio je obostran i trajan. **Zaključak.** Bolesnike sa naglo nastalom audiovestibularnom disfunkcijom uvek treba dife-

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rencijalno dijagnostički posmatrati kao Cogan-ov sindrom. Na vreme započeta terapija glukokortikoidima može sprečiti gubitak sluha i razvoj sistemskih manifestacija bolesti. Među imunosupresivnim lekovima prednost treba dati metotreksatu.

Ključne reči:
coganov sindrom; vaskulitis; vestibularni aparat, bolesti; keratitis; kortikosteroidni hormoni; imunosupresivi.

Introduction

In 1945, David Cogan [1], an ophthalmologist, was the first who identified and described the clinical entity in which the major manifestations include interstitial keratitis (IK) and audiovestibular dysfunction that is similar to that of Ménière's disease. In 1980, Haynes et al. [2] defined the diagnostic criteria for the typical and atypical Cogan's syndrome. With the development of medical knowledge, Cogan's and Behcet's syndrome were classified into a special category of systemic vasculitides [3]. Cogan's syndrome is a rare disease. Over 250 cases have been described in literature so far [4–6]. Patients with IK have red eyes, photophobia and pain [2, 4, 6]. In most of the patients both eyes are affected. In the atypical forms, other structures of the eye are affected, in isolation or in conjunction with IK. Episcleritis or scleritis, retinitis, optic neuritis, glaucoma, papilloedema, central retinal artery occlusion, ptosis, exophthalmus and other manifestations may occur [6]. Audiovestibular manifestations in Cogan's syndrome are similar to those of Ménière's syndrome [1, 2, 4]. Hearing loss is progressive. According to literature, bilateral hearing loss occurred in up to 43% of such patients [4, 6]. Approximately, two thirds of the patients had systemic manifestations (febrility, headache, arthritis, large vessel vasculitis, etc.). Cogan's syndrome can occur in people of all ages, but usually it affects young adults. It equally affects both sexes. The etiology of the disease is unknown. Infections and autoimmune disorders are cited as being the predisposing factors of the disease. In favour of the immunological theory there are findings of antibodies in cornea, anticochlear antibodies (anti-HSP70), antiendothelial antibodies, antinuclear antibodies (ANA), rheumatoid factor (RF) and antineutrophil cytoplasmic antibodies (ANCA) [7–10]. The basis of the treatment comprise glucocorticoids (GCs) [11, 12]. In patents with systemic vasculitis, it is necessary to administer immunosuppressive drugs alongside GCs [13, 14].

The aim of our work is to present our experience in diagnosing and treating 7 patients suffering from Cogan's syndrome.

Case report

A retrospective analysis included 7 patients diagnosed and treated at the Clinic for Rheumatology and Clinical Immunology of the Military Medical Academy, Belgrade, Serbia, between 2004 and 2016. The patients' demographic characteristics, audiovestibular, ophthalmological and systemic manifestations of the disease, the effects of the applied therapy and course of the disease were analysed. The Cogan's and Hayne's original criteria were used for the classification of Cogan's syndrome into typical and atypical [1, 2]. The typical form of the disease is characterised by: interstitial keratitis, audiovestibular symptoms akin to those of Ménière's disease including hearing loss and the interval between the onset of eye disease and audiovestibular manifestations shorter than 2 years. The atypical form of Cogan's syndrome is characterised by: absence of IK, the absence of audiovestibular manifestations akin to those of Ménière's syndrome in patients with IK and the interval between the onset of eye disease and ear disease longer than 2 years.

Out of 7 patients, 5 were women and 2 men, with the average age at the onset of the disease being 39 ± 13 (25–65) years. One patient had the typical form of the disease (Table 1). From the onset of the first symptoms until the diagnosis of the disease passed on average 4.3 years (2 months to 15 years). The first manifestation of the disease in 6 patients was the audiovestibular dysfunction similar to that of Ménière's syndrome. Only in 1 female patient the audiovestibular dysfunctions and eye disease were preceded by systemic manifestations. Only the female patient with the typical form of the disease had interstitial keratitis. Three patients had scleritis (Figures 1 and 2), 2 conjunctivitis and 1 patient had uveitis.

Table 1

Patients' characteristics

Patient No	Type of disease	Age (yrs)*	Sex	First manifestation	Changes to the eye	Interval** (month)	Until diagnosed
1	Typical	39	F	Vertigo, bilateral hearing loss	Interstitial keratitis	1	2 months
2	Atypical	24	F	Vertigo, unilateral hearing loss	Uveitis	12	15 years
3	Atypical	26	F	Systemic manifestations, unilateral mild hearing loss	Scleritis	24	6 years
4	Atypical	43	M	Vertigo, bilateral hearing loss	Scleritis	2	6 months
5	Atypical	35	F	Vertigo, unilateral mild hearing loss	Scleritis	3	4 months
6	Atypical	22	M	Vertigo, unilateral mild hearing loss	Conjunctivitis	10	4 years
7	Atypical	65	F	Vertigo, unilateral hearing loss	Conjunctivitis	5	1 years

***At the onset of disease; **The period between vestibular dysfunction and eye disease.**

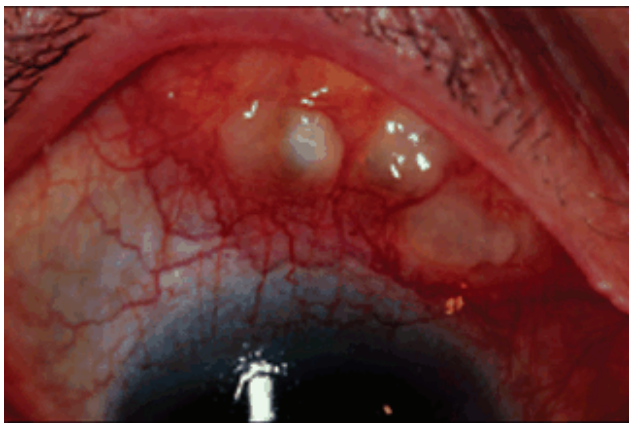

Fig. 1 – Scleritis in one of the patients with Cogan's syndrome.

All 7 patients had systemic manifestations (Table 2). They all had polyarthritis, 4 had headaches, 3 had fever, 1 had lymphadenopathy and splenomegaly and 1 patient had auricular chondritis. The female patient with the typical form of the disease developed aortitis with consequent aortic insufficiency and malignant arrhythmia (Figure 3). Six patients had an accelerated erythrocyte sedimentation rate during the active phase of the disease, (Table 3). The average rate was equal to 69 ± 39 mm/h (8–129). Two patients had anaemia. ANA were detected in 4 patients, ANCA in 3 patients, and rheumatoid factor RF in 1 patient.

All patients were treated with GCs locally and systemically before being diagnosed (Table 4). The treatment would be stopped after the symptoms and signs of ear and eye disease subsided. In the female patient with the typical form of the disease who developed bilateral hearing loss systemic administration of glucocorticoids was commenced one month after the onset of audiovestibular symptomatology. From the moment the patients were diagnosed with Cogan's syndrome, they were all treated with GCs at an initial dose of 0.5–1 mg/kg body weight (BW) of prednisone per day.

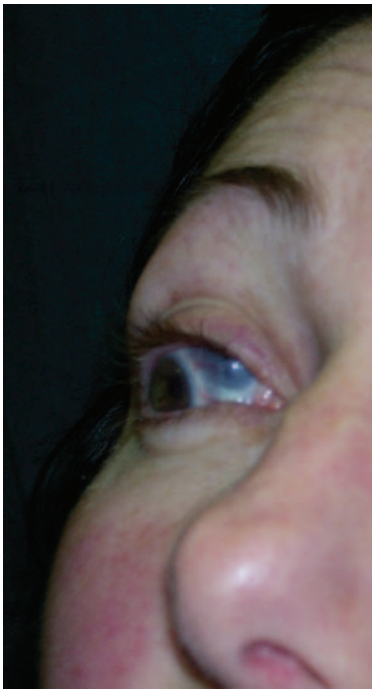

Fig. 2 – The consequence of scleritis.

Table 2

Systemic manifestations of the disease

Patient No	Type of disease	Systemic manifestations
1	Typical	Fever, headache, arthritis, aortitis
2	Atypical	Arthritis
3	Atypical	Fever, headache, arthritis, lymphadenopathy, splenomegaly, chondritis
4	Atypical	Arthritis
5	Atypical	Fever, headache, arthritis
6	Atypical	Arthritis
7	Atypical	Arthritis, headache

Table 3

The results of laboratory tests

Patient No	Type of disease	Erythrocyte sedimentation rate (ESR) mm/h	Anaemia	Autoantibodies
1	Typical	92	+	RF, ANA, ANCA
2	Atypical	8	-	-
3	Atypical	80	+	ANA, ANCA
4	Atypical	87	-	ANCA
5	Atypical	48	-	-
6	Atypical	45	-	ANA
7	Atypical	129	-	ANA

RF – rheumatoid factor; ANA – antinuclear antibodies; ANCA – antineutrophil cytoplasmic antibodies.

Table 4

Treatment

Patient No	Type of disease	Treatment	Outcome	Follow-up (year)
1	Typical	GCs, CyP, AZA, CyA, MTX	Remission after introduction of MTX Permanent bilateral hearing loss	12
2	Atypical	GCs, MTX	Remission Hearing improvement	1
3	Atypical	GCs, MTX, CyP	Remission after introduction of CyP Hearing improvement	3
4	Atypical	GCs, MTX	Remission Hearing improvement	2
5	Atypical	GCs, MTX	Remission Hearing improvement	3
6	Atypical	GCs, MTX	Remission Hearing improvement	4
7	Atypical	GCs, MTX	Remission Hearing improvement	2

GCs – glucocorticoids; MTX – methotrexate; CyP – cyclophosphamide; CyA – cyclosporine A; AZA – azathioprine.

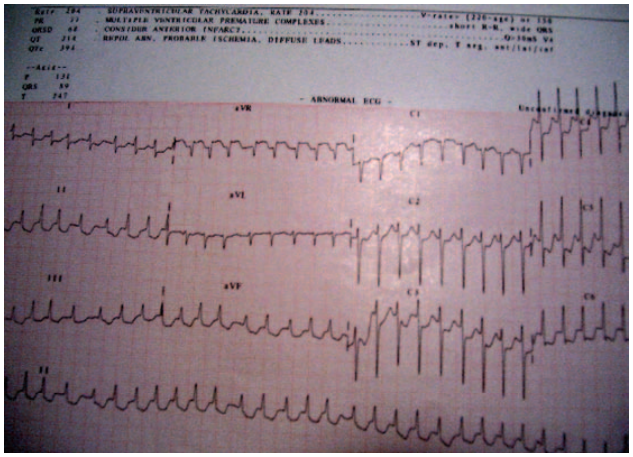

Fig. 3 – Paroxysmal supraventricular tachycardia in the patient who developed aortitis, aortic insufficiency and malignant arhythmia.

The female patient with the typical form of Cogan's syndrome was administered 3 pulse doses of methylprednisolone when signs of aortitis manifested. The maintenance dose was between 5–10 mg of prednisone *per* day. Methotrexate alongside GCs were administered to all patients. The maximum administered dose was 20 mg once *per* week. In 1 female patient, cyclophosphamide was introduced after methotrexate due to a lack of drug effect and was administered in pulse doses. This treatment resulted in remission. In the female patient with the typical form of the disease the treatment was started with GCs and pulse doses of cyclophosphamide. Due to a lack of drug effect cyclophosphamide was replaced with cyclosporine A. After the absence of effect of cyclosporine A, achieving remission was attempted with azathioprine. Remission was achieved only with methotrexate at a dose of 20 mg *per* week. In the female patient with the typical form of Cogan's syndrome bilateral hearing loss was permanent. Regarding the implantation of cochlear implant, recurrence of the disease occurred. In the remaining 6 patients, the treatment led to improvement of hearing.

Discussion

Cogan's syndrome is a rare variable vessel vasculitis. The typical form is characterised by IK and audiovestibular dysfunction similar to that of Ménière's disease [1, 2, 4]. A whole range of systemic manifestations has been described in patients with Cogan's syndrome [6]. The most common cardiovascular manifestation, described in approximately 10% of the cases, is aortitis with aortic insufficiency [15–17]. It is difficult to differentiate aortitis in Cogan's syndrome from Takayasu's arteritis [18]. Large blood vessels are particularly affected. One should keep in mind that arteritis can develop many years after the onset of the disease.

The most common manifestation that will make a patient visit a rheumatologist is arthritis. All our patients were diagnosed after they had been referred to a rheumatologist for a check-up due to arthritis. Individual cases of Cogan's syndrome are described in patients with rheumatoid arthritis, juvenile idiopathic arthritis and ankylosing spondylitis [19]. The central nervous system involvement is presented with hemiparesis or hemiplegia, aphasia, cerebellar symptomatology, myelopathy, meningitis or encephalitis. The peripheral nervous system involvement can manifest as paraesthesia, trigeminal neuralgia, mononeuritis multiplex. All our patients had headaches which are, according to relavant literature data, manifested in approximately 40% of the patients [5, 6, 11, 12]. Manifestations in the gastrointestinal tract such as pain, diarrhoea, and melena are usually the result of arteritis of mesenteric arteries. One should keep in mind the fact that Cogan's syndrome can occur in patients suffering from inflammatory bowel diseases [20]. Hepatomegaly and splenomegaly have been described in separate cases so far. In our group of patients, one female patient had splenomegaly. Extremely rare manifestations in patients with Cogan's syndrome include sinusitis or chondritis. In our group of patients, one female patient had auricular chondritis and it occurred before she was diagnosed and the treatment was started. No laboratory test is specific for Cogan's syndrome. The erythrocyte sedimentation rate is usually accelerated. If any systemic manifestations are pre-

sent, a certain degree of anaemia is usually present as well. Hypocomplementemia and cryoglobulinemia are rarely detected and it is difficult to determine their relevance. The presence of rheumatoid factor (RF), antinuclear antibodies (ANA), and antineutrophil cytoplasmic antibodies (ANCA) point to the possible autoimmune nature of the disease. However, the pathogenic significance of these antibodies is not clear [9, 12, 21]. We established the presence of RF in 1 patient, ANCA in 3, and ANA in 4. In literature, an even greater importance is attached to the antibodies to the corneal antigens and the structures of the inner ear such as anti-Hsp70 [7, 8, 10, 22]. We could not determine these antibodies. It is still not clear whether anti-Hsp70 are pathogenic or they are indicative of progressive hearing loss.

The course of Cogan's syndrome varies. The most serious complication of ear disease is hearing loss that is quite often bilateral. In 6 of our patients ear and eye disease flares occurred at different time intervals but they all had systemic manifestations in between the flares. In the female patient with the typical form of the disease that started acutely and simultaneously to spread to the eye and ear, hearing loss promptly occurred and systemic manifestations were quite severe.

Glucocorticoids are efficient at disease management. When complete hearing loss ensues, it is usually irreversible. All our patients were treated with GCs and 6/7 of them experienced improved hearing. The female patient with permanent bilateral hearing loss was embedded a cochlear implant after achieving remission [23, 24]. Immediately after the surgical intervention a mild recurrence of the disease ensued. In literature, bone trauma is cited as the possible disease trigger [25]. To all our patients immunosuppressive therapy alongside GCs was administered. In all patients, a stable remission of disease is managed with methotrexate that was administered at a maximum dose of 20 mg once per week. Only in one female patient the absence of methotrexate effect was registered. Our experience with metotrexate administration is in conformity with the data found in literature [26, 27]. Other immunosuppressive drugs could be administered as well. Based on our experience, a precedence should be given to cyclophosphamide over azathioprine and cyclosporine A.

Conclusion

The diversity of the Cogan's syndrome manifestations makes diagnosing more difficult. The correlation between ocular and audiovestibular manifestations need to attract attention to this disease. Diagnosing Cogan's syndrome provides a challenge and it calls for a multidisciplinary approach. There are no clear treatment guidelines. Early administration of glucocorticoids can prevent permanent hearing loss and the occurrence of severe complications. In addition to glucocorticoids in patients with systemic manifestations immunosuppressive drug should be administered. Priority should be given to methotrexate.

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Received on December 9, 2016.
Accepted on December 28, 2016.
Online First January, 2017.

CASE REPORT

UDC: 616.9-022.913:616.428
https://doi.org/10.2298/VSP161223035G

Tick-borne lymphadenopathy acquired in Serbia – report of two cases

Ubodom krpelja uzrokovana limfadenopatija – prikaz dve bolesnice zaražene u Srbiji

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Abstract

Introduction. Acronym tick-borne lymphoadenoathy (TIBOLA (*Dermacentor*-borne necrosis erythema and lymphoadenopathy – DEBONEL, scalp eschar associated with neck lymphoadenopathy – SENLAT) comprises clinical diagnosis of tick-borne symptoms of cervical or occipital lymphadenopathy with inoculation eschar at the site of tick bite on scalp. Since the first description, it was proved to be associated with several infectious agents, most frequently *Rickettsia slovaca*, or less often other spotted fever group *Rickettsiae* (*Rickettsia raoulti* and *Rickettsia rioja*), and gained an emerging infectious disease status in Europe. *Dermacentor* ticks serve as vectors and possible natural reservoir. The course is in most cases benign and infection is limited. Doxicycline is the recommended initial treatment, both for adult and most cases in children. **Case report.** Two subjects who acquired the desease caused by tick bites in Vojvodina region of Serbia are presented. Both patients are females. A tick was removed from the scalp, and several days later doxicycline treatment started because of the inflammatory symptoms of lymph node enlargement. Diagnostic eschar appeared in both patients during doxicycline treatment. After a switch to ciprofloxacine, inflammatory symptoms subsided, but the complete healing of scalp necrosis took longer than one month, with residual cicatricial alopecia. **Conclusion.** Although rare, it is necessary to include TIBOLA in a spectrum of epidemiologic risks in cases of tick bites.

Key words:
tick borne diseases; dermacentor; rickettsia; diagnosis; treatment outcome; serbia.

Apstrakt

Uvod. Akronim *tick-borne lymphoadenoathy* (TIBOLA) (*dermacentor borne necrosis erythema and lymphoadenopathy* – DEBONEL, *scalp eschar associated with neck lymphoadenopathy* – SENLAT) podrazumeva kliničku dijagnozu simptoma vratne limfadenopatije i inokulacione eshare na koži poglavine uzrokovanih ubodom krpelja. Od prvih opisa dokazana je uzročna uloga nekoliko infektivnih agenasa, najčešće *Rickettsia slovaca*, ređe drugih rikecija *spotted fever grupe* (*Rickettsia raoulti* i *Rickettsia rioja*). U Evropi je stekla status infekcije u nastajanju. *Dermacentor* krpelji su vektori i verovatni prirodni rezervoar ove grupe rikecija. Tok je obično nekomplikovan i infekcija je ograničena. Doksiciklin je preporučena terapija za odrasle i pedijatrijske bolesnike. **Prikaz bolesnika.** U radu su prikazana dva slučaja TIBOLA stečene ubodom krpelja u Vojvodini i Srbiji. Ubod krpelja kod obe bolesnice bio je u predelu poglavine. Krpelj je uklonjen od strane lekara. Kod obe bolesnice je nakon par dana započeta terapija doksiciklinom zbog uvećanja limfnih čvorova vrata i potiljačne regije. Dijagnostička eshara pojavila se kod obe bolesnice tokom terapije doksiciklinom. Nakon uvođenja ciprofloksacina u terapiju upalni simptomi su se povukli, ali za potpuno zaceljivanje nekroze kože poglavine bilo je potrebno više od mesec dana, sa rezidualnom ožiljnom alopecijom. **Zaključak.** Mada je TIBOLA retka infekcija, mora se prepoznati kao epidemiološki rizik u slučaju uboda krpelja.

Ključne reči:
bolesti, ubodom krpelja izazvane; dermacentor; rickettsia; dijagnoza; lečenje, ishod; srbija.

Introduction

Since initial descriptions of cases with scalp necrosis and lymphadenopathy after tick bite as a clinical syndrome caused by *Ricckettsia slovaca* in 1997, the desease gained an emerging infectious disease status in Europe, and reports were observed worldwide [1,2]. Terminology of this clinical syndrome is still not unified; most cases are referred to as tick borne lymphadenopathy (TIBOLA), *dermacentor*-borne necrosis erythema and lymphadenopathy (DEBONEL), or scalp eschar associated with

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neck lymphadenopathy after a tick bite (SENLAT). Clinical picture characteristics are: several days after a tick bite enlargement of retroauricular or cervical lymph nodes on the scalp occurs, with skin inflammation and formation of scalp necrosis (inoculation eschar) at the spot of the tick bite. In most cases, the course is benign and infection is limited.

Two patients infected by tick bites in Vojvodina region are presented, being the first cases of this clinical entity recorded in Serbia.

Case report

Case one

A fifty-eight-years-old female patient, with unremarkable previous medical history had tick bite in October 2014, in Bačka Palanka region. A tick attached on the scalp (left crown region) was noticed the next day while combing, and removed in primary care. A week later, the patient experienced left-sided headache, and the following day, she noticed enlarged lymph node at the left mandible angle. Doxicycline 200 mg *per os* daily started on the day 10 after the bite, but without improvement neither in pain nor lymph node enlargement and on the day 12, the patient noted a crust at the site of the tick bite. On the day 14, during dermatological examination, the patient presented with 2 cm scalp eschar, palpable cervical lymph node near left mandible angle (Figure 1). Due to presumed lack of response to doxicycline, ciprofloxacin 1g *per os* daily was added with topical antiseptic gel (octenidine hydrochloride), and a headache subsided in 3 days. Dual antibiotic treatment continued, doxicycline from 10th–30th day from the tick bite and ciprofloxacin from 14th–24th day. The demarcated scalp eschar was removed on the day 30 after the bite as well as residual erosion covered with hydrocolloid dressing, but on the day 38 eschar of smaller size reappeared. The patient did not appear for further follow-up and no data were available about the time needed for the complete healing of a skin lesion and size of residual alopecia.

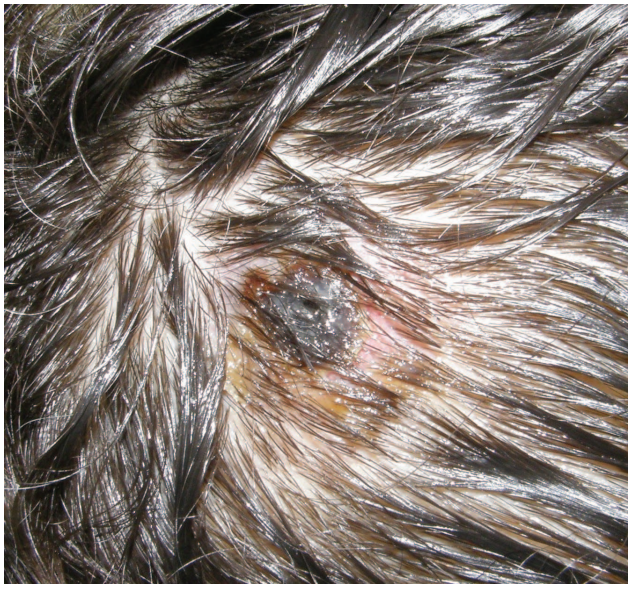

Fig. 1 – Scalp eschar of patient 1.

Case two

A fourty-five-year-old female patient, with unremarkable previous medical history. A tick bite in May 2016 in Vrbas region had a tick was removed from the central-frontal scalp region one day later On the day 2, the patient noticed enlargement of left retroauricular lymph node and started treatment with doxicycline 200 mg *per os* daily (Figure 2). On the day 9 after a tick bite, swelling of forehead appeared, which spread to lower eyelids on the day 10, and due to suspect doxycycline angioedema, the patient was referred to a dermatologist. On the day 10, the spot of a tick bite was inflamed and oozing (Figure 3); doxicycline was discontinued because of presumed inefficacy, and a combination of amoxicillin 2 g and ciprofloxacin 1g *per os* daily continued to be taken for the following 2 weeks. Inoculation eschar was evident 13 days after the bite, determining clinical diagnosis of TIBOLA (Figure 4). Demarcation of eschar was slow during the following month, with residual atrophic alopecia 1 cm in diameter.

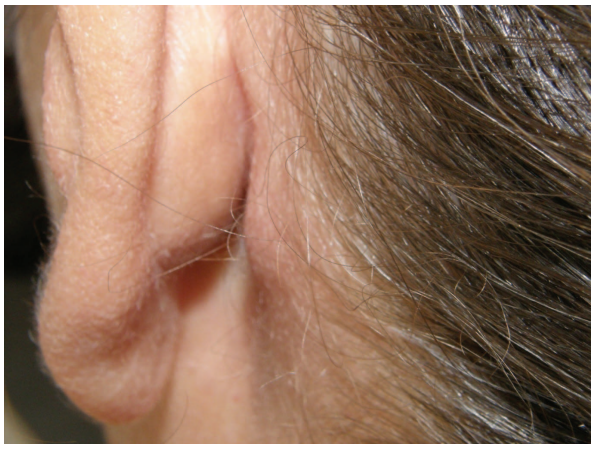

Fig. 2 – Enlarged retroauricular lymph node of patient 2.

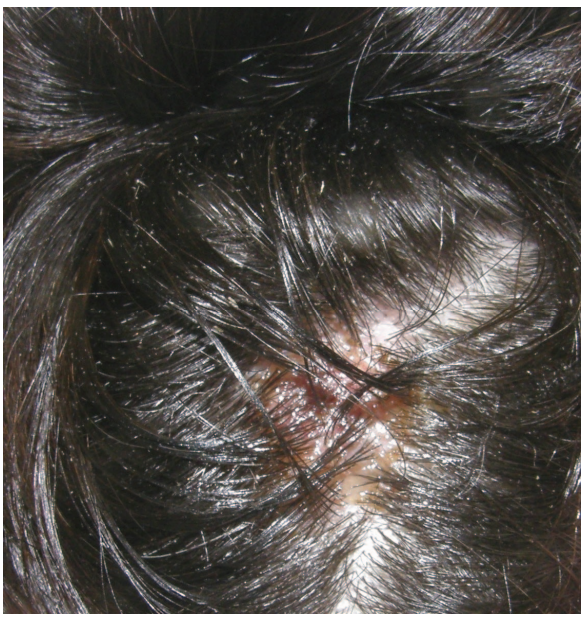

Fig. 3 – Tick bite spot inflammation, 3 days before the eschar in patient 2.

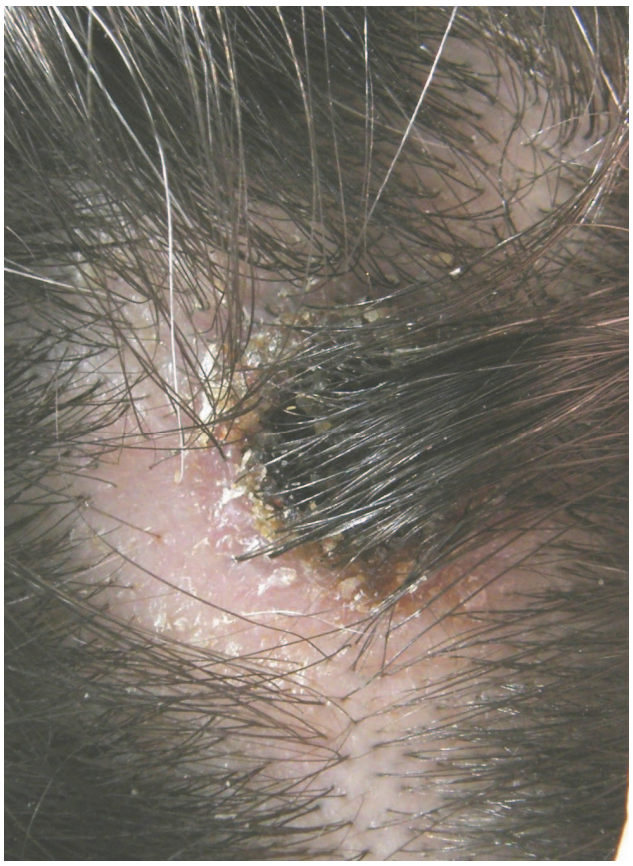

Fig. 4 – Eschar during demarcation phase in patient 2.

In both patients basic laboratory analyses (erythrocyte sedimentation rate – ESR, blood cell count, C reactive protein, fibrinogen level, liver enzymes) were normal, aerobe and anaerobe bacteriological cultures of eschar swab were negative, and *Borrelia burgdorferi* ELISA serology, as well. Serological analyses for *Rickettsia* species (ELISA and Weil Felix test), cultivation of *Rickettsia* and molecular detection of *Rickettsial* DNA were not available. Both patients did not approve skin biopsy and could not report any activity in nature that had preceded tick bite.

Discussion

Acronym TIBOLA (DEBONEL, SENLAT) comprises clinical diagnosis of tick-borne symptoms of cervical or occipital lymphadenopathy with inoculation eschar at the site of a tick bite on the scalp. During 2 decades since the first description, it proved to be associated with several infectious agents, most frequently *Ricckettsia slovaca*, or less often other spotted fever group *Ricckettsiae* (*Rickettsia raoulti* and *Rickettsia rioja*)[3]. Other bacteria were rarely involved (*Fransciscella tularensis, Bartonella henselae, Coxiella burneti*)[4,5]. *Dermacentor* ticks (*Dermacentor marginatus* and *Dermacentor reticulatus*) are vectors specific for the transmission of infectious agents of TIBOLA which are also a possible natural reservoir host for *Ricckettsia slovaca*. *Dermacentor* ticks differ from *Ixodes* species in larger unfed adult body size (> 5 mm vs. 2–4 mm of *Ixodes*), better tolerance to dry and cold environment, longer activity period during colder months, longer feeding time (*Dermacentor* spend attached couple of days vs. 7–9 hours of *Ixodes*) and natural habitat (*Dermacentor* is more frequent in steppe meadows, while *Ixodes* prefer more humid environment in higher vegetation, forests and bush). *Dermacentor* ticks frequently carry *Rickettsia* species of spotted fever group, sometimes multiple *Ricketsia* strains, or *Coxiella burnetti, Franciscella tularensis*, and are not optimal vectors for *Borrelia burgdorferi* transmission[6].

Typical TIBOLA patients are women and children with *Dermacentor* tick bite on the scalp, what is hypothesized to be a consequence of parasitizing habit of adult *Dermacentor* ticks, for hiding in a shelter of long hair-bearing areas (tail and mane) while feeding on animal hosts. Recent study from Hungary demonstrated that a contact with horses is independent risk factor for TIBOLA infection. Possible differences between contact with horses and other large domestic animals were not specified (no patient marked contact with cattle, goats, sheep)[7]. Although outnumbered in nature by *Ixodes* species, spatial distribution of *Dermacentor* ticks has been increasing in recent decades, together with more frequent human and animal infection with *Dermacentor*-specific pathogens[8,9].

Diagnostic hallmarks of TIBOLA are inoculation eschar on scalp and cervical lymphadenopathy, sufficient for the clinical diagnosis. For the precise identification of infectious agent serological tests (ELISA) are frequently not sensitive enough, due to low titer in localized infection and cross reactivity within spotted fever group *Rickettsia*. Weil-Felix agglutination test with OX19 and OX2 antigens, as non expensive and widely used screening diagnostic for scrub typhus in the Middle and Far East countries, can be expected to be positive for TIBOLA, but with even lower sensitivity and specificity[10]. Cultivation of *Rickettsia* is difficult and not routinely done in most microbiological laboratories.

Molecular diagnosis (polymerase chain reaction – PCR) is the most specific method, and can be performed on patient samples (serum, skin biopsy, eschar swab, lymph node aspirate) or tick tissue[11,12]. The course of the disease is usually benign. Incubation period from the tick bite to the appearance of first symptoms is 4–7 days. In the acute phase, apart from inoculation eschar and lymphadenopathy, patients can experience headache, mild infection signs (malaise, moderate fever) or localized inflammatory symptoms (facial oedema, pain of skin or lymph nodes).

It is of diagnostic importance that there is a lack of clinical signs of other Rickettsial diseases, i.e., Mediterranean spotted fever [eschars on other extracranial body sites, multiple eschars, maculopapular centrifugal exanthema (hands and feet), purpura, high fever]. Healing of eschar takes one to two months, and in up to one third of the patients residual cicatricial alopecia is permanent.

In both patients in this report, clinical symptoms were developing during the doxicycline therapy, which is recommended as the first line treatment for TIBOLA, and other *Ricckettsioses*. The role of antibiotics in TIBOLA were previously investigated and it can be concluded that early treatment with doxicycline or azithromycin can shorten the course of the disease[13]. Although the infective cause in both cases in this report could not have been precisely characteri-

zed, it can be hypothesized that presumed inefficacy of doxicycline on the appearance of eschar is the consequence of Rickettsial tropism for endothelial cells, causing localized increase of inflammatory and procoagulant factors mediating tissue injury.

Also, the question of post-exposure antibiotic prophylaxis in TIBOLA has not been concluded in literature, but our cases suggest that if not administered immediately after tick bite, doxicycline cannot prevent eschar appearance.

Presented cases are the first ones described as acquired at the territory of Serbia. It is likely that the TIBOLA (DEBONEL, SENLAT) in Serbia is more unrecognized and underreported than absent, because in neighboring countries disease is not considered to be rare. In Hungary, a country of origin of first TIBOLA cases, patient data are followed in registry of medical institution specialized for the treatment and surveillance of tick borne diseases.

This approach enables more active and precise actions, especially in the light of that since the description of *Borrelia burgdorferi* in 1982 as the causal agent of Lyme disease, eleven more tick-transmissible pathogens were defined in Europe [14]. Recent study from Bosnia and Herzegovina revealed range of 6%–66% positivity to *Dermacentor* ticks (*marginatus* and *reticularis*) for *Rickettsia slovaca* and *Riccketsia raoulti* [15].

Conclusion

Although TIBOLA is rare infecton, it is necessary to include it in a spectrum of epidemiologic risks in case of tick bite in Serbia. It is of public importance in Serbia to educate patients for self- help in case tick bite occurs (not to postpone tick removal until visiting a physician for a proper mechanical tick removal technique without the use of chemicals), with special emphasis on preventive measures for frequent skin and scalp check-ups during outdoor activities in regions with abundant tick population.

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Received on December 23, 2016.
Revised on February 06, 2017.
Accepted on February 07, 2017.
Online First March, 2017.

CASE REPORT

UDC: 616.314-089.23:616.716-007-089
https://doi.org/10.2298/VSP161227044C

Multidisciplinary treatment of complex skeletal class III malocclusion

Multidisciplinarno lečenje kompleksne skeletne malokluzije III klase

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Abstract

Introduction. Skeletal malocclusions, especially those with a prominent vertical component, always present a challenge for the interdisciplinary approach to their treatment planning. The aim of this report is to present a patient with a complex skeletal deformity in all three directions (vertical, sagittal and transverse). **Case report.** A twenty-four year old female patient with a skeletal Class III malocclusion, open bite and laterognathia, was firstly treated by orthodontic fixed appliances, whereas the dental decompensation of dentoalveolar structures was carried out and adjusted to their bone structures, thus enabling an adequate and sufficient reposition of the jaw. A surgical correction included bi-maxillary osteotomy due to pronounced vertical cephalometric parameters, necessitating a posterior maxillary intrusion and mandibular repositioning. In that manner, the relapse was prevented and a long-term stable result obtained. In the retention period, the patient wore removable bi-maxillary retention devices. **Conclusion.** The combined orthodontic-surgical treatment provided the Class I occlusion with aesthetic and functionally satisfactory results which were envisioned by the treatment plan.

Key words:
malocclusion, angle class III; open bite; orthognathic surgery; orthodontics, corrective; treatment outcome.

Apstrakt

Uvod. Skeletne ortodontske anomalije, pogotovu one sa naglašenom vertikalnom komponentom, uvek predstavljaju izazov za interdisciplinarni pristup u planiranju njihovog lečenja. Cilj ovog rada je da se prikaže pacijentkinja sa teškom skeletnom anomalijom u sva tri pravca (vertikalnom, sagitalnom i transverzalnom). **Prikaz slučaja.** Pacijentkinja stara 24 godine sa malokluzijom III klase, otvorenim zagrižajem i laterognatijom, lečena je najpre ortodontskim fiksnim aparatima, pri čemu je izvršena dekompenzacija dentoalveolarnih struktura, čime su one postale usaglašene sa svojim koštanim bazama. Tako je omogućena adekvatna i dovoljna repozicija vilica. Hirurška korekcija je podrazumevala bimaksilarnu osteotomiju, jer su kod ove pacijentkinje postojali naglašeni vertikalni kefalometrijski parametri, te je bila neophodna intruzija posteriorne maksile, kao i repozicija mandibule. Time je sprečen recidiv i dobijen dugogodišnji stabilni rezultat. U retencionom periodu pacijentkinja je nosila bimaksilarne mobilne retencione aparate. **Zaključak.** Kombinovanim ortodontsko-hirurškim lečenjem obezbeđena je okluzija I klase sa estetski i funkcionalno zadovoljavajućim rezultatima predviđenim planom lečenja.

Ključne reči:
malokluzija, klase III; zagrižaj, otvoreni; hirurgija, ortognatska, procedure; ortodoncija, korektivna; lečenje, ishod.

Introduction

Skeletal cranio-dental facial anomalies, especially those with a prominent vertical component, are most often of the heritable etiology and always represent a challenge for the interdisciplinary approach to their treatment planning. During the growth of these malformations and their development, the disharmony of the size of the jaws becomes more and more pronounced and reflects to the soft facial tissues, creating facial asymmetry, which additionally complicate a clinical picture and the treatment of these patients. A combined surgical-orthodontic treatment is preferable, and this is the only way to obtain a stable functional occlusion, normal skeletal inter-maxillary relation and the aesthetically harmonious tissue profile.

These patients require an adequate differential diagnosis, a detailed clinical examination, careful analysis and a

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treatment plan provided by professionals of different specialties as well as the defined indications and contraindications for the different treatment modalities in order to determine the preferable treatment [1, 2].

The most frequent reason for coming for advice is that of aesthetic nature, but at the same time it is a difficult mastication function. Temporomandibular dysfunctions are also frequently represented in the clinical picture in these patients, especially in those with a facial asymmetry and psychological and social handicaps of different kinds [3].

The purpose of this report is to present the results of a multidisciplinary treatment of a patients with cranio-dental facial deformities in all three direction-sagittal (Class III), vertical (open bite) and transverse (cross bite and facial asymmetry).

Case report

A twenty-four-years-old female patient with a facial asymmetry and the increased lower third of the face had a facial asymmetry of functional type with a slightly concave profile and the anterior lower third of the face. Clinical findings confirmed dental Class III with a narrow maxilla, open bite, crowding in both sets of teeth, increased vertical dimensions of the face and lateral cross-bite on the right side. The cephalometric analysis of angular variables (ANB, SNA, SNB) revealed skeletal Class III (ANB = -1), maxillary retrognatism (SNA = 79) (SNB = 80), increased vertical facial parameters, suggesting divergent Class III (B = 42, 45 = SNMP, Bjork = 407). In addition, the presence of dentoalveolar compensatory mechanism was found in terms of the upper protrusion and retrusion of lower incisors (Figure 1a and 1b).

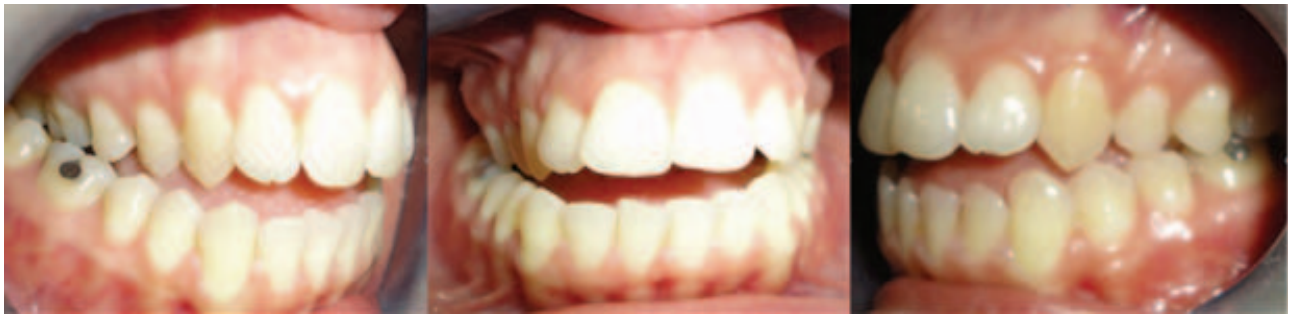

Fig. 1a – Pre-treatment intraoral photographs

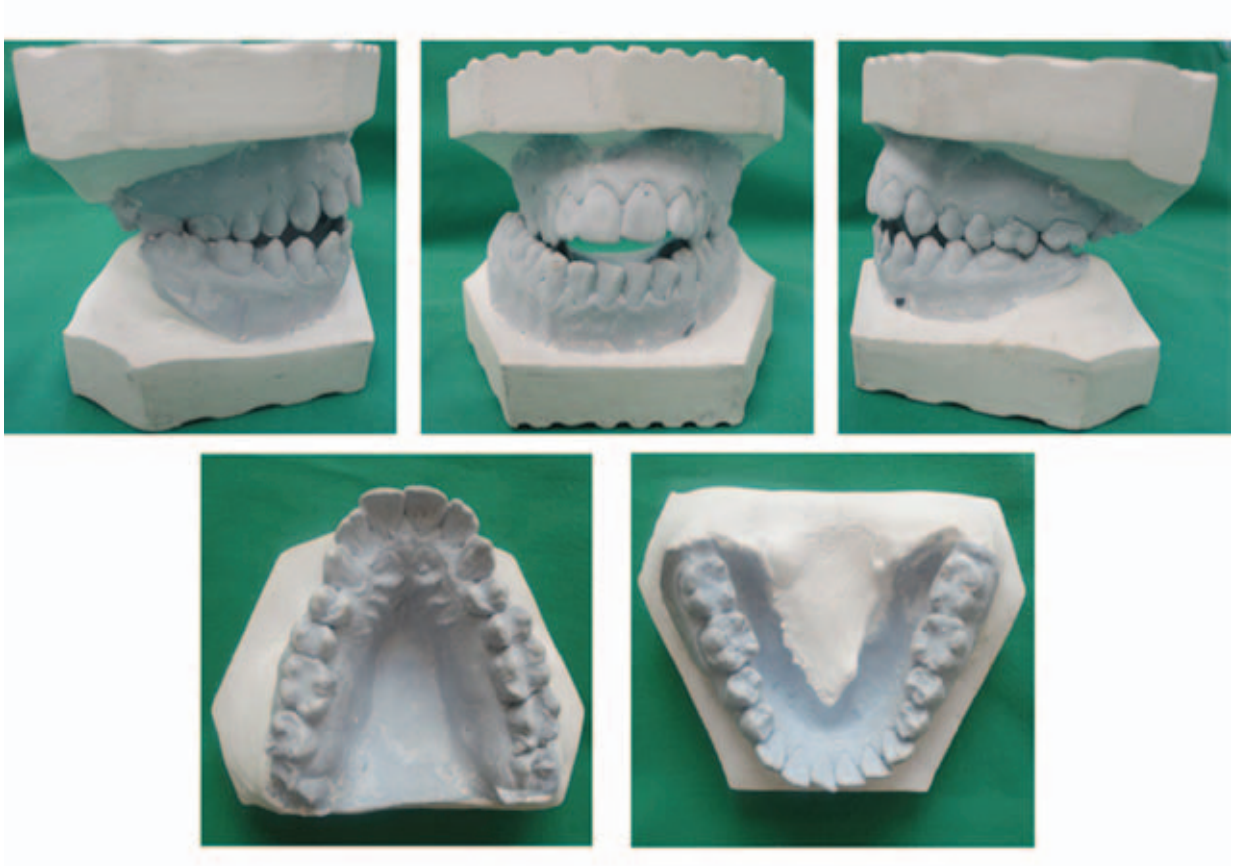

Fig. 1b – Pre-treatment dental models.

The global objective of orthodontic pre-surgical treatment was to correct inter-maxillary relationships in all three directions (sagittal, vertical and transversal), and later, to close surgically the bite and reduce the lower third of the face, remove the facial asymmetry and regulate the sagittal inter-maxillary relationships into the position of Class I. Prior to this, it was necessary to expand the upper jaw and thereby remove the interference that created the cross-bite and the facial asymmetry, solve the problem of dental anxiety and thus provide conditions for surgical correction.

In pre-surgical orthodontic phase of the treatment, decompensation of dentoalveolar arches was carried out, which means that the upper jaw was expanded, the upper incisors were retruded, whereas the lower incisors were protruded (rating of perceived exertion – RPE + Roth .0.022"). By expansion of the upper jaw and protrusion of the lower incisors, the space in both sets of teeth was obtained in order to resolve a dental anxiety. In this manner, we provided conditions for the adequate and sufficient surgical reposition of the jaws.

During the orthodontic treatment phase, the control models were occasionally made in order to check the possibilities of a surgical repositioning and when that possibility was established, the surgical arches 0.019 × 0.025 stainless steel (SS) were placed with the surgical hooks, which were used for a tighter inter-maxillary fixation. Based on cephalometric assessments, two splints were made to position the jaws during surgery.

A surgical correction included the ante-positioning of the maxilla, intrusion of the maxillary posterior segments, the counterclockwise rotation and posterior placement of the mandible, which required a bi-maxillary surgical procedure (Le Fort I, bilateral sagittal split osteotomy – BSSO).

The postsurgical orthodontic treatment enabled us to delicately fix the problem by inter-maxillary rubber bands, causing a state of the maximum intercuspation as one of the most important factors of the reliability of the results. In this way, the relapse was prevented and the long-term reliable results were obtained. In the retention period, the patient wore the bi-maxillary mobile retention appliances.

Functionally and aesthetically satisfactory occlusion and normal horizontal and vertical overbite were achieved, the middle point of dental arches was corrected and the skeletal Class I ratio was obtained (Figure 2). By removing the facial asymmetry as well as vertical and sagittal skeletal disharmony, the facial aesthetics was significantly improved whereas the normal function was enabled. The most cephalometric parameters measured before and after the treatment showed differences and were closer to the reference values (Figure 3a, 3b and 3c, and Table 1).

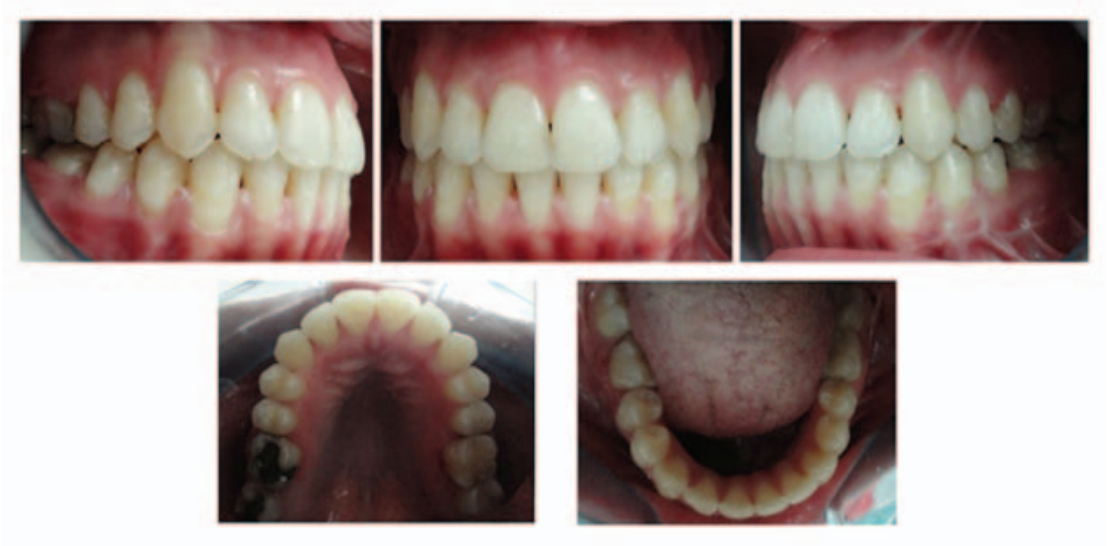

Fig. 2 – Post-treatment intraoral potographs.

Table 1

Comparison of pre- and post-treatment cephalometric values

Measurement	Mean	Pre-treatment	Post-treatment
SNA	82	79	77
SNB	80	80	76
ANB	2	-1	1
B	25–30	42	35
Bjork	396	407	405
MP-NS	32	45	44
Upper incisor to PP	75	115	113
Lower incisor to MP	90	81	81
U6- posterior maxillary hypoplasia		26	23
IOTN-Index Orthodontic treatment need			
Dental Health Component		5	1
Aesthetic Component		9	1

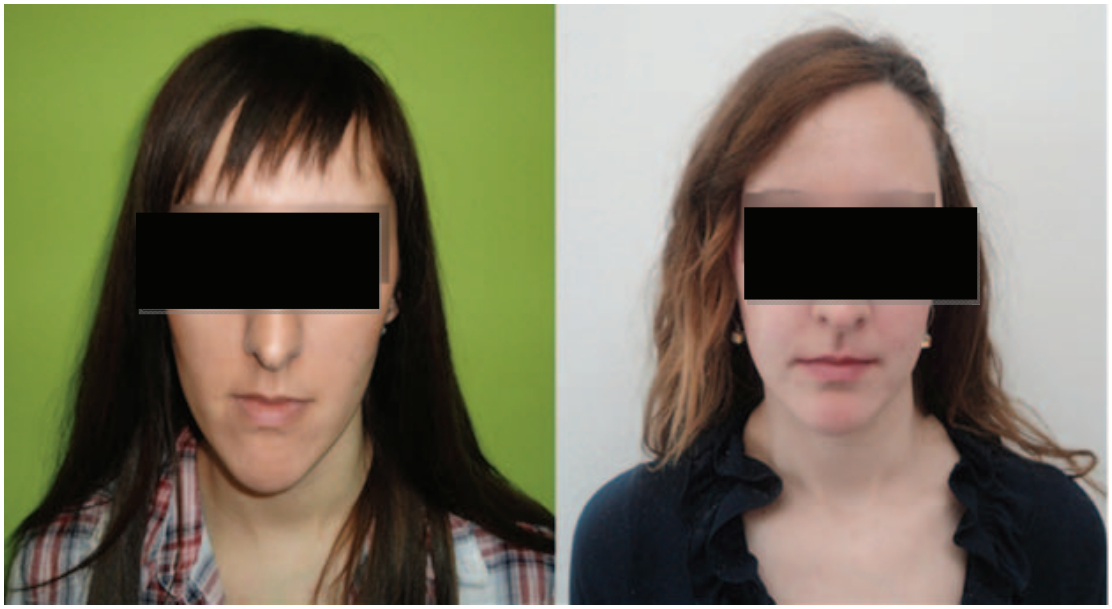

Fig. 3a – Pre-treatment and post-treatment *en face* photographs show good facial and esthetic balance.

Fig. 3b – Pre-treatment and post-treatment profile photographs show good facial and esthetic balance.

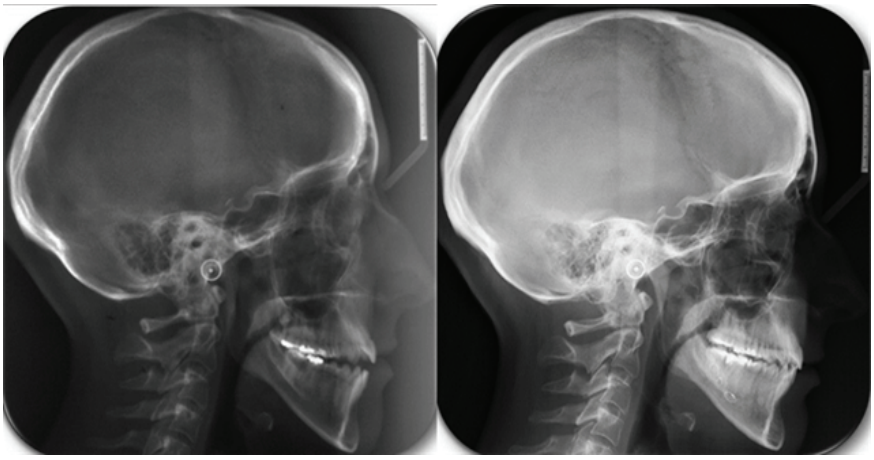

Fig. 3c – Pre-treatment and post-treatment lateral cephalometric radiographs.

Skeletal Class III malocclusion with the open bite and the divergent growth pattern is one of the most difficult skeletal anomalies to treat. The best results are achieved by the usage of bi-maxillary surgical procedure and repositioning of both jaws, which results in reduction of both, sagittal and vertical discrepancies [4, 5]. The persistence of the results is achieved by reduction of the posterior maxillary hyperplasia which is always present in patients with a divergent facial type. Therefore, the intrusion of the maxillary posterior segments enables the mandible to be rotated around its axis when the bite is surgically closed and thus the lower third of the face is reduced [5, 6], as it was the case in the patient presented in this report.

It is necessary to establish the exact localization of the problem in patients with skeletal Class III and a facial asymmetry. The etiology of facial asymmetry is often unclear and may be multifactorial. Therefore, it is very important to make an accurate assessment at the beginning of the treatment. It can be congenital, skeletal or developed later due to some local etiological factors or simply dental [7].

Class III malocclusions which are not part of the syndrome and the cleft facial asymmetry are most often the result of narrowness, usually a hypoplastic maxilla or the simple mandible deviation due to its higher growth potential [8]. As dental compensation is present in Class III patients in the form of protrusion of the upper and retrusion of the lower incisors in the sagittal direction, there is also the dental compensation in the form of molar inclination, the shape of dental arches and the lateral overjet in the transverse direction in patients with the facial asymmetry and deviation of the mandible [7, 9–11].

In the patient presented in this report, the facial asymmetry was caused by the narrowness of the upper jaw, whereas the abovementioned transversal dental compensation was also present and had to be corrected before surgical repositioning in order to obtain a correct and stable occlusion. The results shown in Figure 2 were made two years after the completion of the entire orthodontic-surgical treatment, which supports the reliability of the results.

Conclusion

The orthodontic surgical therapy is required in patients with the complex cranio-dental facial deformities in all three directions. The surgical phase of the treatment should be planned after the completion of growth. Thus, the postsurgical subsequent growth would not compromise the obtained results because these deformities are of developmental nature. The success and particularly the reliability of the results depend on the correct diagnosis, clinical experience and the treatment planning.

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Received on December 27, 2016.
Revised on March 02, 2017.
Accepted on March 03, 2017.
Online First March, 2017.

ISTORIJA MEDICINE

UDC: 355/359:616-036,22]::61(091)
https://doi.org/10.2298/VSP180801151C

„Srpska, prva faza" suzbijanja epidemija 1914. i 1915. godine

"Serbian, first phase" of the suppression of epidemics in 1914 and 1915

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Ključne reči:
epidemije; istorija medicine, XX vek; prvi svetski rat; tifus, pegavi; dezinfekcija; srbija.

Key words:
disease outbreaks; history, 20th century; world war I; typhus epidemic louse-borne; disinfection; serbia.

„I kroz nedaće se mora proći na putu do uspeha"

Uvod

Važno je za dalja razmatranja prepoznati dve faze tokom trajanja epidemije tifusa u prvim godinama Velikog rata u Srbiji: prvu, pre dolaska Britanske vojnosanitetske misije koju je predvodio pukovnik dr Vilijam Hanter, i drugu, nakon njenog dolaska 4. marta 1915. godine. Takođe, važno je utvrditi da li je prva, tzv. „srpska faza", dala značajan doprinos u suzbijanju epidemije i koliki je taj doprinos bio za svetsku medicinu.

Na osnovu Vukšićeve analize iz 1989. godine, kod nas je došlo do prekretnice u istorijsko-medicinskoj proceni napora Hanterove misije i angažovanja Sanitetskog odeljenja Vrhovne komande srpske vojske. Ocenjeno je da je srpskoj vojsci 1915. godine bila vraćena borbena gotovost [1]. Odlika druge faze bila je saradnja između pukovnika dr Lazara Genčića (načelnika saniteta Vrhovne komande srpske vojske u periodu 1912-1916) i dr Hantera (komandanta Misije kraljevskog vojnosanitetskog korpusa Velike Britanije) i oni su od 4. marta 1915. radili združeno. Na kraju, preduzete mere su rezultirale uspehom, uz poseban doprinos dr Hantera.

U Srbiji 1915. godine, kod suzbijanja epidemija pegavca i rekurensa (povratnog tifusa, povratnice), bilo je nekoliko nivoa hitnosti koje je trebalo rešavati. Vojni ciljevi imali su prioritet i bilo je potrebno da se odmah reše. Oni su se odnosili na pitanja: da li je srpskoj vojsci vraćena borbena gotovost i da li su moguće ratne operacije u uslovima postojanja pegavca na području borbenih dejstava [2, 3]. Naučni ciljevi su bili drugog reda hitnosti (npr. dokazivanje hipoteze o prenošenju pegavog tifusa). Treći red hitnosti bile su istorijske i medicinske procene (u čemu se sastoji doprinos i čiji je) koje su mogle da se sprovedu i u kasnijem periodu.

U rešavanju problema suzbijanja epidemija, važno je uočiti uzajamnu povezanost postupaka u postizanje zadatih ciljeva. Da li je suzbijanje epidemije tifusa 1914–1915. godine [1, 2] dovelo je do efikasnog taktičkog sredstva i da li je to sredstvo moglo biti upotrebljeno u eventualnoj kontraofanzivi?

Problemi u suzbijanju epidemije tifusa 1915. godine

Godine 1915. bilo je potrebno ispuniti nekoliko uslova da bi Srpski sanitet bio uspešan. Prvi je bio postavljanje dijagnoze pegavog tifusa. Tokom epidemije, mogla se postaviti samo klinička dijagnoza na osnovu opservacije bolesnika, dok je sigurnija dijagnoza bila moguća jedino autopsijom umrlih od pegavog tifusa. Drugi je bio da se pravilnim izborom strategije izabere efikasno taktičko sredstva za suzbijanje epidemije [2, 3].

Prva „srpska" faza

Postavlja se pitanje, da li su srpski lekari tada znali da su vaši od značaja za pojavu pegavog tifusa? [1]

[1] Ocena istoričara medicine (1965) je da Batut u popularnoj „knjižici" O pegavom tifusú, prvi put kod nas objavljuje da su Šarl Nikol i Konsej, francuski lekari u Tunisu, utvrdili 1909. godine da je prenosilac pegavca bela vaš sa rublja [Stanojević V. Epidemija pegavog tifusa u našoj vojsci (njene žrtve, iskustva i pouke). U: Zbornik neobjavljenih radova saopštenih u sekciji, knj. 2. Beograd: Srpsko lekarsko društvo; 1965. s. 63-91.:77]. Međutim, isto tako je poznato da je Kujačić objavio seriju članaka od maja do septembra 1913. o Nikolovim ogledima. Potom izdaje knjigu 31.01.1914. godine. [Čukić G. Vek od objavljivanja priloga Jovana Kujačića. U: Zbornik radova sa V Naučno-stručnog skupa istorije medicine, farmacije, veterine i narodna zdravstvena kultura. Knj. 4. Zaječar; 2013. s. 111-24. s. 115]. Načelnik saniteta dr Genčić je znao da vaš prenosi pegavac bar od 15.01.1915. [Referat pov. Br. 8257 od 15.01.1915 dr L. Genčića Načelniku štaba Vrhovne komande, VA, p3a,k101,f3,d14]

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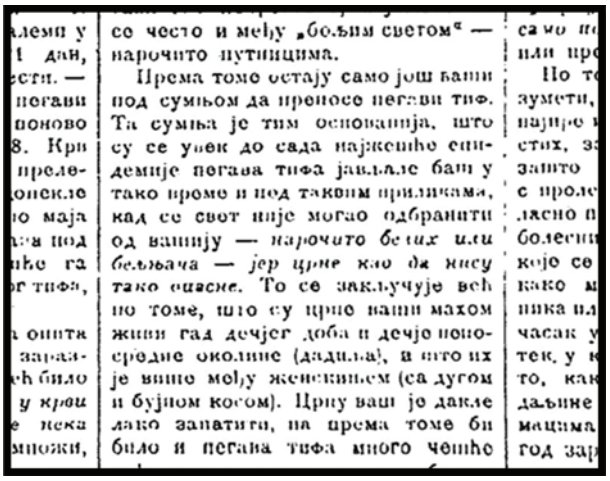
Prema Vukšićevim istraživanjima dr Milan Jovanović Batut je saznao od Konseja da pegavac prenose vaši i „objavio 29. januara 1915. Članak" [1]. Batut je preporučio nekoliko metoda depedikulacije [5, 6]. „Te metode su bile neadekvatne za katastrofalnu epidemiju pegavca (peglanje, zatrpavcanje odeće u zemlju, depedikulacija odeće u pećima za pečenje hleba, itd)" [1].

Tokom istraživanja događaja iz 1915. zaključeno je da je 29. januar 1915. važan datum za istoriju medicine u Srbiji jer je tada srpska javnost, navodno, prvi put obaveštena o Nikolovom istraživanju [1].

Dr Milan Jovanović-Batut je dobio dozvolu za štampanje knjige „Pegavi tif" pre štampanja novinskog članka. Ministarstvo prosvete i crkvenih poslova je 25. januara 1915. izdalo dozvolu za objavljivanje 8 000 primeraka knjige i naložilo da se odmah odštampa. Predata je u štampu 27. januara 1915. [6]. Batutovu knjigu, istog sadržaja, izdala su dva izdavača, u Nišu i na Cetinju [7].

Srpske novine objavile su obiman članak 28. januara 1915. godine (Slika 1) [8, 9].



со често и међу „бољим светом" — нарочито путницима.

Према томе остају само још ваши под сумњом да преносе пегави тиф. Та сумња је тим основанија, што су се увек до сада најжешће епидемије пегава тифа јављале баш у тако проме и под таквим приликама, кад се свет није могао одбранити од вашију — *нарочито бетих или бољьача — јер црне као да нису тако опасне.* То се закључује већ по томе, што су црне ваши махом живи гад дечјег доба и дечје поносредне околине (дадиља), а што их је више међу женскињем (са дугом и бујном косом). Црну ваш је дакле лако запатити, па према томе би било и пегава тифа много чешће

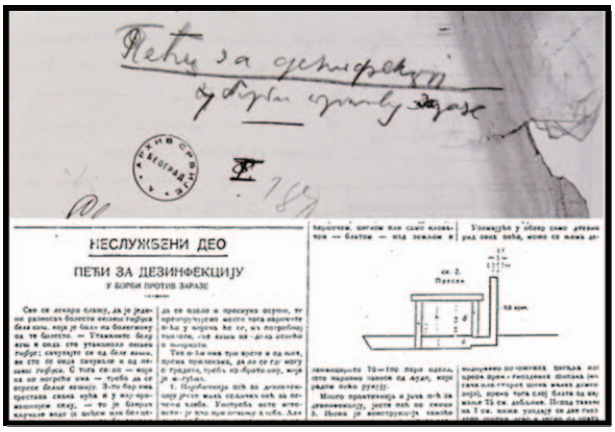
Sl. 1 – Šta prenosi epidemijski tifus? Prema Batutu: „ostaju samo još vaši pod sumnjom da prenose pegavi tif". „Pegavi tifus, kako se širi i suzbija", Srpske novine, 28. januar 1915, deo s. 3.

Batutov tekst o pegavom tifusu odnosi se na pravilan izbor strategije (da se uništavaju vaši) i na karakteristike dobro izabranih protivepidemijskih mera (treba ih primenjivati i kod bolesnih i kod zdravih osoba, među vojskom, građanstvom i zarobljenicima). Takođe, u tom tekstu navode se sredstva za uništavanje vaši, predlaže se korišćenje toplog, suvog vazduha (furune) i toplog, vlažnog vazduha (autoklav). Ove preventivne mere su se pokazale kao bitne protiv epidemijskog tifusa u Srbiji nakon završetka druge faze.

Barem od 15. januara Genčić je znao da su vaši prenosioci nepoznatog uzročnika pegavca, zbog čega je depedikulaciji dao strateški značaj. On je definisao taktiku upotrebom autoklava. Tim povodom, on se obraća Sanitetskom odeljenju Ministarstva vojske zahtevajući izveštaj da li su pristigli iz Švajcarske naručenih 20 autoklava smatrajući ovaj broj nedovoljnim: „Za dezinfekciju odela vojske treba da ima vrlo mnogo pokretnih dezinfekcionih aparata – na svaki puk bar po jedan (...). Tih aparata treba više poručiti, i 100 nije mnogo" [11]. M. Grba u svom predgovoru prevoda rada dr Hantera navodi da se dr Genčić obratio 27. januara vojvodi Putniku, načelniku štaba Vrhovne komande dostavivši predlog najhitnijih potreba za vojsku: „15 bakteriologa sa pokretnim laboratorijama i laborantima, 15 epidemiologa, 1 000 bolničara i bolničarki specijalizovanih za zarazne bolesti i 200 parnih dezinfektora za operativnu vojsku" [2].

Dana 10. februara 1915. godine dr Vojislav Subbotić je predložio na sastanku Srpskog lekarskog društva u Nišu da se za iskorenjivanje vaši koristi ukopana komora koja je radila po principu suvog, toplog vazduha. Komora je imala karakteristike prototipa (zidovi od cigala zadržavali su toplotu, beli papir služio je za merenje temperature, itd) [9, 11]. O tome je pisao „Ratni dnevnik" od 12. februara 1915. godine u prilogu „O predohrani od pegavca" u kome poziva „tehničare" da daju nacrte komora.

Mašinski inženjer Velisav Vulović [2], predsednik Državnog odbora za suzbijanje zaraznih bolesti, verovao je u naučnu medicinu i to nije dovodio u pitanje. Za desetak dana nakon objavljivanja Subbotićeve prezentacije, napravio je nacrte komora sa dnevnim kapacitetom obrade od 300 do 600 odela. Potom je objavio planove i ponudio ih kao sredstvo za depedikulaciju/razvašljivanje. Preporučeno je masovno korišćenje komora stanovništvu i bolnicama, za vojnike, civile i zarobljenike. Ovo je bilo veoma značajno jer je ovaj Odbor bio veoma uvažen u Srbiji, a Vulovićeve preporuke objavljene su u zvaničnim državnim novinama („Srpske novine") koje su imale uticaj na javnost (Slika 2) [9].



НЕСЛУЖБЕНИ ДЕО

ПЕЋИ ЗА ДЕЗИНФЕКЦИЈУ

У БОРБИ ПРОТИВ ЗАРАЗЕ

Sl. 2 – Naslov članka „Peći za dezinfekciju" u „Srpskim novinama", 24. februar 1915. (rukopis članka deponovan u Arhivu Srbije).

U Nišu je ubrzo izgrađena komora. Počela je da radi početkom marta 1915. godine [11]. Komora u Nišu imala je dnevni kapacitet obrade 4-5 hiljada odela. „Dezinfekciona centrala" je imala komoru i kupatilo koje je u jednom danu

[2] Velisav Vulović (1865–1931) je preteča sanitarnih inženjera u Srbiji koji je usavršio prototip dr Subbotića, izradom nacrta četiri tipa dezinfekcionih komora na suvi, topli vazduh sa dnevnim kapacitetom od 300 do 600 obrađenih odela. One dobijaju odliku protivepidemijskog sredstva namenjenog zdravima i bolesnima: bolnicama, vojsci i građanstvu.

moglo da zbrine više od hiljadu korisnika. Dok su se korisnici kupali, odeća je oslobađana od parazita. Takvim aktivnostima, dezinsekcija je postala korisna protivepidemijska mera. „Dezinfekcionu centralu" su koristili: bolesnici, vojska, građani i zatvorenici, kojih je bio veliki broj u Nišu [11].

Državni odbor za suzbijanje zaraznih bolesti se pridržavao Batutovih principa. Ovo nije bilo javno rečeno, međutim, to se vidi iz sadržaja tekstova u novinama. Odbor je imao glavne aktivnosti nakon štampanja Batutovog članka 28. januara 1915. Oni koji nisu verovali u Nikolovu teoriju da vaši prenose pegavi tifus su grešili. Ove rezultate je trebalo prihvatiti ili ih, pak, proveriti ogledom.

Sličnost u fazama suzbijanja epidemije pegavog tifusa u Srbiji ogleda se u sledećem: cilj Vojnog saniteta Srbije, kao i Hanterove misije, bio je da se srpskoj vojsci povrati borbena spremnost koja je bila narušena pojavom epidemija. Ono što odgovara Programu u devet tačaka za suzbijanje epidemije pegavog tifusa se primenjivalo pojedinačno u Srbiji u prvoj fazi. Ali, to nije dovelo do smanjenja epidemija.

Pre dolaska Hanterove sanitetske misije, sanitet Srbije se odlučio za strategiju uništavanja vaši. Bila je namenjena za masovnu upotrebu u bolnicama, ali i za zdrave. Za ovu svrhu je puštena u rad komora na suvi, topli vazduh. Ovo sredstvo razvašljivanja je bilo efikasno u sprečavanju širenja epidemijskog tifusa doprinoseći tako „specijalnoj prevenciji". Radom tzv. „dezinfekcione centrale" početkom marta 1915. godine Srbija je dala značajan doprinos svetskoj medicini u suzbijanju pegavog tifusa [9, 11].

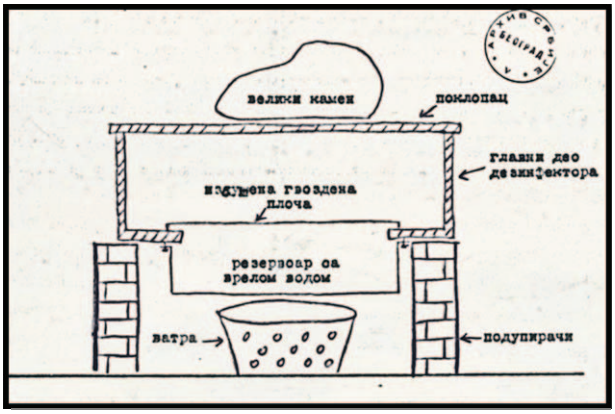
Druga faza

Hanterovim Programom su ozvaničene i unificirane preventivne mere [3], a on ih je, kao organizator, svojim aktivnostima podigao na viši organizacioni nivo: kumulativnim delovanjem, preciznim sprovođenjem itd. Preduzete mere su na kraju uspele da suzbiju epidemije pegavog tifusa i rekurensa. Tako je, zahvaljujući radu sanitetskih timova, srpskoj vojsci vraćena borbena gotovost. Dr Hanter je pronašao rešenje i za drugi vojni cilj – kako da se bori u uslovima kada postoji pegavi tifus na terenu. Posebno u ratu, odluke moraju biti brze, tačne i precizne, jer jedna od zaraćenih strana ima prednost ako raspolaže odgovarajućim saznanjima. Dr Hanter je već 1915. ocenjen da je uspešno obavio zadatak [3]. Ovo se realizovalo dobro odabranom strategijom i adekvatnim taktičkim sredstvima koja su se već pokazala efikasnim u toku suzbijanja epidemijskog tifusa [3]. Vojni sanitet Srbije učestvovao je u realizaciji suzbijanja epidemija tokom obe faze.

Hanterov doprinos je ubrzo našao primenu u završnoj fazi Velikog rata, u kontraofanzivi savezničke vojske 1918. godine, koja je mogla da dejstvuje na području na kome je moguća pojava karantinske bolesti pegavca zahvaljujući preventivnim merama i posedovanjem sredstava koja su bila proverena tokom prethodnog suzbijanja epidemija.

Da li je Hanterov rad bio nastavak prve, „srpske" faze? Ne, obe faze su bile potpuno nezavisne do dolaska Hanterove misije. Hronološki označena druga faza bila je značajnija, ali neki od njenih elemenata potiču iz prve faze. Npr. u drugoj fazi korišćene su komore na suvi, topli vazduh, o čemu evedoče Hiršfeld, Strong i Genčić [11]. Ni Hanterove procene nisu u potpunosti razmatrale „prvu, srpsku fazu". On zna za prototip komore dr Subbotića [2, 3], ali ne i za inovacije Vulovića.

Hanterova misija je prvih dana po dolasku u Srbiju napravila Program za suzbijanje epidemija i ponudila Ministarstvu vojnom prototip, improvizaciju autoklava. Predloženi Stamersov prototip bio je u obliku „drvenog sanduka" (Slika 3), premda se u tekstu predloga pominju burad, drvena i metalna [11].



Sl. 3 – Predloženi prototip sredstva za razvašljivanje je skica komore „drvenog sanduka", a u tekstu se ukazuje na komore od buradi, drvenih i metalnih [11].

Postojale su dve vrste „srpskih buradi": tip 1 - „Srpsko bure" napravljeno od metalnog bureta. Poreklo naziva objašnjava Protić: radi korišćenja u Srbiji, stranci angažovani u Srbiji, nazvali su ga „srpsko bure" [12]. Isto je smatrao i Hanter. To znači da se ovaj naziv odnosi na Stamersovo bure, improvizaciju od metalnih buradi koja su prepravljana u radionicama Vojnotehničkog zavoda (VTZ) u Kragujevcu; tip 2 – „Srpsko bure" napravljeno od drvenog bureta.

Komisija za suzbijanje izarazne bolesti [4] koju je 21. februara 1915. formirao dr Genčić dva puta je izdala brošuru pod nazivom „Kratak vodič za dezinfekciju", prvu 30. marta 1915. godine i, potom, još jedno uvećano izdanje 30. juna 1915. godine. Ponuđena skica improvizovanog autoklava napravljenog od drvenog bureta (Slika 4) nije nazvana „Srpsko bure". Ovo ime nije koristila Komisija i nije poteklo iz brošure. Zamena autoklava je nazvana „prilagođeni (improvizovani) uređaj"[13].

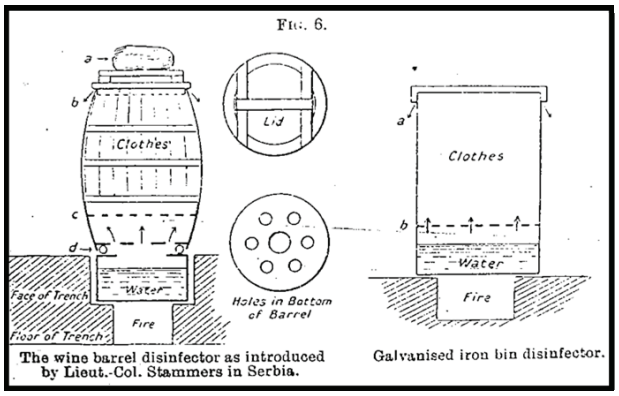
Izvan zvaničnih institucija koristilo se ime „srpsko bure", pa i kao narodno ime. Odjek stranog porekla bio je usvojen u narodu, među stanovnicima na selu i u gradovima, kao i među domaćim lekarima.

Oni koji su želeli prikazati sliku „(improvizovanog) uređaja" morali su napraviti sopstvenu verziju uređaja, koristeći sliku Stamersovog prototipa drvene kutije kao pola-

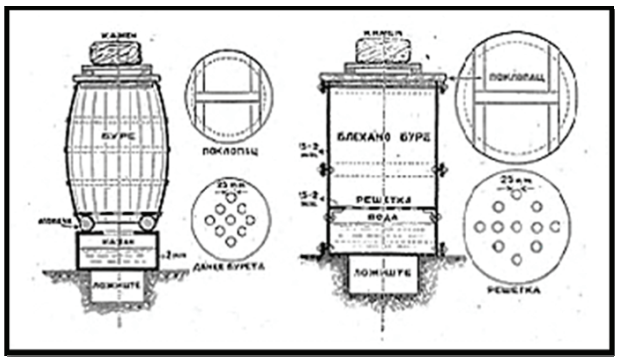
[3] http://history.amedd.army.mil/booksdocs/HistoryofUSArmyMSC/chapter3.html

[4] Osnivački dokument Komisije, VA p3a, k60, f6, d19/7

znu (Slika 3). Ona je bila prototip za prikaz skica svih vrsta improvizovanih autoklava napravljenih od metalnih i drvenih buradi (Slike 4 i 5) [13].



Sl. 4 – Skica improvizovanog autoklava (*The Lancet*, 1918) [14].



Sl. 5 – Skice improvizovanog autoklava iz brošure „Kratak vodič za dezinfekciju" [13]: Prototip sredstva, koji je na početku predložen kao „drvena kutija", modifikovan je posle 6. marta 1915. godine od strane Komisije za suzbijanje zaraznih bolesti, i u narodu je poznat kao „srpsko bure".

Stamersova skica improvizovanog autoklava (drvene kutije) predata je 6. marta. Napravljena je u VTZ od 9. do 11. marta [2, 3]. Nakon uvida u u skicu, Vulović, kao mašinski inženjer i poznavalac parnih kotlova, je tražio: „Naredite jednom mašinskom inženjeru da odmah snimi jednu od parnih perionica" [5]. Isto je uradio i sa Subbotićevim prototipom. To je bilo radi poboljšanja dnevnog kapaciteta i kvaliteta uređaja (tehničkih karakteristika, boljeg korišćenje pare itd.). „Parne perionice" su napuštene, pretpostavlja se iz kurtoazije. Razvoj prototipa kretao se u nekoliko pravaca: a) „Stamersovo bure "; b) „srpsko bure" (Komisija za suzbijanje zaraznih bolesti) (Slika 4), c) sanitarni voz (Hanter) [2, 3, 13, 14], i d) lokomobila [13].

„Stamersovo bure" je drugi predlog Stamersa od 14. marta: improvizacija autoklava napravljena od metalnog bureta [13]. Bilo bi opravdano nazvati ga po autoru zato što je Stamers doprineo i njegovom poboljšanju. On je dao značaj uskladištenim praznim buradima koja su bila prvobitno korišćena za druge namene, a inicirao je njihovu upotrebu za pravljenje improvizovanih autoklava. Njegov predlog je razmatran po drugi put, jer je uređaj već bio proveren kao „drveni sanduk" i bio prihvaćen. Prepravljanja buradi su vršene od 18. marta, dinamikom od 50 do 100 sedmično u radionicama u Kragujevcu i Nišu [2, 3] . „Stamersovo bure" distribuirano je prvenstveno vojnim jedinicama.

Drveno „srpsko bure" nastalo je na sličan način. Inicijalna skica Stamersa je propraćena komentarom da se drvena kutija može zameniti drvenim buretom. Sve ostalo što je bilo učinjeno tokom novog skiciranja improvizovanog autoklava bilo je rezultat aktivnosti saradnika Komisije tokom njihovog rada na brošuri. Bilo je potrebno uskladiti Stamersov prototip sa mogućnostima srpskih seljaka, koje je Komisija uzela u obzir (što nije moralo da bude poznato Stamersu), tako da su seljaci mogli da ih naprave od materijala koji su već imali u domaćinstvu. Konačna izrada prepuštena je seljacima jer se očekivala samoinicijativa (na primer, seljaci su imali iskustvo u pravljenju rakije gde „para ne sme pobeći") [6]. Drvena burad su bila prvenstveno namenjene srpskom selu. Na skici je bilo nepokretno ložište, a iznad kazana za zagrevanje vode je stavljano drveno bure (Slika 5).

Povećanje kapaciteta prepušteno je Hanterovoj misiji. Prema Hanterovim uputstvima napravljen je i sanitarni voz [2, 3, 14].

Uzroci epidemije

Koji je bio glavni uzrok uvećanju epidemije? Nemar (greška) nije bila od najvećeg značaja za uvećanje i posledice epidemije 1915. godine. Pre svega, svetska medicina je bila nemoćna kada je u pitanju način suzbijanja epidemije pegavog tifusa. U to vreme, bilo bi pogrešno neuspeh pripisati rukovodiocu dr Genčiću [15]. Dakle, tačnija procena bi bila da su epidemija i njene posledice „tragičan događaj" u ratu, gde su lekari, potaknuti patriotizmom, doprineli njenom saniranju i pri tome u velikoj meri bili i njene žrtve [15].

Razlozi za medicinski uspeh bili su nedogmatično shvatanje doktrinarnih stavova, kao i visprenost koordinatora. To potvrđuje valjanost odluka dr Genčića i ispravnost odluke onih koji su ga postavili da bude načelnik saniteta.

Bilo bi očekivano da je onaj ko je prvi izvršio procenu rada na suzbijanju epidemije, odmah nakon 1915. godine, napravio grešku. Saveznička komanda ocenila je Hantera uspešnim 1915. [7], dok njegov srpski saradnik (dr Genčić), „pretpostavljeni" ili bar njegova „desna ruka", koga je Hanter redovno obaveštavao i koji je uvek izlazio u susret njegovim opravdanim zahtevima, „nije zadovoljio" pa je zato „kritikovan". Nešto nije u redu, jedan od ocenjivača je pogrešio ili 1915. ili 1925. godine.

Tko je ocenjivao Genčića: suveren, vojna komanda, ili ...?

Dr Genčić je posle rata negativno ocenjen od svog „okruženja". Njegov rad je „kritikovan" [16, 17]. Procenu su izvršili akteri koji su nakon rata bili u funkciji istoričara me-

[5] AS, MUD-S TO 38/1915.

[6] Za zaptivanje, predložena je „kobasica od peska". Za zatvaranje šupljina seljaci su koristili blato ili testo.

[7] http://history.amedd.army.mil/booksdocs/HistoryofUSArmyMSC/chapter3.html

dicine. Zabeleženi su njihovi komentari na sastancima. Kako su govorili za svog načelnika, tako su i zapisali, a time su indirektno i sebe negativno ocenili.

Kritike na račun načelnika saniteta, pukovnika dr Genčića opravdavane su činjenicom da je pre Hanterovog dolaska bio veliki broj obolelih i umrlih od pegavca [16]. Stanojević je komentarisao: „Moramo proći i kroz nedaće da bi postigli uspeh" [18]. Medicinski cilj se uspešno postiže samo ako doprinosi savladavanju bolesti. Da li je Vojni sanitet Srbije svojim doprinosima u suzbijanju epidemije bio uspešan u tome?

Dr Vukšić razmatrajući epidemije ocenjuje rad dr Hantera (englesko angažovanje, druga „engleska faza") uspešnim, ali „prva faza" ostala mu je po strani jer nije razmatran rad Vojnog saniteta i Državnog odbora i njihov doprinos suzbijanju epidemije. Negativna ocena zasnovana na registrovanom velikom broju hospitalizovanih i umrlih 1915, odnosila se samo na „prvi, srpsku" fazu.

Da li je Genčić sprečio bilo koga da doprinese pronalaženju rešenja tokom epidemije? Apsolutno ne! [15]. Podržao je sve one koji su mu se obratili i dali dokaze da bi njihove sugestije doprinele suzbijanju epidemije. Hanter je u više navrata kontaktirao Genčića. Genčić je uvek podržao Hanterove predloge [2, 3]. Njihova saradnja je bila uspešna, a ovo je takođe važna karakteristika druge faze.

Podržao je Genčić i domaće predlagače i sarađivao sa Državnim odborom, razmatrao predloge pristigle u Ministarstvo vojno, a potom upućene njemu, itd. [9].

Genčićevi uspesi, organizacioni i praktični, nisu bili prepoznati do 2009. godine. Njegova smena sa položaja načelnika saniteta ne može se opravdati nedostatkom rezultata u suzbijanju zaraza 1915. godine [15].

Mogući uzrok Genčićeve „smene" utvrdio je Dimitrijević [19]. Po njemu, to se dogodilo zbog političkih interesa: zajedništva, tj. potrebe krajnje odmerenog reagovanja i tolerantnog dijaloga. Drugi uzrok može biti kadrovska zamena cele vrhovne komande koja je izvršena u Skadru 1916. godine na zahtev Prestolonaslednika i Vlade [20]. Znači, ni to nije bilo radi Genčićevog neuspeha u suzbijanju epidemija 1915. godine.

Bivši načelnik saniteta dobio je 1929. godine odlikovanje najvišeg ranga, pri čemu su istoričari medicine ostali sami u svojim naporima da opravdaju svoje negativne ocene. Genčić, bez obzira što je bio usamljen u odnosu na njegovu opoziciju, primer je koji pokazuje da većina ne mora da bude u pravu: „(...) Nije retko da zajedničke interesi i krajnje vrednosti zajednice bolje zastupa nekonformistička manjina nego što to čini konformistička većina" [21].

Učesnici, istraživači epidemije 1915. godine trebalo je da bolje upoznaju prvi fazu u posleratnim godinama, jer bi onda videli da li postoje sličnosti sa drugom, uspešno završenom fazom u kojoj su epidemije suzbijene.

Iako sama epidemija nije bila suzbijena u prvoj fazi, trebalo bi da istoričari medicine zapaze da je prva faza uspešna zbog sledećih razloga: Vojni sanitet Srbije je dijagnostikovao pegavi tifus i rekurens i tako pomogao Hanterovoj misiji pre njenog dolaska u Srbiju; istoričari medicine nisu, takođe, primetili „nemoć medicine", pa nisu mogli konstatovati da je treba rešavati; izabrana je ispravna strategija u borbi protiv epidemije koju je za javnost ozvaničio dr Milana Jovanovića-Batut, čiji je članak objavljen 28. januara 1915. godine u službenim novinama. Sa istom činjenicom je dr Genčić 15. januara upoznao vojvodu Putnika; već 10. februara, dr Subbotić je predložio prototip ukopane peći na principu suvog toplog vazduha; 24. februara 1915. predsednik Državnog odbora, ing. Vulović, je predložio četiri vrste komora; početkom marta 1915. godine rad je započela „Dezinfekcioni centrala" u Nišu sa dnevnim kapacitetom od 4-5 hiljada razvašljenih odela (imala je kupatilo koje je moglo da primi 1 200 ljudi dnevno, a da se dok se oni kupaju, obavlja depedikulacija njihove odeće); pukovnik dr Hanter i njegova sanitetska misija su bili od posebne važnosti kada je u pitanju uspeh 1915. godine.

Radovi iz 1989. [1] i 2009. godine [15] pokazuju moguće promene u ranijim istorijskim/medicinskim procenama. Očekuje se nova istorijska/ medicinska procena prve faze suzbijanja epidemije pegavog tifusa u 1915. godini. Sigurno je sačuvan deo arhivskog materijala Sanitetskog odeljenja srpske vojske jer cela arhiva nije uništena pri povlačenju. Bilo je objavljeno 1922. godine da je iz ratnih godina sačuvan arhivski materijal, a taj materijal je prikazan u tabelarnom izveštaju 1924. godine [22]. Arhiva je sačuvana i u sledećem ratu. Ona omogućava preispitivanje događaja iz 1915. godine.

Zaključak

Analize prve faze suzbijanja epidemije tifusa u Srbiji tokom 1915. godine pokazale su da je tokom nje srpski sanitet imao vrlo značajnih poduhvata. To se, pre svega, odnosi na primenu komora na suvi, topli vazduh koji se koristio za uništenje vaši, prenosnika uzročnika tifusa, što predstavlja značajan doprinos srpskog saniteta svetskoj medicini, uopšte, koja u vreme početka Velikog rata nije raspolagala saznanjima da je epidemije pegavca i rekurensa moguće suzbiti sredstvima pogodnim za masovno razvašljivanje. Za to veliku zaslugu ima načelnik saniteta srpske vojske u tom periodu, dr Lazar Genčić koji je, u saradnji sa Državnim odborom primenjivao specijalne mere prevencije tokom celog perioda trajanja epidemija. Od dolaska Britanske vojnosanitetske misije u Srbiju njen doprinos je bio od presudnog značaja za suzbijanje epidemije. Apsurd predstavlja činjenica što su Hanterove aktivnosti ocenjene kao uspešne, dok je njegov saradnik dr Genčić proglašen neuspešnim. Potrebno je da se domaće istorijsko-medicinske ocene događanja 1915. godine preispitaju uzimajući u obzir „nemoć" medicine tog vremena u suzbijanju epidemija pegavca i rekurensa, a da se primećeni nemar objektivizira i da mu se vidi složenost usled vođenja tzv. „totalnog rata", ratne nemaštine, itd..

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Received on August 1, 2018.
Accepted on September 14, 2018.
Online First September, 2018.

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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 i članci iz istorije medicine ne smeju prelaziti 16 stranica (bez priloga); aktuelne teme – deset, seminar praktičnog lekara – osam, kazuistika – šest, prethodna saopštenja – pet, a komentari i pisma uredniku – tri, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina (sem mm Hg i °C).

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Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** rada, zahvalnost (po želji), literatura, prilozi.

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a) Poželjno je da naslov bude kratak, jasan i informativan i da odgovara sadržaju, podnaslove izbegavati.

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Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/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 za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz bolesnika** i **Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

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Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

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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

U radu literatura se 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 *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 pristupa tim podacima.

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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 fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

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.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

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