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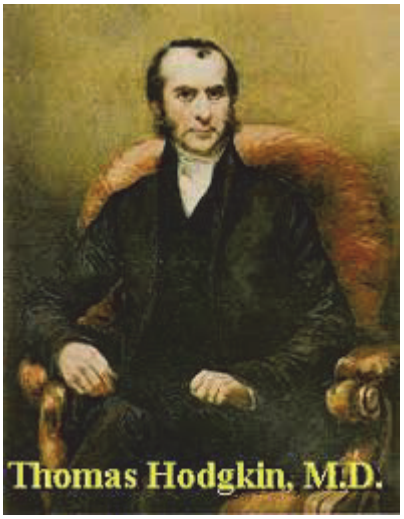

Thomas Hodgkin (17 August, 1798 – 5 April, 1866), a British physician, one of the most prominent pathologist and a pioneer in preventive medicine, who, in 1832, was the first to describe one of two common types of cancers of the lymphatic system, later named after him - Hodgkin`s disease.
This year, on 17th August, 220th anniversary of his birthday will be marked.

Tomas Hočkin (17. avgust, 1798 – 5. april, 1866), britanski lekar, jedan od najistaknutijih patologa svog vremena i pionir preventivne medicine, prvi je, 1832. godine, opisao jedan od dva najčešća oblika karcinoma limfatičkog sistema, kasnije nazvanog po njemu – Hočkinova bolest.
Ove godine, 17. avgusta, obeležiće se 220 godina od njegovog rođenja.

ORIGINAL ARTICLES

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Characteristics of suicide in the city of Niš within the period 2000–2010

Karakteristike samoubistva u gradu Nišu u periodu 2000–2010. godine

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Abstract

Background/Aim. Suicide is a significant public health problem worldwide. Numerous factors contribute to suicide. The aim of this study was to investigate the characteristics of suicide in the city of Niš in the period 2001–2010. **Methods.** The retrospective study consisted of 608 persons divided into three groups: suicide committers with mental disorders, somatic disorders or without registered disorders. Data on socio-demographic characteristics, previous suicide attempts, methods of suicide and presuicidal syndrome were obtained from the Police Directorate for the city of Niš, Ministry of Internal Affairs, Republic of Serbia and from the Statistical Office of the Republic of Serbia. **Results.** Persons with mental disorders were the most prevalent group of people who committed suicide (54.3%), followed by persons without registered disorders (31.9%) and with somatic disorders (13.8%). Persons with mental disorders who committed suicide were most often divorced, with high school education, monthly salary and with at least one previous suicide attempt. The hanging was the most frequent method of committing suicide in all investigated groups, followed by self-poisoning using drugs or liquid substances. The presuicidal syndrome was significantly more frequent among persons with mental disorders compared to persons with somatic disorders or without registered disorders others (45.8% vs. 16.7%, and 45.8% vs. 28.4% respectively $p < 0.001$). **Conclusion.** Although the persons with mental disorders are in the greatest risk of suicide, they are under medical care. In this regard, the prevention programs should be directed towards persons with severe somatic disorders and to old persons without registered disorders.

Key words:
suicide; risk factors; comorbidity; mental disorders; psychophysiologic disorders; serbia.

Apstrakt

Uvod/Cilj. Suicid je značajan svetski zdravstveni problem. Mnogobrojni faktori doprinose nastanku samoubistva. Cilj ovog istraživanja bio je ispitivanje karakteristika suicida na teritoriji grada Niša u periodu 2001–2010. godine. **Metode.** Ovo retrospektivno istraživanje obuhvatilo je 608 osoba koje su izvršile samoubistvo, podeljene u tri grupe: osobe sa psihičkim poremećajima, osobe sa fizičkim poremećajima i osobe bez registrovanog poremećaja. Podaci o sociodemografskim karakteristikama, prethodnim pokušajima i načinima izvršenja samoubistva i prisustva presuicidnog sindroma prikupljani su iz Ministarstva unutrašnjih poslova Republike Srbije, Policijske uprave grada Niša i Zavoda za statistiku Republike Srbije. **Rezultati.** Suicid su najčešće izvršile osobe sa psihičkim poremećajima (54,3%), zatim osobe bez registrovanog poremećaja (31.9%), a najmanje osobe sa somatskim smetnjama (13.8%). Osobe sa psihičkim poremećajima najčešće su bile razvedene, sa srednjim obrazovanjem, mesečnim primanjima i bar jednom su prethodno pokušale samoubistvo. Najčeši metod suicida bio je vešanja, a zatim samotrovanje lekovima. Presuicidni sindrom najčešće je uočen kod osoba sa psihičkim poremećajima u poređenju sa osobama sa somatskim poremećajima ili sa osobama bez registrovanih poremećaja (45,8% prema 16,7%, i 45,8% prema 28,4%, redom, $p < 0.001$). **Zaključak.** Iako su

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osobe sa psihičkim smetnjama pod najvećim rizikom da izvrše suicid, one su pod psihijatrijskom kontrolom, stoga bi program prevencije suicida trebalo da bude usmeren na osobe sa teškim somatskim smetnjama i invaliditetom, kao i na stare, usamljene osobe bez dijagnostikovanih poremećaja.

Ključne reči:
samoubistvo; faktori rizika; komorbiditet; psihički poremećaji; psihofiziološki poremećaji; srbija.

Introduction

Suicide is defined as an act of deliberately killing oneself. There is no single explanation of why people commit suicide. Moreover, there is no single factor responsible for suicide death. It is a complex psychopathological phenomenon that is influenced by several interacting factors, such as personal, social, psychological, cultural, biological and environmental [1].

Suicide still represents a significant public health problem worldwide. Despite global increase in population between 2000 and 2012, the absolute number of suicides fell about 9%. The World Health Organization (WHO) estimated that 804,000 suicide deaths occurred worldwide in 2012 represented an annual global age-standardized suicide rate of 11.4 per 100,000 population (15.0 for males and 8.0 for females). In 2012 suicide accounted for 1.4% of all deaths worldwide, making it the fifteenth leading cause of death. Additionally, it is the second leading cause of death in population aged 15–29 years[2].

The prevalence, characteristics and methods of suicide vary widely according to different geographic regions, different communities and ethnic origin, gender, age and time. Although the age-standardized rate of suicide is slightly higher in high-income countries (HICs) than in low-income and middle-income countries (LMICs), 12.7 vs. 11.2 per 100,000 people, 75.5% of all global suicides occur in LMICs. Significantly more men than women die by suicide. In HICs male-to-female suicide ratio is higher compared to LMICs (3.5 vs. 1.6). In most countries, the highest suicide rates is recorded among elderly people. With regard to the age, the highest suicide rates are noted among persons aged 70 years or over, both of men and women in almost all regions of the world. However, over the past 50 years, suicide rates have risen among young people [1–3].

In Serbia, in the period from the early 1950s to the mid-2010s, the total number of suicides exceeded 75,000. In the mid-2010s, the average age-standardized mortality rate was 16 suicides per 100,000 inhabitants. The differences in suicide deaths according to gender and age in Serbia followed the world trend. In the last two decades, out of a total number of suicides, 70.7% were committed by males and only 29.3% by females.

In addition, 48.1% of persons who committed suicide were aged 60 years or over. Although downward trend in suicide mortality in the last two decades was observed, Serbia is still significantly above the world and slightly above the European average [4–6].

It has been reported that numerous factors contribute to suicide. In general, these factors can be categorized as state-dependent, or, proximal and trait-dependent, or distal factors. The distal factors increase predisposition, whereas the proximal ones act as precipitants. Mental and somatic disorders, psychosocial crisis, availability of means and exposure to models are common proximal risk factors. On the other side, genetic loading, personality characteristics, restricted fetal growth and perinatal circumstances, early traumatic life events and neurobiological disturbances are recognized as distal risk factors [7, 8]

Years of research on suicide show that people with current mental disorders are the most common group dying by suicide. Previous studies reported that mental disorders are present in about 80%–90% of persons who kill themselves and contribute with 47%–74% to population risk of suicide. Specific disorders associated with suicide include mood disorders, substance use, anxiety, impulse control disorders, personality and psychotic disorders [9, 10]. In addition, previous findings confirmed that persons with more than one mental disorder are at a higher risk of suicide, especially those with both depressive and substance use disorders [11]. Furthermore, suicide is associated with poor physical health and disabilities [12].

Recent studies reported the suicide mortality of Serbian population in different time periods related to age and gender [5, 6, 13]. On the other side, there are no previously reported findings of suicide among the persons with mental or somatic disorders. Therefore, this study aimed to investigate the suicide deaths at the territory of the city of Niš in the period 2001–2010. Furthermore, this study investigated the possible differences in age, gender, socio-demographic characteristics and presuicidal syndrome among the persons with mental and somatic disorders who committed suicide.

Methods

This retrospective, psychological autopsy study [14] consisted of 608 persons who committed suicide at the territory of the city of Niš, Republic of Serbia in the period within 2001–2010. Data on persons who died of suicide and self-inflicted injury [site codes X60-X84 revision 10th of the International Statistical Classification of Diseases and Related Health Problems (ICD-X)] [15] were obtained from the Police Directorate for the city of Niš, Ministry of Internal Affairs, the Republic of Serbia and Statistical Office of the Republic of Serbia, Department of Demography. We included only those reports where death occurred from self-inflicted or intentional self-harm.

The study sample was divided into three groups. The first group consisted of 330 persons, with previously diagnosed mental disorders, who committed suicide. The eighty-four suicide committers with previously diagnosed somatic disorders were included in the second group. Others were in-

cluded in the third group which consisted of 194 persons, without any diagnosed disorder, who committed suicide (Table 1). From each family interview, medical and police record, we extracted the details of age, gender, marital status, educational level, source of income, current diagnosed mental or somatic disorder, history of previous suicide attempts and methods of suicide. Additionally, we made a record of any reported presuicidal syndrome in the period before the suicide in all included patients. The presuicidal syndrome was defined as any change in the usual behavior of person during the lifetime before the suicide (i.e., sadness, tiredness, reticence, nervousness, worry, indecisiveness, poor concentration, etc.) [16].

Statistical analyses were performed by IBM SPSS Statistics for Windows Software (Version 20.0 < IBM Corp, Armonk, NY, USA). Results were presented as frequencies and percentages. The χ^2 analysis was conducted to assess a statistical significance of categorical data. All p values less than 0.05 were considered significant.

Results

A total of 608 (293 males and 315 females) suicide deaths occurred in the city of Niš during the investigated period. Persons with mental disorders were the most prevalent group among those who committed suicide (330, 54.3%), followed by persons without registered disorders (194, 31.9%) and with somatic disorders (84, 13.8%) (Table 1). The number of suicide deaths increased annually in all groups during the investigated period. Although the number of suicides in persons with mental disorders were higher compared to other groups, we did not observe any significant differences in the trend of suicide deaths among them (p = 0.088) (Figure 1).

The average age of suicide committers in the groups with mental and somatic disorders was as follows, 49.86 ± 18.10 and 49.69 ± 15.39 years. On the other hand, an average age of persons who committed suicide in the group of others without registered disorder were 55.82 ± 25.27 years.

Table 1

Distribution of suicide committers included in the study

Study group	Type of disorder	n
Persons with mental disorders	Depressive episodes (ICD-V-F 32)	104
	Disorders of adult personality (ICD-V-F 60-63)	103
	Disorders due to use of alcohol (ICD-V-F10)	69
	Disorders due to use of opioids (ICD-V-F 11)	33
	Schizophrenia (ICD-V-F-20-21)	21
Persons with somatic disorders	Malignant neoplasms (ICD-II-C-00-97)	61
	Invalidity (ICD-VI-G-10-14,35; IX-I-69)	23
Others (without disorders)		194
Total		608

ICD – International Classification of Diseases; n – number of persons.

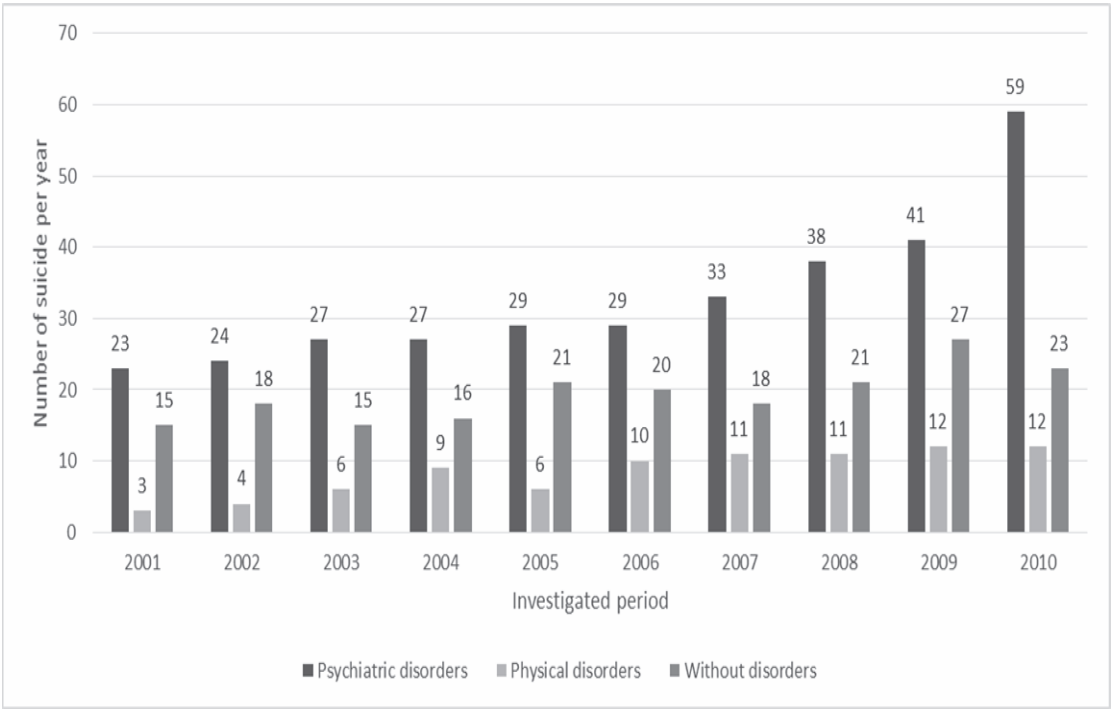

Fig. 1 – The number of suicides within 2001–2010 period.

The observed difference was statistically significant the groups ($p = 0.002$). Additional Bonferroni correction analysis revealed that a significant difference existed only between the group of persons with mental disorders and the one without registered somatic disorders ($p = 0.002$).

Most of the suicide committers with mental disorders were divorced (92; 27.9%) or single (87; 26.4%). Moreover, most of the suicide committers with somatic disorders were married (35; 41.7%) and persons without registered disorders were widowed (58; 29.9%) or single (57; 29.4%). Observed marital status differences were statistically significant ($p < 0.001$) (Table 2). In all investigated groups, the high school was the most frequent level of education (50.9%, 48.8%, and 53.1%, respectively). The frequencies of this type of education among suicide groups were significantly different compared to other education levels ($p < 0.001$) (Table 2). In regard to their source of income, most persons with mental and somatic disorders who committed suicide had a monthly salary (49.1%, and 63.1%, respectively). On the other side, the majority of persons without registered disorders were financially dependent (87, 44.8%). These observed differences were statistically significant ($p < 0.001$), (Table 2).

We did not record the previous suicide attempts in a majority of people with somatic disorders and without registered disorders (91.7%, and 86.7%, respectively) (Table 2). Moreover, the majority of persons with mental disorders had one previous suicide attempt recorded (42.4%). We observed a significant difference in previous suicide attempts between investigated groups ($p < 0.001$) (Table 2).

The hanging was the most frequent method of committing suicide in all investigated groups (35.5%, 33.3%, and 34.5%, respectively), followed by self-poisoning using drugs or liquid substances (25.2%, 27.4%, and 26.3%, respectively). However, differences between methods of suicide were not statistically significant ($p = 0.296$) (Table 3).

Most of suicide committers in all investigated groups did not have presuicidal syndorme (54.2%, 83.3%, and 71.6%, respectively). On the other hand, presuicidal syndrome was more frequent among the persons with mental disorders compared to the persons with somatic disorders or to those without registered disorder (45.8% vs. 16.7%, and 45.8% vs. 28.4%, respectively). These differences were statistically significant ($p < 0.001$) (Table 4).

Table 2

Sociodemographic characteristics

Sociodemographic status	Persons with mental disorders, n (%)	Persons with somatic disorders, n (%)	Persons without disorders, n (%)	*p* value [a]
Marital status				
single	87 (26.4)	16 (19.0)	57 (29.4)	
married	75 (22.7)	35 (41.7)	41 (21.1)	
extramarital community	26 (7.9)	14 (16.7)	16 (8.2)	$p < 0.001$
divorced	92 (27.9)	12 (14.3)	22 (11.3)	
widowed	50 (15.2)	7 (8.3)	58 (29.9)	
Education level				
no school	10 (3.0)	0 (0)	13 (6.7)	
elementary school	24 (7.3)	9 (10.7)	45 (23.2)	
high school	168 (50.9)	41 (48.8)	103 (53.1)	$p < 0.001$
faculty	128 (38.8)	34 (40.5)	33 (17.0)	
Source of income				
salary	162 (49.1)	53 (63.1)	35 (18.0)	
pension	61 (18.5)	6 (7.1)	52 (26.8)	
social care	21 (6.4)	3 (3.6)	20 (10.3)	$p < 0.001$
dependent	86 (26.1)	22 (26.2)	87 (44.8)	
Previous suicide attempts				
no	135 (40.9)	77 (91.7)	170 (86.7)	
yes, once	140 (42.4)	7 (8.3)	19 (9.8)	$p < 0.001$
yes, more than once	55 (16.7)	0 (0)	5 (2.6)	

[a] χ^2 test; n – number of persons.

Table 3

Methods of suicide

Methods of suicide (ICD – X code)	Persons with mental disorders, n (%)	Persons with somatic disorders, n (%)	Persons without disorders, n (%)
Self-poisoning by drugs and by exposure to liquid substances (X60-65, X68-69)	83 (25.2)	23 (27.4)	51 (26.3)
Hanging (X70)	116 (35.2)	28 (33.3)	67 (34.5)
Drowning and submersion (X71)	31 (9.4)	10 (11.9)	30 (15.5)
Firearm and explosive material (X72-75)	60 (18.2)	9 (10.7)	28 (14.4)
Jumping from a high place (X80)	40 (12.1)	14 (16.7)	18 (9.3)

ICD-X – International Statistical Classification of Diseases and Related Health Problems, 10th revision; n – numbers of persons.

Table 4

The presence of presuicidal syndrome in investigated groups

Altered behavior	Persons with mental disorders, n (%)	Persons with somatic disorders, n (%)	Persons without disorders, n (%)	*p* value [a]
Yes	151 (45.8)	14 (16.7)	55 (28.4)	
No	179 (54.2)	70 (83.3)	139 (71.6)	$p < 0.001$

[a] χ^2 test; n – number of persons.

Discussion

Suicide is a complex and heterogeneous condition. It is known that numerous factors contribute to suicide, which is never the consequence of one single cause or stressor.

Previous psychological autopsy case-control studies showed a strong relationship between suicide and mental disorders [17, 18]. They have shown that mental disorders are present in 80%–90% of persons who kill themselves. Moreover, Harris and Barraclough [19] reported that the risk of suicide increased 5-fold to 15-fold in persons with mental disorders. Previous meta-analyses suggested that specific mental disorders were associated with a higher risk of suicide [9, 20]. They reported that mood disorders [summary odds ratio (SOR) = 13.4], substance-related disorders (SOR = 5.2), personality disorders (SOR = 4.5) and psychotic disorders (SOR = 6.6) were the most common mental disorders among the persons who committed suicide. These results are in accordance with the study of Ferrari et al. [21] who stated that the relative risk of suicide in an individual with major depressive disorder was 19.9 (OR = 9.5–41.7), with schizophrenia 12.6 (OR = 11.0–14.5), and with alcohol use 9.8 (OR = 9.0–10.7).

The results of our study are in accordance with previous reports. More than a half of persons who committed suicide in the city of Niš during the period between 2000 and 2010 had diagnosed mental disorders. The most frequently diagnosed disorders were depression episodes (34.3%), disorders of adult personality (34%) and disorders due to the use of alcohol and opioids (33.7%). Depression is recognized as a leading diagnosis associated with suicide, occurring in almost two-thirds of the cases [22]. It is disturbing that around three-quarters of suicide occurred to individuals who were never examined at the second care services [23]. Recent systematic review of Hawton et al. [24] identified the following suicide risk factors in persons with depressions: male gender, family history of suicide or mental disorders, history of attempted suicide, hopelessness and comorbid disorders such as anxiety, personality disorder, misuse of drugs, and alcohol abuse. Additionally, Coryell and Young [25] reported that clinical predictors of suicide in persons with major depressive disorder include a history of attempted suicide, high levels of hopelessness and high ratings of suicidal tendencies.

For every suicide committed, there are significantly higher number of people who attempt suicide every year. Significantly, a previous suicide attempt is the single most important risk factor for suicide in the general population [1]. We showed that the persons with mental disorders who committed suicide more often previously tried to commit suicide compared to persons with somatic disorders and those without registered disorders (42.4% vs. 8.3%, and 42.4% vs. 9.8% respectively; $p < 0.0001$). These findings are in agreement with the previously reported results [24, 25]. Some investigations showed that besides depression, bipolar disorder, borderline personality disorder, opioid use, schizophrenia, anorexia nervosa and alcohol use disorder showed significantly increased rates of suicide compared with general population [10, 26, 27]. On the other hand, Cho et al. [28] reported that studies from East Asia had a significantly lower mean prevalence of mental disorders among persons who committed suicide [69%, 95% confidence interval (CI) = 56.8–80.0] than those in North America (88.2%, 95% CI = 79.7–93.5) and South Asia (90.4%, 95% CI = 71.8–97.2). The authors suggest that the sociocultural factors in different geographic regions may have a possible role in suicide occurrence [28].

Hawton and van Heeringen [7] stated that about 10% of individuals who died by suicide in most countries had no apparent mental disorder. Moreover, suicide is also associated with several somatic disorders, including cancer, multiple sclerosis, spinal cord injury and pain [29–32]. Our results are in accordance with findings of Fegg et al. [32] who observed that cancer was the most prevalent somatic disorder in persons who committed suicide. Patients with cancer are more emotionally distressed, with depressed mood and with suicidal ideation [31]. Vyssoki et al. [31] reported that suicide risk increases with cancer severity. Deeper feeling of hopelessness with disabilities and lack of willingness to think that situation will improve are overwhelming feelings of those people. Recent findings showed suicidality and depression as the important predictors of suicide acceptability regardless occurrence of somatic disability [33, 34]. Additionally, several studies reported that patients with cancer were more prone to develop depressive episodes, which is additional comorbidity to mental disorders and suicide death [35, 36].

The previous study of suicidal behavior in Dutch primary care, conducted in 30-years period, noted that more males than females committed suicide. Also, the trend of such behavior was increasing in men, while it was continuously declining in women [37]. These findings are in accordance with other studies showing larger suicide risk in men than women as found for suicide in general [7]. Such results were explained by economic recession and threats of losing a job or unemployment that affected HICs during this period of research. On the other hand, our study on 608 people who committed suicide showed similar frequencies between genders. This could be due to different socioeconomic status between our and West European countries. Also, our study

covered the population of only one city area with specifically observed gender distribution.

The meta-analysis found unemployment as a risk factor for suicide after adjusting for prior mental disorder [38]. Likewise, our results showed that majority of persons without registered mental or somatic disorders who committed suicide were financially dependent. Feeling of hopelessness, defeat, entrapment and lack of future prospective, were recognized as risk factors for suicidal behavior, arising from long-term unemployment, job loses, incapability of work due to chronic illness or handicap [39, 40].

Our findings reveled significantly greater percentage of people who are single, divorced or widowed regardless the presence of mental disorders. Living alone is a well-known suicide risk factor [41]. Therefore, general practitioners should provide closer attention to this group and to the group of old people.

The variations in suicide methods are observed in relation to different region, gender, age, urban versus rural residence, etc. [3] According to WHO mortality data, there are three suicide methods which are the most frequently used – hanging, pesticide poisoning and using firearms [42]. The findings of this study are similar to the ones published in previous Serbian investigations [5, 6] regarding the most frequently used suicide methods. Ilic et al. [5] reported that the leading methods in Serbia were suicides by hanging and firearms. In addition, similar findings were reported by Dedic [6]. Our results are in line with these results. Thus, we reported that the most frequent method of suicide was hanging, which is followed by self-poisoning. These results are also in agreement with international evidence, which, in general, describe a superiority of suicide by hanging [43–45].

Conclusion

Our study showed that different mental disorders were the most frequently registered among suicide committers in the city of Niš in the period 2000–2010. Presuicidal syndrome was significantly more frequent in suicide committers with mental disorders compared to those with somatic disorders or in others. It is important to emphasize that the city of Niš is the third largest city – in the Republic of Serbia and this results may be also representative of the national level.

Although there is no way to predict who would commit suicide, recognition of early signs of presuicidal syndrome should be the basis for future prevention. It is important to emphasize that any change in usual behavior in persons with mental and somatic disorders might be an indicator for the possible suicide. Although the persons with mental disorders are in the greatest risk for suicide, in most cases they are under medical care. In this regard, the prevention programs should be directed towards people with severe somatic disorders and to the old ones without registered disorders. That is why it is necessary to establish the national suicide prevention strategies with specific targets for suicides reduction.

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Assessing temporomandibular disorders: mouthpiece design considerations

Procena temporomandibularnih poremećaja u odnosu na dizajn ronilačkog usnika

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Abstract

Background/Aim. Scuba diving is one of the sports with the fastest growing popularity. Nowadays doctors of dental medicine meet divers-patients in their offices more often. Treatment of these patients has some specific features that are related to difficulties in the temporomandibular joint, masticatory muscles and soft tissues of the oral cavity. A set of those complaints represent a condition called „diver's mouth syndrome". Most scuba divers complain of temporomandibular joint and the masticatory muscles pain; inadequate mouthpieces can exacerbate temporomandibular dysfunction (TMD) even when its symptoms are not present in everyday life. The aim of this research was to find a decent substitute for a fully customised mouthpiece, that are not present at our market, to discover the qualities of a good mouthpiece and establish prevalence of TMD among divers. **Methods.** This study included 30 scuba divers. Scuba divers filled out the questionnaire before diving, then dived twice with each of the 3 different commercial mouthpieces provided for this research (Mares Universal, Seac sub, Mares LiquidSkin (Universal Mares, Seac sub, Mares LiquidSkin). After diving, they filled out the second part of the questionnaire and so they performed an assessment of the mouthpiece and gave insight into the prevalence of TMD symptoms caused by using the mouthpiece. **Results.** According to the average score of satisfaction (the least present symptoms such as pain, fatigue, and numbness of the masticatory muscles and the jaw joint), this research proved Mares LiquidSkin mouthpieces to be the best out of the 3 commercial mouthpieces. For its use, average satisfaction score among participants was 7.07 (out of 10) and none of the divers reported jaw and muscle stiffness during and after the dive with this mouthpiece. The smallest percentage of participants reported pain in the orofacial region and discomfort while swallowing when used it in comparison with other mouthpieces. The anatomy and material of the mouthpieces turned out to be an extremely important factor. **Conclusion.** Several factors contribute to a good mouthpiece design; the choice of material, its elasticity and softness, the thickness and length of the interdental bite platform and the width of the oral screen that is inserted into the vestibule. The preferred material should be soft silicone. The interdental bite platforms should support the posterior teeth and the oral screen should fit the jaws and not be too wide.

Key words:
diving; equipment and supplies; temporomandibular joint disorders.

Apstrakt

Uvod/Cilj. Ronjenje sa bocama je sport čija se popularnost izuzetno povećava. Danas se stomatolozi u svojim ordinacijama sve češće susreću sa pacijentima-roniocima. Prilikom tretmana pacijenta koji roni, stomatolozi bi trebalo da budu upoznati sa stanjem zvanim „sindrom ronilačkih usta", koji uključuje bol u zglobu i zubima, oštećenje desni ili hiperplaziju gingive – zubnog mesa. Većina ronilaca se žali na bol temporomandibularnog zgloba i mastikatornih mišića; neadekvatan usnik može pogoršati temporomandibularne poremećaje (TMP) čak i kada u svakodnevnom životu ne postoje njeni simptomi. Cilj ovog istraživanja bio je da se pronađe pristo-

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jna zamena za potpuno prilagođen usnik, koji nije prisutan na našem tržištu i da se otkrije koji su kvaliteti dobrog usnika i kolika je rasprostanjenost TMP među roniocima. **Metode.** U ovom istraživanju učestvovalo je 30 ronilaca sa bocama. Ronioci su ispunjavali upitnik nakon ronjenja po dva puta sa svakim od tri različita komercijalna usnika obezbeđenih za ovo istraživanje (Mares Universal, Seac sub, Mares LiquidSkin). Oni su procenjivali usnike i davali uvid u rasprostranjenost simptoma TMP-a izazvanih korišćenjem usnika. **Rezultati.** Prema prosečnoj oceni zadovoljstva (najmanje prisustvo simptoma kao što su bol, umor i ukočenost mastikatornih mišića i zgloba vilice), ovo istraživanje je pokazalo da je usnik Mares LiquidSkin najbolji od tri posmatrana komercijalna usnika. Prosečna ocena zadovoljstva je bila 7.07 (od ukupno 10) i pokazani su izuzetni rezultati – nijedan ronilac se nije žalio na ukočenost vilice i mišića za vreme i posle ronjenja sa ovim usnikom, a u poređenju sa drugim usnicima, on je kod manjeg broja ispitanika izazivao bol u orofacijalnoj regiji i nelagodnost prilikom gutanja. Anatomija i materijal usnika pokazali su se kao ekstremno važan faktor. **Zaključak.** Nekoliko faktora čine dobar dizajn usnika: izbor materijala, njegova elastičnost i mekoća, debljina i dužina interdentalne platforme ugriza i širina platforme koji se stavlja u vestibulum. Idealan materijal je mekani silikon, interdentalna platforma ugriza bi trebalo da podržava zadnje zube i širina bi trebalo da odgovara širini čeljusti.

Ključne reči:
ronjenje; oprema i pribor; temporomandibularni zglob, poremećaji.

Introduction

In the past years scuba diving became more popular, not only as a recreational, but also as a professional sport. Rather than focusing only on the impact of underwater high pressure on the human body, researchers have become more interested in scuba diving equipment design. As the number of scuba divers increases, doctors of dental medicine have the opportunity to treat such patients in their offices more often. For that reason, doctors of dental medicine should be educated to recognize and treat symptoms and complications in the orofacial region that are sometimes present among diver population [1]. These symptoms, known as „diver's mouth syndrome", include temporomandibular pain, tooth pain and gingival hyperplasia and can be caused by the mouthpiece [1–3].

Stomatognathic system is a very complex system which consists of temporomandibular joint (TMJ), teeth, orofacial muscles, facial bones and jaw, oral mucosa, nerves, blood and lymph vessels [4] and it enables the performance of the functions of mastication, speaking, breathing and deglutition. For the functioning of the stomatognathic system, it is essential that all elements are functioning properly, otherwise, disturbance of one of the factors can cause disruption of other factors of the system.

Temporomandibular dysfunction (TMD) represent a very serious health problem and occupy a leading place when it comes to the disease of the musculo-skeletal system in general [5]. Those disorders are present in a large percentage in general population and there is data in the literature showing that the symptoms are often present in females [6]. Temporomandibular disorders are caused by different etiological factors such as genetic factors, trauma, malocclusion, parafunctions, emotional stress, factors of inflammatory and noninflammatory nature and so on. A diagnosis is very complex and it is followed by a long-term therapy often requiring multidisciplinary approach (dentist, surgeon, physiatrist, otolaryngologist).

The association between TMD and scuba diving is the subject of many research and it is believed that these disorders is on the rise with the increasing interest in training for scuba diving certification [7].

The acronym SCUBA stands for self-contained underwater breathing apparatus, yet it is used as a noun. Mouthpieces are part of scuba diving equipment, inserted in the mouth, gripped by the teeth and held in place. They enable air flow from a regulator whilst ensuring a watertight seal. Main part of a mouthpiece are an airway tube connected to the demand valve which delivers breathing gas, oral screen inserted in the vestibule of the mouth, interdental bite platforms into which the diver bites and palatal lugs. Scuba diver mouthpiece is held by teeth. Mouthpiece is a part of the second stage of the regulator, which is connected by a hose with a scuba tank loaded with air. Except allowing air consumption, the shape and position of mouthpiece in the mouth vestibulum prevent water entry. There are plenty of different commercial mouthpieces on the market. Main parts of the mouthpiece are: connector tube to the demand valve of the second stage, vestibular shield, interdental bite platform and palatal flange (Figure 1).

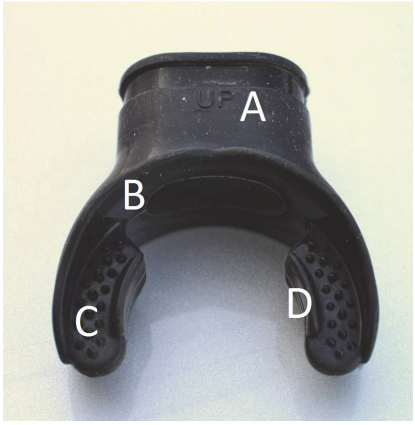


Fig. 1 – Mouthpiece parts.

The aim of our research was to determine the qualities of a good commercially available mouthpiece, ensuring both comfort while diving and causing minimal or no symptoms related to the temporomandibular joint. Other researchers took into consideration semi-customized and fully-custom-

ized mouthpieces as well. Fully-customized mouthpieces are not available in Croatia, considering the complexity of their fabrication in dental laboratories and price, while semi-customized mouthpieces can only be seldom found.

Methods

In this study, 30 scuba divers, from Croatia and Slovenia, aged 26–36 years, participated voluntarily and anonymously after signing the informed consent. The research included a questionnaire consisting of two parts. The first part was filled in before the test dive and it consisted of the issues related to oral health of the subjects (tooth status, problems related to TMJ and masticatory muscles), frequency and length of practicing scuba diving as well as mouthpieces that they used when diving (Appendex 1).

The study participants filled out the second part of the questionnaire after the test dives and it was related to comfort, discomfort and occurrence of different potential symptoms in the orofacial region during and after the dive (Appendix 2).

Scuba divers had been given 3 different mouthpieces to try out. They dived twice with each of them.

Mouthpieces chosen for this research were Mares universal mouthpiece (mouthpiece No 1), SEAC SUB mouthpiece (mouthpiece No 2) and Mares LiquidSkin Mouthpiece (Figures 2 and 3).

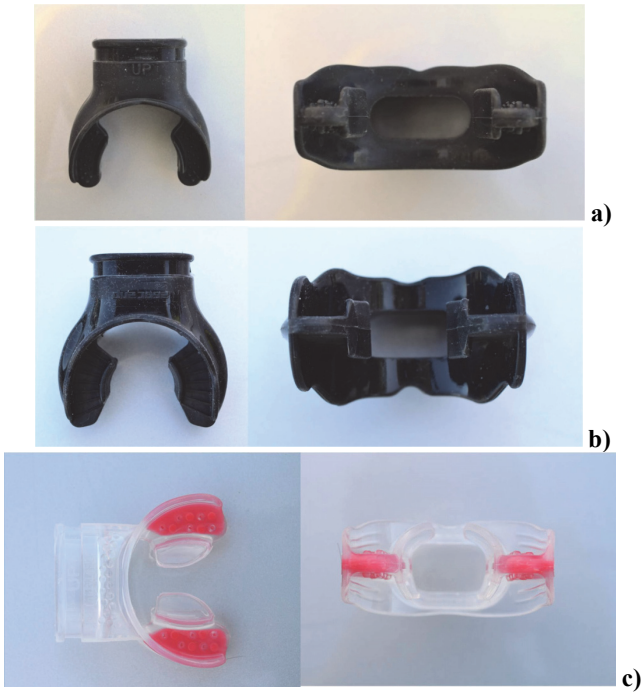


Fig. 2 – Mouthpieces used in the study: a) Mares universal mouthpiece – mouthpiece No 1; b) SEAC SUB mouthpiece – mouthpiece No 2; c) Mares LiquidSkin mouthpiece – mouthpiece No 3.

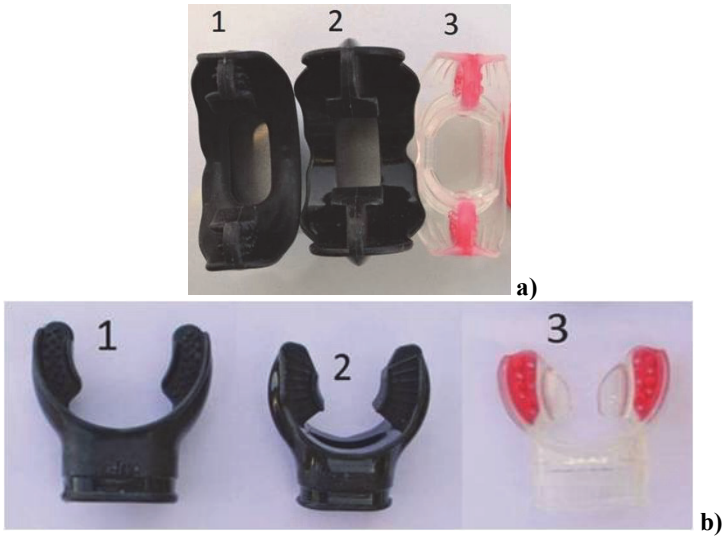


Fig. 3 – Comparison of mouthpieces used by a) size, and b) width.
1 – Mares universal mouthpiece; 2 – SEAC SUB mouthpiece; 3 – Mares LiquidSkin mouthpiece.

Scuba divers could use fully-customized and commercial mouthpieces. Commercial mouthpieces are the most common on the market. They are made of rubber or silicone and their shape is designed to fit each scuba diver. Fully-customised mouthpieces are made of termoformical material, which, after being in hot conditions, becomes soft when a scuba diver puts it in the mouth and takes a gentle bite. In this way the user forms a shape that fits him/her. Our criteria for choosing these 3 mouthpieces were their presence on the market and popularity among divers.

Results

Three commercially available mouthpieces were tested after 180 dives; 30 divers dived twice with each of the chosen mouthpieces.

Rigidity and stiffness of face and jaw muscles during and after the dive with all 3 mouthpieces are shown in Figure 4a. The divers ranked mouthpiece No 3 as the best one, with no pain noticed during or after the dive. On the contrary, mouthpiece No 2 was given the lowest rating – 16.67% of examinees felt pain in the course of diving while 20% felt pain after the dive.

Experienced jaw and muscle pain during and after the dive was presented in Figure 4b. As shown, mouthpiece No 3 caused the least, or to be more precise, no pain after the dive while 6.67% of the divers felt pain during the dive. Both other mouthpieces, No 1 and No 2, caused greater pain during and after the dive.

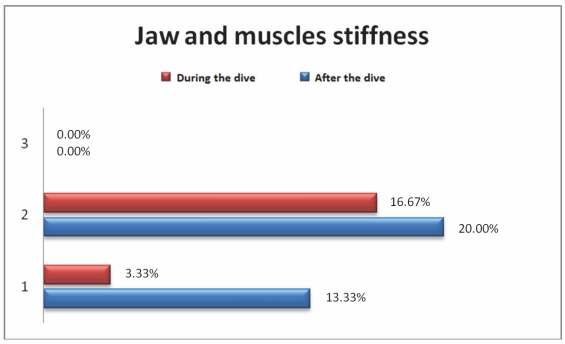
The occurrence of discomfort while swallowing saliva is shown in Figure 4c. The least percentage of divers felt discomfort while diving with the mouthpiece No 3 and none after the dive while the biggest percentage of discomfort occurred both during and after diving with the mouthpiece No 1.

All in all, the divers reported to be most pleased with the mouthpiece No 3, with an average satisfaction score of 7.07. The mouthpiece No 1 was ranked as the second best and the mouthpiece No 2 as the third one (Figure 5).

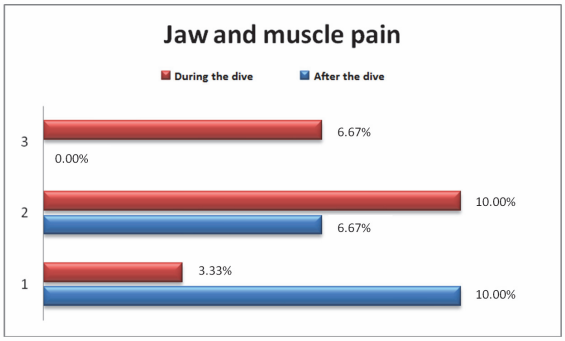
Discussion

Data collected during this study were obtained from a relatively small sample of 30 examinees, yet, in studies similar to this one, the number of examinees ranged from only 6 to a maximum of 72 examinees [1, 8, 9]. Despite this relatively small sample of examinees, valuable information about the prevalence of TMD symptoms and the importance of the mouthpiece design can be drawn from the collected data. The highest average grade, considering satisfaction with the used mouthpiece was given to Mares LiquidSkin mouthpiece (mouthpiece No 3). Mouthpiece No 3 showed the best results considering seldom occurrence of TMD symptoms which makes it the best ranked mouthpiece in this research.

A point of this study was to determine the qualities of a good mouthpiece and not to promote any specific brand. Results of different studies have shown that pain and jaw stiffness are strongly connected with the type of material and the length, thickness and position of interdental bite platforms.



a)



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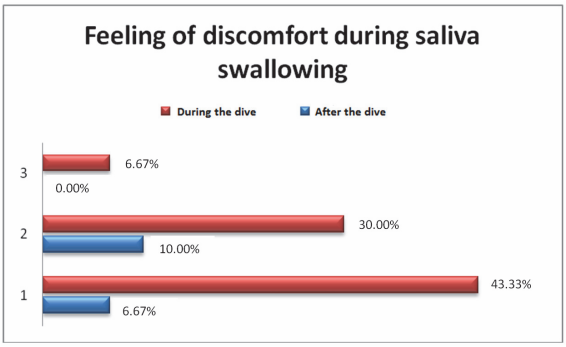

c)

Fig. 4 – Comparison of complaints caused by monthpieces tested:
a) Rigidity and stiffness of face and jaw muscles;
b) Jaw and muscle pain;
c) Discomfort during swallowing.
1 – Mares universal mouthpiece;
2 – SEAC SUB mouthpiece;
3 – Mares LiquidSkin mouthpiece.

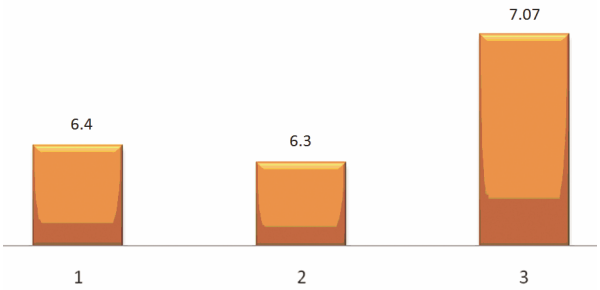

Fig. 5 – Average satisfaction score with monthpieces tested.
1 – Mares universal mouthpiece;
2 – SEAC SUB mouthpieces;
3 – Mares LiquidSkin mouthpiece.

Commercially available mouthpieces used in this study are all made out of silicone which is thought to be a better material choice than rubber. While other two mouthpieces tested in this study, No 1 and No 2 (Mares Universal and Seac Sub, respectively) are stiffer, which was often mentioned in the fulfilled questionnaires, Mares LiquidSkin is, according to the manufacturer, made out of two kinds of very soft silicone. Interdental bite platforms are made out of softer silicone while the rest of the mouthpiece is also soft and very flexible and because of that they can adapt to the vestibule of the mouth easily. It seems that the main reason behind such good ranks of mouthpiece No 3 (Mares LiquidSkin) is its softness. Very strong bite into the interdental platforms is usual during the dive – it can be caused by the cold water, exhilaration or anxiety and it puts high pressure on the TMJ. Biting into a softer material to keep the mouthpiece in place causes less muscle tension, less jaw fatigue, pain, muscle and jaw stiffness and less discomfort during saliva swallowing making in this way a dive more pleasant.

Another important factor in the design of a mouthpieces is the length of the interdental bite platforms and its position. Interdental platforms in many, if not almost all commercially available mouthpieces, reach from canines to second premolars or mesial surface of the first molar, and thus do not provide support for the posterior teeth. Increased pressure, specifically on canines and premolars distributes inadequately the occlusal force to the TMJ and masticatory muscles which can cause the inflammation of the TMD. The distribution of the occlusal force is greatest at the molar region – molars have the largest occlusal and root surface.

Some authors recommend that interdental platform should stretch from premolars to molars [8, 10–12], while some had an opinion that it was necessary to include canines, premolars and molars to reduce stress on the temporomandibular joints. Increased pressure on the interdental platform was due to efforts to keep that part in the mouth during the dive [13] and it was considered that this caused higher prevalence of TMD in inexperienced divers [7]. In a study conducted on a large number of participants, it was found that in 44.1% of subjects, who were without disorders before dive, symptoms in stomatognathic system occured as a result of a strong bite on the interdental platform [14].

Oral screen, which gets inserted in the vestibule, can cause discomfort during the dive. Oral screen should not be wide, or it could interfere with the upper and lower frenula and cause pain and discomfort. Mouthpiece No 2 (Seac Sub), ranked as the last, has an oral screen a few mm wider than others which has largely contributed to its poor rank. The scuba divers also noted that this mouthpiece was too big and too wide. Wide and stiff oral screens can lead to gingival injuries and irritations which could later cause wounds and gingival hyperplasia [15]. Although fully customized mouthpieces are considered to be the best, it is possible to find an adequate alternative. A well designed commercially available mouthpiece would have to fulfil several requirements: type of material, its softness and flexibility, length and thickness of the interdental platform, width of the oral screen and its overall size.

It is believed that it is necessary that dentists perform periodic monitoring of the situation in the mouth of the divers in order to prevent the consequences that may appear in the joints, muscles and other tissues in the oral cavity. For the health of the oral structure, a design of scuba diving equipment is very important, especially the part in the mouth of divers, which, if is inadequate, can lead to deterioration of oral health [16].

Conclusion

It is essential that every scuba diver, whether it is engaged in professional or recreational scuba diving, try more mouthpieces which are available in the market and choose for themselves the most appropriate ones. The further research are of great importance in order to improve the design of the mouthpiece with the aim to reduce the possibility of occurrence and progressive disease of the stomatognathic system and thus contribute to maintaining the health of the individual.

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Appendix 1

THE FIRST PART: QUESTIONNAIRE BEFORE DIVING

Participant's number: _____
Name, Surname: _____
Date of birth: _____
Professional qualifications, profession: _____
Gender: M F

1. Status of teeth

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

T - intact tooth
A - anodontia
EX - extraction
RR - radix relicta
F – filling plomba
CR - crown
P - pontic
C – cantilever
IN - inlay
OV - overlay
D - dentures tooth
CL - clasp
SC - surveyed crown
DC - double crown
Ca - caries
S - sealant
IM - implant

The difference in height of rest position and maximum intercuspation	___ mm
The width of the upper incisors	___ mm
The front width of the dental arch 4-4	___ mm
The rear width of the dental arch 6-6	___ mm
Front length of the dental arch	___ mm
Front length of the dental arch	___ mm
The back length of the dental arch	___ mm
Height palate	___ mm

2. How many years have been you diving? __ __ year(s) __ __ month(s)

3. How often do you dive?

1–10 per year	1
11–20 per year	2
21–30 per year	3
31–40 per year	4
41–50 per year	5
51–60 per year	6
61–70 per year	7
71–80 per year	8
More than 80 per year	9

4. Do you dive over the whole year or only in season (summer time)?

over the whole year	1
in season (summer time)	2

5. Do you use commercial (standard) or personal (adjusted) mouthpiece ?

commercial	1
personal (adjusted)	2

6. (For those who use personal (adjusted) mouthpiece) Have you ever used a commercial (standard) mouthpiece ?

No	0
Yes	1

7. How long have you been using commercial mouthpiece ? ___ year(s) ___ month(s)

8. Rate on the scale 0 to 10 how much you feel satisfied with your mouthpiece, where 0 – very displeased, 10 – very pleased

Very displeased Very pleased

0 1 2 3 4 5 6 7 8 9 10

Questions	Every day	During the dive	After the dive
9. Have you ever felt problems in temporomandibular joint (TMJ)?	No 0 Yes 1	No 0 Yes 1	No 0 Yes 1
10. Have you ever felt pain in the face?	No 0 Yes 1	No 0 Yes 1	No 0 Yes 1
11. Have you ever felt a headache?	No 0 Yes 1	No 0 Yes 1	No 0 Yes 1
12. Have you ever felt a neck pain?	No 0 Yes 1	No 0 Yes 1	No 0 Yes 1
13. Have you ever felt a ear pain?	No 0 Yes 1	No 0 Yes 1	No 0 Yes 1
14. Have you ever felt buzzing in the ears?	No 0 Yes 1	No 0 Yes 1	No 0 Yes 1
15. Have you ever felt pain in chewing muscles?	No 0 Yes 1	No 0 Yes 1	No 0 Yes 1
16. Have you ever felt pain in jaws?	No 0 Yes 1	No 0 Yes 1	No 0 Yes 1
17. Have you ever felt of face/jaws stiffness?	No 0 Yes 1	No 0 Yes 1	No 0 Yes 1
18. Have you ever felt face/jaws muscule fatigue?	No 0 Yes 1	No 0 Yes 1	No 0 Yes 1
19. Have you ever had problems to open your mouth as you wish, due to jaws joint/chewing muscule pain?	No 0 Yes 1	No 0 Yes 1	No 0 Yes 1
20. Have you ever had problem with opening your mouth due to jaw joint/chewing muscule pain?	No 0 Yes 1	No 0 Yes 1	No 0 Yes 1
21. Have you ever noticed “clicks” in jaws joint?	No 0 Yes 1	No 0 Yes 1	No 0 Yes 1
22. Have you ever had problems or feel discomfort while (when) chewing food, due to jaw joint pain?	No 0 Yes 1	No 0 Yes 1	No 0 Yes 1
23. Have you ever felt discomfort while swallow saliva?	No 0 Yes 1	No 0 Yes 1	No 0 Yes 1
24. Have you ever felt dry mouth?	No 0 Yes 1	No 0 Yes 1	No 0 Yes 1

The study participants filled out the second part of the questionnaire after the test dives and it was related to comfort, discomfort and occurrence of different potential symptoms in the orofacial region during and after the dive, (Appendix 2).

Appendix 2

THE SECOND PART: QUESTIONNAIRE AFTER TESTING THE MOUTHPIECE

Participant's number:

1. Rate on the scale 0 to 10 how much you feel satisfied with your mouthpiece, where 0 – very displeased, 10 – very pleased

Very displeased Very pleased

0 1 2 3 4 5 6 7 8 9 10

Questions	During the dive	After the dive
2. Have you ever felt pain in chewing muscles?	No 0 Yes 1	No 0 Yes 1
3. Have you ever felt pain in jaws?	No 0 Yes 1	No 0 Yes 1
4. Have you ever felt pain of face/jaws or stiffness?	No 0 Yes 1	No 0 Yes 1
5. Have you ever felt face/jaws muscule fatigue?	No 0 Yes 1	No 0 Yes 1
6. Have you ever felt discomfort while swallow saliva?	No 0 Yes 1	No 0 Yes 1
7. Have you ever felt dry mouth?	No 0 Yes 1	No 0 Yes 1

8. How easy can you breath with this mouthpiece compared to the commonly used one?

Smaller Same Bigger
1 0 2

9. Have you had problems with ear compensation while diving with this kind of mouthpiece?
Yes 1 No 0

10. Please write any suggestion and idea you may have after mouthpieces testing

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Risk factors for developing fear of falling in the elderly in Serbia

Faktori rizika od nastanka straha od pada kod starih osoba u Srbiji

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Abstract

Background/Aim. After a fall, the elderly can develop a fear of falling which can be more frequent and more serious problem than the fall itself because it represents the main factor limiting an older person in his/her everyday functioning. The aim of this study was to identify and examine, in a more detailed way, risk factors for developing fear of falling triggered by the history of their falls over the previous year in the elderly in Serbia who live in houses or apartments. **Methods.** The cross-sectional study was conducted on 400 people, mean age 75.04 years (min. 65, max. 94), randomly selected from the register of patients in the Primary Health Center of Niš (164 men and 236 women), in the period January-June 2014. Socio-demographic questionnaire – Elderly Fall Screening Test (EFST), Multi-Factor Questionnaire Falls (MFQ) and Falls Efficacy Scale International (FESI) were used in this study. **Results.** After applying the multivariate binary logistic regression, it was found that significant predictors for developing fear of falling were as follows: female gender (OR = 2.599; p = 0.009), age 75–79 years (OR = 4.637; p = 0.009) and over 80 years (OR = 3.830; p = 0.001), increase in household members (OR = 1.206; p = 0.033), people who estimate their health as average (OR = 0.268; $p < 0.001$) and good (OR = 0.059; $p < 0.001$), number of falls higher than 2 (OR = 2.761; p = 0.003), presence of injuries during the fall (OR = 2.483; p = 0.028), periodical and repeating situations of "near-fall" (OR = 3.830; p = 0.001), limited activity (OR = 2.124; p = 0.007) problems with sight (OR = 3.006; $p < 0.001$), cognitive problems (OR = 2.296; p = 0.005) and problems with balance (OR = 3.809; $p < 0.001$). **Conclusion.** The results of this study can be used for planning promotional programs for falls and a fear of falling prevention, as well as prevention of their consequences.

Key words:
aged; aged, 80 and over; accidental falls; fear; risk factors; serbia.

Apstrakt

Uvod/Cilj. Nakon pada, stare osobe mogu da razviju strah od pada koji može biti učestaliji i ozbiljniji problem od pada jer postaje glavni faktor ograničavanja stare osobe u svakodnevnom funkcionisanju. Cilj ove studije bio je da se otkriju faktori rizika za nastanak straha od pada kod starih osoba u Srbiji koje žive u kući ili stanu u odnosu na istoriju pada u poslednjih godinu dana. **Metode.** Studija preseka sprovedena je na 400 osoba odabranih putem slučajnog uzorka iz registra Doma zdravlja Niš (164 muškaraca i 236 žena), prosečne starosti 75,04 godina (min. 65, maks. 94 godine) u periodu januar-jun 2014. godine. U istraživanju je korišćen: socio-demografski upitnik, Skrining test za pad kod starih osoba – *Elderly Fall Screening Test* (EFST), upitnik za procenu više faktora rizika za pad – *Multi-factor Falls Questionnaire* (MFQ), skala za procenu straha od pada – *Falls Efficacy Scale International* (FESI). **Rezultati.** Multivarijantnom binarnom logističkom regresijom kao značajni prediktori nastanka straha od pada izdvojili su se: ženski pol (OR = 2.599; p = 0.009), godine starosti od 75-79 (OR = 4.637; p = 0.009) i preko 80 godina (OR = 3.830; p = 0.001), porast broja članova domaćinstva (OR = 1.206; p = 0.033) osobe koje procenjuju svoje zdravlje kao prosečno (OR = 0.268; $p < 0.001$) i dobro (OR = 0.059; $p < 0.001$), broj padova veći od 2 (OR = 2.761; p = 0.003), prisutne povrede prilikom pada (OR = 2.483; p = 0.028), povremene i česte situacije „blizu" pada (OR = 3.830; p = 0.001), ograničenje aktivnosti (OR = 2.124; p = 0.007), problemi sa vidom (OR = 3.006; $p < 0.001$), kognitivni problemi (OR = 2.296; p = 0.005) i problemi sa ravnotežom (OR = 3.809; $p < 0.001$). **Zaključak.** Rezultati ove studije mogu da posluže za planirane promotivnih programa prevencije padova i straha od pada, a samim tim, i njihovih posledica.

Ključne reči:
stare osobe; stare osobe, 80 i više godina; padovi, slučajni; strah; faktori rizika; srbija.

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Introduction

Since the early 80s, researchers have believed that fear of falling is a result of psychological trauma due to a fall, which was known as "post-fall syndrome" or "ptophobia" (phobic reaction to standing or walking) [1]. A post-fall syndrome was first described in 1982 by researchers Murphy and Isaacs who noticed that the elderly developed intense fear and walking difficulties after the fall [2]. Since then, the fear of falling has been perceived as one of the main symptoms of falling occurences and considered a special health problem in the elderly [3]. Some studies showed that the fear of falling may also be present in the elderly who did not experience a fall itself [1, 3–5].

The presence of fear of falling in the elderly can cause serious emotional, psychological, medical and social changes such as reduced or limited functional activity, loss of autonomy and independence concerning the performance of daily living activities, reduced social activities as well as the feeling of weakness and insecurity [6, 7]. Moreover, a very old person, after the fall, may limit his/her everyday activities because of the fear of another fall and reduce the activities recommended as a protective measure by his/her family and health workers. In this way he/she can only cause a loss of independence and reduce social interactions, which, in turn, lead to physical inactivity and reduced quality of life [8–10]. Kang [11] also confirmed this in his work stating that the old people, who have experienced a fall, reported that they did not have the will to perform daily activities because of the presence of fear of falling. He further explained that limited mobility deteriorated sustainability of muscle strength, balance, and therefore increased a risk of fall, which eventually resulted in a vicious circle that was difficult to get out of.

That is to say, an old person who suffers fall may subsequently develop a fear of further falls [12]. On the other hand, the fear of falling was identified as an independent risk factor for reduced quality of life, limiting activities, loss of independence, the leading cause of falls and injury caused by falls, morbidity and mortality [13]. Thus, the development of one syndrome can initiate "cascading changes", where the accumulated effects lead to the spread of the effects on all levels of functioning [14] and result in the escalation of the risk of functional decline and other adverse outcomes [15]. Moreover, the fear of falling is more common in older people living in a house or apartment, with an assessment of the frequency of falls within the range between 29% and 77% [13]. Up to 55% of elderly people in the general population report they fear of falling and the prevalence is higher among the elderly female patients with previous history of falls as well as among people with certain comorbidity [16].

Fear of falling and fall are not directly related and they result from the action of basic common risk factors including socio-demographic factors [17], history of falls [14], medical conditions (e.g., arthritis [18], osteoporosis [19], the visual problems [20, 21], problems with urination [22], balance and walking disorders [10, 23] cognitive status [24], orthostatic hypotension [25]).

In this respect, if the fall and fear of falling share the risk factors listed, this means that old people with these characteristics are at an increased risk of fall and developement of fear of falling. This may be of a particular importance to the primary health care doctors and visiting nurses, so that they can identify the elderly with the aforementioned risk factors as well as to the implementation of targeted programs of prevention of falls and developing fear of falling.

Methods

Respondents

The sample represents 400 respondents aged 65 years or more (164 men and 236 women) who live in the city of Niš.

At the time of this research, there was no relevant information on the incidence we were examining to determine the maximum size of the sample. We started from the variability of occurrence of 50% [26]. Taking this into account as well as the fact that based on a comparative review for 2009 of Patronage service of the Primary Health Center of Niš, the total number of people on the territory of the city of Niš was 255,479 and 44,378 people were 65 years old, or more), we came to the information that the sample of 384 respondents was sufficient for the necessary research. The respondents were selected randomly, using a table of random numbers from the registry of patients in the Primary Health Center of Niš. The criteria for being involved into the study were: age 65 and over, living in a house or apartment, the ability to understand and follow instructions, being mobile with or without the aid. The criteria for exclusion from the study were: age under 65, inability to understand and to follow instructions and immobility. The criterion for exclusion of a patient from the study was the consent of a respondent to be excluded from the research.

Study design

This was the cross-sectional study in which data was collected by interviewing the respondents. Data collection was conducted by a researcher through home visits and in the presence of visiting nurses. The research was conducted in the period of January–June 2014.

Instruments

The general Socio-demographic questionnaire contains 8 questions which refer to age, gender, place of living, marital status, level of education, satisfaction with income, assessment of health and number of household members.

Fall screening test for the fall in the elderly over 65 years of age – Elderly Fall Screening Test (EFST) is designed to detect a risk level of a fall and it contains 5 items: history and the number of falls, injuries caused by falling, 'near-fall' experience and current walking function (estimation of gait and the form of walking) [27]. In a study done by Cwikel et al. [28], it was found that the sensitivity of this test reached 83% and specificity 69%. The values of each question (0 points – there is no risk of a fall or 1 point – there is a risk of a fall) were summed up giving a total score between 0

and 1 which categorized people as with no or low risk of fall while a score ≥ 2 categorized people as with moderate or high risk for fall. Fall Screening Test in this study showed satisfactory reliability of low intensity (Cronbach's α = 0.76).

In order to get an insight into disorders of health conditions, the questionnaire for the assessment of multiple risk factors regarding a fall – Multi-factor Falls Questionnaire (MFQ) was used [28]. Total MFQ result was calculated as the sum of points obtained from each group of the risk factors with a fall involved. If the total score was ≤ 3, a respondent was categorized with the low risk of fall, and if the result was more than 3, it was considered that a person was at high risk of falling. In this study, MFQ did not show a satisfactory reliability (Cronbach's α = 0.48).

For the assessment of fear of falling the International scale of concern regarding a fall – Falls Efficacy Scale International (FES-I) was used and its reliability was proved in studies conducted by Delbaere et al. [29]. Each item was evaluated on a scale from 1 (not at all concerned) to 4 (very concerned). In order to obtain a total score for the FES-I, the scores on all items were simply added up together and the total value ranged from 16 (no concern about falling) to 64 (seriously concerned about falling). In the examined sample, the FES-I showed satisfactory reliability of high intensity (Cronbach's α = 0.99).

Independent variables

Socio-demographic data taken into consideration in this study were gender, age, marital status, place of residence, education level, income satisfaction, health assessment and community life. The four age groups were set as follows: a) 65 to 69, b) 70 to 74 c) 75–79 and d) 80 years and more. Based on the questions from the EFST about the history of falls, a variable "number of falls in the last 12 months: 0–1 and 2 or more injuries due to falls and 'near-fall' situations was constructed. Regarding health disorder condition, the following variables were assessed: limitation of activity due to falling, vision problems, symptoms of cognitive problems, balance problems, walking / mobility problems, arthritis, osteoporosis, orthostatic hypotension, the use of aids and micturition problems.

Ethics

This study by the Ethical Committee of the Faculty of Medicine, University of Novi Sad, Ethical Committee of Primary Health Center Niš, and the managing director of the Primary Health Center Niš.

Statistical analysis of the data

The obtained data was processed in the software package, Statistical Package for the Social Sciences 20.00, version 20 (SPSS). The Cronbach's α coefficient was used to determine the reliability of the tests. Descriptive statistics and statistics for conclusion were used to analyse the data. Techniques of descriptive statistics, that is, arithmetic mean and standard deviation were used to determine the severity of basic research variables. The logistic regression analysiswas used to identify predictors of fear and the results were presented as odds ratio (OR) with 95% confidence interval (95% CI) and *p*-value. All parameters were analyzed in the univariate model; the statistically significant ones were included in a multivariate analysis. Statistical significance was set on the level of 0.05.

Results

The study included 400 respondents, out of whom there were 41% of men and 59% women. The age of respondents was between 65 and 94 years, with an average 75.04 years (SD ± 5.85). The average age of men was 74.81 years (SD ± 5.77), while of the women was 75.20 years (SD ± 5.91). Most of the respondents were married (66%) and lived in the city (53.5%) while more than a half of the participants reported that they lived alone (52.8%). Depending on the education, the most frequent category of respondents had elementary education, and the fewest number of the respondents had university education. Assessment of health status ranged from poor – 38.8%, average – 45.8% and good –15.5%. More than a half of the participants (79%) said that they were not satisfied with the income, i.e., it did not meet their needs.

With the aim of determining independent socio-demographic risk factors for developing the fear of falling using the univariate logistics regression analysis, the following variables were identified as important: gender, age, marital status, permanent residence, place of living, level of education, number of household members, satisfaction with the income and health assessment (Table 1). Results showed that the probability for developing the fear of falling was 2.5 times greater in women (OR = 2.586; $p < 0.001$) and that it was significantly increasing with age: from 70–74 years of age the probability increased more than two times (OR = 2.438; $p = 0.005$), from 75–79 years (OR = 5.839; $p < 0.001$) and over 80 years (OR = 5.676; $p < 0.001$) even over 5 times. The probability for fear of falling was 1.7 times greater in the widowed (OR = 1.737; $p = 0.016$), in comparison to people who had a marital partner. Living in the countryside was predictors of developing the fear of falling (OR = 0.579; $p = 0.007$) as well as primary school education (OR = 0.289; $p < 0.001$). With every household member the probability of developing the fear of falling increased by 14.8% (OR = 1.148; $p = 0.048$). People who were not satisfied with their income feel 2.3 times greater fear of falling compared to those who were satisfied (OR = 2.307; $p = 0.007$). People who estimated their health as average (OR = 0.227; $p < 0.001$) and good (OR = 0.041; $p < 0.001$) were at a lower risk of falling than people who estimated their health as bad.

The listed independent socio-demographic variables were included in the multivariate logistics model with fear of falling as an dependent variable (Table 2). The whole model with all predictors was statistically significant ($\chi^2 = 148.56$, $p < 0.001$) and it explained, in whole, the variances of fear of falling between 31.0% (Cox and Snell R-Squared) and 41.6% (Nagelkerke R-Squared).

Table 1

Sumary of univariate regression for socio-demographic predictor variables

Variable	No fear n (%)	Fear n (%)	OR	95% CI	*p*
Gender					
[men]	95 (53.7)	69 (30.9)	/	/	/
women	82 (46.3)	154 (69.1)	2.586	1.716–3.895	< 0.001
Age (years)					
[65–69]	61 (34.5)	24 (10.8)	/	/	/
70–74	49 (27.7)	47 (21.1)	2.438	1.313–4.527	0.005
75–79	37 (20.9)	85 (38.1)	5.839	3.173–10.746	< 0.001
≤ 80	30 (16.9)	67 (30.0)	5.676	2.996–10.755	< 0.001
Marital status					
[married]	132 (74.6)	139 (62.3)	/	/	/
not married	0 (0.0)	1 (0.4)	1.369	0.005–3.658	0.965
divorced	4 (2.3)	8 (3.6)	1.899	0.559–6.475	0.304
widower/widow	41 (23.2)	75 (33.6)	1.737	1.109–2.5722	0.016
Residence					
country side	69 (39.0)	117 (52.5)	/	/	/
city	108 (61.0)	106 (47.5)	0.579	3.888–0.864	0.007
Place of living					
[house]	156 (88.1)	205 (91.9)		/	/
flat	21 (11.9)	18 (8.1)	0.652	0.336–1.266	0.207
Level of education					
[primary school]	92 (52.0)	176 (78.9)	/	/	/
> primary school	85 (48.0)	47 (21.1)	0.289	0.187–0.447	< 0.001
Number of household members, mean ± SD	2.35 ± 1.25	2.65 ± 1.67	1.148	1.002–1.316	0.047
Satisfaction with income					
[yes]	31 (17.5)	20 (9.0)	/	/	/
no	127 (71.8)	189 (84.8)	2.307	1.259–4.226	0.007
partially	19 (10.8)	14 (6.3)	1.142	0.469–2.782	0.770
Assessment of health					
[bad]	30 (16.9)	125 (56.1)	/	/	/
average	94 (53.1)	89 (39.9)	0.227	0.139–0.372	< 0.001
good	53 (29.9)	9 (4.0)	0.041	0.018–0.092	< 0.001

[] – reference group; SD – standard deviation; OR – odds ratio; CI – confidence interval.

Table 2

Sumary of multivariate regression for socio-demographic predictor variables

Variable	OR	95% CI	*p*
Gender			
female	2.093	1.716–3.895	0.009
Age (years)			
[65–69]	/	/	/
70 –74	1.823	1.313–4.527	0.099
75–79	4.637	3.173–10.746	< 0.001
≤ 80	3.830	2.996–10.755	0.001
Marital status			
widower/widow	1.223	1.109–2.5722	0.489
Permanent residence			
[countryside]	/	/	/
city	0.630	3.888–0.864	0.079
Place of living			
house	/	/	/
flat	0.652	0.336–1.266	0.207
Level of education			
[primary school]	/	/	/
> primary school	0.581	0.187–0.447	0.070
Number of household members	1.206	1.002–1.316	0.033
Satisfaction with income			
[yes]	/	/	/
no	1.159	1.259–4.226	0.653
Health assessment			
[bad]	/	/	/
average	0.268	0.139–0.372	< 0.001
good	0.059	0.018–0.092	< 0.001

[] – reference group; OR – odds ratio; CI – confidence interval.

The following variables gave a unique statistically significant contribution to the mode: female gender (OR = 2.599; p = 0.009), people older than 75–79 years (OR = 4.637; p = 0.009) and over 80 (OR = 3.830; p = 0.001), an increase in household members (OR = 1.206; p = 0.033), people who estimated their health as average (OR = 0.268; p < 0.001) and good (OR = 0.059; p < 0.001).

Results of the EFST showed that the entire population of 400 respondents was processed. One third of the respondents, that is, 149 (37.70%), reported that a fall occurred two or more times, 246 (61.50%) respondents had an injury after the fall while 271 (67.80%) respondents was periodically or frequently in a 'near-fall' situation.

With the univariate logistics regression analysis, with fear of falling as an dependent variable, the probability of potential independent risk factors from the EFST was analyzed: history of falls, number of falls, injuries during a fall and situations "near-fall" (Table 3). All tested variables showed high statistical significance: people with a positive history of falls were almost in 5 times greater risk of developing a fear of a new fall (OR = 4.637; p < 0.001) while people who had two or more falls fear had 6 times more higher risk for developing a fear of a new fall (OR = 6.365; p < 0001) than people who had a smaller amount of falls or did not have them at all. People who injured themselves during a fall were in 4 times greater risk of developing a fear of a new fall (OR = 6.365; p < 0.001) whereas people who were periodically or frequently in a 'near-fall' situation are in a 5 times greater risk for fear of a new fall (OR = 5.036; p < 0.001).

In the multivariate logistics model, all statistically significant individual variables for the development of fear of falling were included and analyzed from the univariate analysis (Table 4). The whole model with all predictors was statistically significant (χ^2 = 95.10, p < 0.001) and in whole explained variances of fear of falling between 21.2% (Cox and Snell R-Squared) and 28.3% (Nagelkerke R-Squared). The following variables gave a unique statistically significant contribution to the mode: number of falls higher than 2 (OR = 2.761; p = 0.003), presence of injuries during a fall (OR = 2.483; p = 0.028) and periodical and frequent situations of 'near-fall' (OR = 3.830; p = 0.001).

Results from the MFQ showed that the fear of falling was present in a high percentage of respondents who had limited their activities (64.80%), had problems with sight (54.40%), had indications of cognitive problems (72.60%), problems with balance (79%), gait (70.50%), arthritis (39.10%), osteoporosis (6.76%), orthostatic hypotension (77%), used walking aids (59%), and had problems with urination (50.20%).

Table 3

Sumary of univariate regression for predictor variables from the Elderly Fear Screening Test

Variable	No fear n (%)	Fear n (%)	OR	95% CI	p
History of falls					
[negative]	117 (66.1)	64 (28.7)	/	/	/
positive	60 (33.9)	159 (71.3)	4.845	3.166–7.414	< 0.001
Number of falls					
[0–1]	147 (83.1)	97 (43.5)	/	/	/
≥ 2	30 (16.9)	126 (56.5)	6.365	3.964–10.220	< 0.001
Injuries during a fall					
[no]	142 (80.2)	104 (46.6)	/	/	/
yes	35 (19.8)	119 (53.4)	4.205	1.984–8.914	< 0.001
Situations 'near-fall'					
[never or rarely]	90 (50.8)	38 (17.0)	/	/	/
periodically and often	87 (49.2)	185 (83.0)	5.036	3.189–7.953	< 0.001

[] – reference group; OR – odds ratio; CI – confidence interval.

Table 4

Sumary of multivariate regression for predictor variables from the Elderly Fear Screening Test

Variable	OR	95% CI	p
History of falls			
[negative]	/	/	/
positive	1.662	0.908–3.043	0.100
Number of falls			
[0–1]	/	/	/
≥ 2	2.761	1.422–5.363	0.003
Injuries during a fall			
yes	/	/	/
no	2.483	1.105–5.578	0.028
Situations 'near fall'			
[never or rarely]	/	/	/
periodically and often	2.683	1.616–4.457	< 0.001

[] –reference group; OR – odds ratio; CI – confidence interval.

Table 5

Sumary of univariate regression for predictor variables from the Multi-factor Falls Questionaire

Variable	No fear n (%)	Fear n (%)	OR	95% CI	*p*
Limitation of activities					
[no]	130 (73.4)	73 (32.7)	/	/	/
yes	47 (26.6)	(67.3)	5.683	3.677–8.784	< 0.001
Visual problems					
[no]	150 (84.7)	92 (41.3)	/	/	/
yes	27 (15.3)	131 (58.7)	7.911	4.852–12.898	< 0.001
Indication of cognitive problems					
[no]	103 (58.2)	56 (25.1)	/	/	/
yes	74 (41.8)	167 (74.9)	4.151	2.713–6.350	< 0.001
Balance problems					
[no]	141 (79.7)	53 (23.8)	/	/	/
yes	36 (20.3)	170 (76.2)	8.993	5.283–15.307	< 0.001
Walking problems					
[no]	150 (84.7)	102 (45.7)	/	/	/
yes	27 (15.3)	121 (54.3)	4.467	2.593–7.694	< 0.001
Arthritis					
[no]	131 (74.0)	123 (55.2)		/	/
yes	46 (26.0)	100 (44.8)	2.315	1.510–3.549	< 0.001
Osteoporosis					
[no]	168 (94.9)	182 (81.6)	/	/	/
yes	9 (5.1)	41 (18.4)	5.628	1.978–16.014	0.001
Orthostatic hypotension					
[no]	147 (83.1)	141 (63.2)	/	/	/
yes	30 (16.9)	82 (36.8)	2.850	1.767–4.595	< 0.001
Use of aids					
[no]	165 (93.2)	145 (65.0)	/	/	/
yes	12 (6.8)	78 (35.0)	4.151	2.713–6.350	< 0.001
Urination problems					
[no]	110 (62.1)	100 (4.8)	/	/	/
yes	67 (37.9)	123 (55.2)	2.019	1.350–3.020	0.001

[] –reference group; OR – odds ratio; CI – confidence interval.

Table 6

Sumary of multivariate regression for predictor variables from the multi-factor falls questionaire

Variable	OR	95% CI	*p*
Limitation of activities			
[no]	/	/	/
yes	2.124	1.223–3.689	0.007
Visual problems			
[no]	/	/	/
yes	3.006	1.284–4.106	< 0.001
Indication of cognitive problems			
[no]	/	/	/
yes	2.296	2.080–6.976	0.005
Balance problems			
[no]	/	/	/
yes	3.809	0.913–3.352	< 0.001
Walking problems			
[no]	/	/	/
yes	1.749	0.691–2.197	0.092
Arthritis			
[no]		/	/
yes	1.224	0.913–3.352	0.488
Osteoporosis			
[no]	/	/	/
yes	2.194	0.837–5.753	0.110
Orthostatic hypotension			
[no]	/	/	/
yes	1.660	0.879–3.143	0.118
Use of aids			
[no]	/	/	/
yes	1.749	0.812–3.813	0.152
Urination problems			
[no]	/	/	/
yes	1.053	0.607–1.829	0.854

***p* < 0.001; [] –reference group; OR – odds ratio; CI – confidence interval.**

In order to determine the risk factors for the development of fear of falling from the MFQ, the univariate analysis was applied first (Table 5). All analyzed variables of health conditions represented predictors for the fear of falling: limited activity (OR = 5.683; $p < 0.001$), problems with sight (OR = 7.911; $p < 0.001$), indications of cognitive problems (OR = 4.151; $p < 0.001$), problems with balance (OR = 8.993; $p < 0.001$), problems with gait (OR = 4.467; $p < 0.001$), arthritis (OR = 2.315; $p < 0.001$), osteoporosis (OR = 5.628; $p < 0.001$), orthostatic hypotension (OR = 2.850; $p < 0.001$), the use of walking aids (OR = 4.151; $p < 0.001$) and problems with urination (OR = 2.019; $p = 0.001$).

The listed variables from the MFQ were included in the multivariate logistics model with the fear of falling as a dependent variable (Table 6). The whole model with all predictors was statistically significant ($\chi^2 = 196.570$, $p < 0.001$) and explained, in whole, the variances of the fear of falling between 38.8% (Cox and Snell R-Squared) and 52.0% (Nagelkerke R-Squared). The following variables gave a unique statistically significant contribution to the mode: limited activity (OR = 2.124; $p = 0.007$), problems with sight (OR = 3.006; $p < 0.001$), cognitive problems (OR = 2.296; $p = 0.005$) and problems with balance (OR = 3.809; $p < 0.001$).

Discussion

The need for this research has emerged due to changes in the demographic structure of the population and growing number of old people in our population. The aging of popu-

lation, the growing prevalence of chronic diseases and limited financial resources for health care highlighted the importance and significance of health prevention and the need for longer independence of the elderly, because of the consequences which could lead to overburdened health care system, an increase of costs and the lack of resources [30]. The fear of falling was reported by the elderly in the general population as the most important concern, even more than the fear of being robbed, being in a financial difficulty, or the fear from a serious medical problem [31].

To our knowledge, there are no programs in our country for the prevention of falls and developing a fear of falling in the elderly based on scientific evidence and this was the reason for such a research. In this study the variables, for which it is established that are important predictors for the development of the fear of fall, support the results in literature in other cross-sectional studies. Models of the univariate binary logistic regression identified variables that could predict the development of fear of falling. Even though many risk factors are connected with the fear of falling, results of the multivariate binary logistic regression showed that only a few independent variables could be proven as predictors for the development of the fear of falling; in many studies, it was found that an increase of age was an important risk factor for the development of the fear of falling [8, 32–34]. The results of this study showed that the presence of the fear of falling increased with age and that it was, in its highest percentage (38.1%), present at the age of 75–79 years. A recent study conducted in Brazil showed similar values where the highest frequency of the fear of falling also appeared at the age of 70 to 79 years (47%) [35]. So, the age proved to be a significant predictor for the occurrence of developing the fear of falling at the age of 70–79 and in the group aged 80 years and more. When it comes to gender structure, in this study, across the overall population of those who feel the fear, a large percentage belonged to women (69%), which points out that women are at an increased risk to develop the fear of falling compared to men who were afraid of further falls – and these results match the results in many studies (24%) [9], (69%) [36], (26%) [37]. The increase in number of household members showed to be one of the risk factors for the development of fear of falling. These kind of results are at odds with the results achieved in the world where the elderly who live alone are at a higher risk of falling and developing the fear of falling [38]. The reason for obtaining such results lies in the fact that there are no studies which have included the number of household members in the analysis. Results of this study can be used in further research for several reasons. The Republic of Serbia is a country in transition and there still exists large families with traditional divison of household members roles. The existence of stereotypes about the elderly as "people who cannot work" and their disengagement has, as a consequence, the decrease in needs, withdrawal and decrease in contacts and interactions and all the way to social isolation [39]. The fear of falling and restrictions of activities make the elderly play a role in "transition towards physical weakness" [40]. The results of this study showed that the fear of falling was present in a high percentage in the respondents with limited activities (64.80%), but the role of an elderly person in a large family was not examined. Earlier studies also proved that there was a correlation among self-assessed health, mortality, morbidity, socio-demographic characteristics, chronic diseases and disabilities. It is confirmed in literature that for older people a positive perception of health means a better quality of life [40]. The results of this study showed that the assessment of health was an important risk factor for the development of fear of falling but only in people who estimated their health as bad. These results are in accordance with the results in relevant literature.

Many researchers have so far been consistently associating the history of falls with the occurrence of the fear of falling [8, 9, 37, 41]. Even though in this study the fear of falling was present in more than a half of the respondents with a positive history of falls, the analysis of the results showed that the history of falls was important issue for the development of the fear of falling only in two or more falls and these results were in accordance with the results appearing in most studies where history of falls was noted as an independent risk factor for the development of the fear of falling [42]. Except the number of falls, injuries during a fall in this study presented themselves as predictors for the development of the fear of falling. These results are in accordance with the facts that the older people who had experienced more falls and were injuried then, had a higher risk of developing the fear of falling compared to those who fell only once [37, 43]. When it comes to one fall, it is considered that many older people have this experience in their close surroundings but a history of more falls points out to some chronic problems and increases a risk of other numerous negative outcomes, including injuries and mortality [37]. The fear might not come right after the first fall, but it can begin after more falls [37]. Such is the case in "near-fall" situations that are described as independent factors of future falls and it was confirmed that older people who reported two or more similar situations, were in the twice higher risk for another fall [44]. In this study, older people who periodically or frequently had a "near-fall" situations were in the fear of falling and that means that their "near-fall" situations are predictors for developing the fear of falling. In a recently published research, it has been discovered that the fear of falling and falls themselves share similar set of risk factors [14].

Health conditions are another important predictor for the development of the fear of falling. Many health conditions (eg., arthritis [18], osteoporosis [19], sight problems [20, 21], problems with micturition [22], balance and gait disorders [10, 23], cognitive status [24] and orthostatic hypotension [25]) can increase the risk of falling and fear of falling. Results of this study, based on the MFQ, showed that in a high percentage, the fear of falling was present in the respondents who limited their activities, had problems with sight, showed indications of cognitive problems and problems with balance. In many researches, the achieved results pointed out that respondents with individual health conditions (eg., arthritis, osteoporosis, visual and cognitive damages) often felt fear of falling [14, 30, 41]. This research confirms the facts found in the literature, except in the presence of arthritis, osteoporosis, problems with micturition and orthostatic hypotension. A fear of falling is the main factor which limits older persons in their everyday functioning. They are socially isolated and who experience a loss of independence which, as a consequence, further influence their quality of life [45]. Besides, problems in balance can disrupt functional independence and they can even lead to tem-

porary accommodation in a nursing home. Fear of falling is connected to disorders in balance and can contribute to a high risk while walking, because of the feeling of instability which is, in turn, another important predictor for developing the fear of falling [37]. In this study, balance disorders was shown as a predictor of developing the fear of falling. When we take into account that other predictors for falling occurence and developing the fear of falling showed significance, there is an impression that planned preventive programs for falls and developing the fear of falling in the general population are necessary in public health. It is important to emphasize that planned preventive programs for intervention for the elderly are based on improving physical self-efficacy and the level of mobility obstructed by feeling a strong fear during the performance of activities, and not by reducing the fear of falling [46].

Conclusion

Even though further research on developing a fear of falling needed significant variables were referenced in this study and they point to certain characteristics which identify people who are at a risk of developing the fear of falling.

Observed together, these results suggest that older females, people in the age group of 75–79 and over 80 years, people that live in a household with more family members and assess their health as average or good are at an increased risk of developing a fear of falling. Also, older people that report more than two falls and inflicted injuries, occasional or often "near-fall" situations, people who limit their activities due to a fall, have problems with sight and people with indications of cognitive problems and those with problems with balance have increased risk for developing fear of folling.

The results provide an opportunity to health workers to prevent falls and developing a fear of falling as well as to future researches to clearly define the impact of a fear of falling not only to future falls, but also the impact of the fear on functioning and loss of independence, and, finally, the impact on quality of life of the elderly.

Health workers cannot influence the age of a person, but other risk factors may be influenced in order to prevent the development of the fear of falling.

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Clinical and ultrasonographic features in anterior ischemic optic neuropathy

Klinička i ultrasonografska obeležja prednje ishemičke optičke neuropatije

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Abstract

Background/Aim. Anterior ischemic optic neuropathy (AION) represent a segmental infarction of the optic nerve head which is supplied by the posterior ciliary arteries. There are two types of AION: non-arteritic (NA-AION and arteritic (A-AION), due to giant cell arteritis (GCA). The aim of this study was to investigate the clinical features and ultrasound characteristics of the orbital vessels and superficial temporal and carotid arteries, in patients with unilateral acute AION in order to help differentiate newly diagnosed NA-AION from A-AION. **Methods.** In this prospective comparative, observational study, 62 consecutive patients with clinical suspicion of unilateral acute AION were examined at admission and in the first two months of evolution, following a protocol including color Doppler imaging (CDI) of the orbital vessels. **Results.** We found 12 patients with A-AION, all of them with biopsy-confirmed disease, and 50 patients with NA-AION. A-AION patients presented a combination of a history of amaurosis fugax before acute, painless, and severe vision loss in the affected eye, and a diffuse pale optic disc edema. In these patients, CDI of the orbital vessels indicated high resistance index (RI), with severe diminished blood flow velocities in all orbital vessels, in both orbits. In the NA-AION patients, none of these clinical symptoms were found and blood velocities and RI in posterior ciliary arteries were preserved. Typical sonographic feature in temporal arteritis as part of GCA was the „dark halo" sign. **Conclusions.** The ultrasound investigations enable prompt differentiation between NA-AION and A-AION.

Key words:
optic neuropathy, ischemic; risk factors; giant cell arteritis; diagnosis, differential; ultrasonography, doppler, color.

Apstrakt

Uvod/Cilj. Prednja ishemička optička neuropatija (AION) predstavlja segmentni infarkt papile optičkog nerva vaskularizovanog preko posteriorne cilijarne arterije. Postoje dva tipa AION: arterijska (A-AION), koja je gotovo bez izuzetka, posledica arteritisa dzinovskih ćelija (GCA) i nearterijska (NA-AION). Cilj rada je bio da se istraže klinička i ultrazvučna obeležja orbitalnih krvnih sudova, temporalne superficijalne arterije i karotidnih arterija, koja bi bila od pomoći u diferencijalnoj dijagnostici akutne AION. **Metode.** Prospektivnom, uporednom opservacionom studijom obuhvacena su 62 uzastopna bolesnika sa sumnjom na jednostranu akutnu AION, koji su bili ispitani prilikom prijema i tokom prva dva meseca evolucije bolesti prema protokolu koji je uključivao kolor dopler snimanje orbitalnih krvnih sudova. **Rezultati.** Kod 12 bolesnika je ustanovljena i biopsijom potvrđena A-AION, dok je kod 50 bolesnika ustanovljena NA-AION. Kod bolesnika sa A-AION bolest se manifestovala prisustvom *amaurosis fugax* pre pojave akutnog, bezbolnog gubitka vida teškog stepena u zahvaćenom oku i difuznim bledim edemom optičkog diska. Kolor doplerom orbitalnih krvnih sudova ustanovljen je ozbiljno smanjen protok krvi u posteriornim cilijarnim arterijama izraženiji na zahvaćenoj strani i praćen visokim indeksom rezistencije (IR) u svim retrobulbarnim krvnim sudovima obe orbite. Kod bolesnika sa NA-AION kolor doplerom nisu registrovane opisane promene. Tipično ultrazvučno obeležje u temporalnom arteritisu, kao delu GCA, bio je „dark halo" znak. **Zaključak.** Ultrazvučno ispitivanje omogućava postavljanje brze diferencijalne dijagnoze izmedu A-AION /NA-AION kod bolesnika sa akutnom AION.

Ključne reči:
neuropatija, optička, ishemička; faktori rizika; arteritis, temporalni; dijagnoza, diferencijalna; ultrasonografija, dopler, kolor.

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Introduction

Anterior ischemic optic neuropathy (AION) represent an acute ischemic disorder involving the anterior part of the optic nerve, also called the optic nerve head (ONH), supplied by the posterior ciliary arteries (PCAs) – nasals and temporals [1–7]. Blood supply blockage of the PCAs can occur with or without arterial inflammation. There are two types of AIONs: arteritic (A-AION), caused by a vasculitic mechanism due to giant cell arteritis (GCA), and non-arteritic (NA-AION) [1–7].

GCA is a primary vasculitis that concerns predominantly extracranial medium-sized arteries, especially branches of the external carotid artery (ECA), including the superficial temporal arteries (TAs) [1–8]. The typically predominant extracranial vascular involvement (including the orbital vessels) is explained by the affinity of inflammation to the elastic fibers in the media; as intracranial arteries have less elastic fibers in the media, they are seldom involved [3–8]. The diagnosis of GCA requires, according to the criteria established by the American College of Rheumatology, age more than 50 years at clinical disease onset, new headache in the temporal area, temporal artery tenderness, and/or reduced pulse, jaw claudication, systemic symptoms, erythrocyte sedimentation rate (ESR) exceeding 50 mm/h, elevated C-reactive protein (CRP), and typical histologic findings (granulomatous involvement) in temporal artery biopsy (TAB) [1–8]. Approximately 20% of patients with GCA have ophthalmologic complications, including visual loss secondary to A-AION, or central retinal artery occlusion [1–8]. These are generally early manifestations due to the vasculitic involvement of retrobulbar (orbital) vessels deriving from the ophthalmic artery (OA), more precisely the PCAs and the central retinal artery (CRA) [1–8].

NA-AION is a multifactorial disease that results in hypoperfusion and ischemia of the ONH, with multiple risk factors that contribute to its development (nocturnal arterial hypotension, etc) [1–7]. According to Biousse and Newman [1], the most important contributing factor to NA-AION is represented by congenital and physiologic small cups. They mentioned that the size of the ONH (optic disc) and the physiological cup depend on the size of the scleral canal; a small scleral canal will result in a small cup (with a crowded ONH and a small cup-to-disc ratio) [1, 2, 9]. The presence of a "disk at risk", with structural crowding of nerve fibers (crowded disk) and reduction of the vascular supply will impair perfusion of the ONH to a critical degree [1–6, 9].

The main purpose of our study is to show the essential role of color Doppler imaging (CDI) of orbital vessels in order to quickly differentiate the mechanism of AION (arteritic, versus non-arteritic); the former should be treated promptly with systemic corticosteroids to prevent further visual loss of the fellow eye.

Methods

In this prospective, comparative and observational study, we included 62 consecutive patients who presented, in our ophthalmology and neurology departments from June 2012 through February 2015, with clinical suspicion of acute unilateral AION.

The study was approved by the "Victor Babes" University of Medicine and Pharmacy Local Research Ethics Committee. All patientts gave informed consent and were examined following a complex protocol, including:

1 – a complete history of all previous or current systemic diseases;

2 – an ophthalmological examination, (conducted by an ophthalmologist at the presentation, at 2 weeks, at 1 month and at 2 months), including visual acuity with the Snellen visual acuity chart, visual fields with a Goldmann perimeter, relative afferent pupillary defect, intraocular pressure, slit-lamp examination of the anterior segment, lens and vitreous, direct ophthalmoscopy and color fundus photography (both methods were used at the presentation and repeated at 2 weeks after the onset of visual loss), and fluorescein fundus angiography which was performed during the first 2 days after the presentation (acute stage of AION);

3 – a physical examination (including possible contralateral neurologic signs such as hemiparesis, the inspection and palpation of the TAs) was performed at the presentation by a neurologist and an internist in order to detect an eventual stroke or a temporal arteritis (as part of GCA);

4 – a CDI of orbital vessels was realized with a 10 MHz linear probe for detecting (by color Doppler sonography) and measuring (by spectral analysis pulsed Doppler sonography) the blood flow in the orbital vessels: the OAs, the CRAs, the PCAs (nasal and temporal), and the superior ophthalmic veins. Blood flow towards the transducer was depicted as red and flow away from the transducer was colored blue: a) the CRA was identified just bellow the optic disc (< 1 cm) and had a forward red-coded blood flow; b) the nasal and temporal trunks of PCA were identified along both sides of the optic nerve. The arteries had a forward red-coded blood flow; c) the OA was identified deeper in the orbit usually before crossing the optic nerve. It had a forward red-coded blood flow. The Doppler sample gate placed on the detected vessel was 15 mm. When the orbital vessels were not parallel to the ultrasound beam, we performed an angle correction between 0–60°. Also, a spectral velocity analysis was performed. The peak systolic velocity (PSV) and end-diastolic velocity (EDV) were calculated for each vessel. The resistance index (RI) was automatically calculated according to the following equation: RI = (PSV-EDV)/PSV. Absent signals not corresponding to ipsilateral internal carotid occlusive disease were classified as Doppler sonographic findings typical of GCA of the orbital arteries (occlusion of an orbital artery). Serial CDI examinations of the orbital blood vessels were performed at the presentation, at 1 week, and at 1 month on all AIONs patients.

5 – Extracranial arteries were examined with a 7.5–10 MHz linear array transducer, combining B mode and color coded Doppler/pulsed-wave Doppler ultrasound duplex sonography (EDS), looking for an internal carotid artery's (ICA) source of emboli and with a 10 MHz linear probe for the examination of ECA branches, especially temporal arteries (TAs). Color box steering and beam steering were maximal and the color covered the artery lumen exactly be-

cause using these machine adjustments, sensitivities and specificities of the temporal arteritis diagnosis are higher. We examined both common superficial TAs and their frontal and parietal rami in longitudinal and transverse planes. Concentric hypoechogenic mural thickening (also called halo) was considered as an ultrasonographic finding typical of GCA.

Arterial segment was considered stenosed when PSV was more than twice than in the pre- stenotic segment with wave forms demonstrating turbulence and reduced velocity beyond the stenosis. Acute occlusion was considered if the ultrasound (US) showed hypoechoic material in the artery lumen with absence of color signals.

The first two authors of the study performed both CDI of orbital vessels and EDS. The first investigator, who was blinded to the patients ophthalmological status, looked only for detecting and measuring orbital and extracranial vessels blood flow. Discrepancies were resolved by consensus.

EDS was performed at the presentation, 2 weeks and at 1 month on all A-AIONs patients, and at the presentation on all NA-AION patients.

6 – Electrocardiogram **(**ECG) in all patients, and transthoracic echocardiography (TTE) in selected cases were performed in the first 2 days after the presentation to detect an eventual cardiac source of emboli in selected cases (atrial fibrilation, etc.) were done during the first 2 days after the presentation.

7 – Cranial computed tomography (CT) scanning was performed at the presentation in all AION patients in order to identify a concomitent stroke.

8 – CT-angiography (CT-A) was done at the presentation and after EDS, only in selected cases (it allowed the assessment of the arterial wall and the endoluminal part of the aorta and its branches in selected cases of ipsilateral severe ICA stenosis/occlusion).

9 – a laboratory workup, including ESR, CRP, factor V Leiden, glycemia, etc., was done during the first 2 days after the presentation.

10 – a temporal artery biopsy (TAB) was selectively done when GCA was suspected following the criteria of the American College of Rheumatology. Because of unilateral clinical ocular involvement in all cases, we took a biopsy from the ipsilateral TA representing 2.5 cm of the tender, swollen segment of the affected artery or from the TA's site targeted by the color coded Doppler (TAB was guided by EDS because of segmental/discontinuous TA's involvement in GCA: skip lesions). All TABs were performed on the second day after the presentation.

Data analysis

The evaluation of the duplex color coded Doppler of the orbital vessels quality in order to foresee the A-AION diagnostic was completed by the SPSS v.17 program by using the calculated RI for the clinically affected eye for all orbital arteries. Starting from the receiver operating characteristics (ROC) curve coordinates for each investigated artery, the best threshold value was identified in order to achieve the minimum cost of the test (maximum of the sensitivity – Se + specificity – Sp). Using Microsoft Excel, the classification quality assessment was performed for the following parameters: sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

Results

We found 12 patients with unilateral acute A-AION, all of them with biopsy-confirmed disease (TAB positive), and 50 patients with unilateral acute NA-AION (no TAB).

We obtained the two groups of AION patients, using both clinical features, laboratory data, and results of ultrasound exams.

The comparison of major features of unilateral acute A-AION and NA-AION patients are presented in Table 1.

Table 1

Comparison of major features of arteritic anterior ischemic optic neuropathies (A-AION) and non-arteritic anterior ischemic optic neuropathies (NA-AION) patients

Feature	A-AION	NA-AION
Age (years), mean ± SD	72.3 ± 7.5	59.2 ± 11.9
Sex ratio	Female > male (8 : 4 patients)	Female = male (26 : 24 patients)
Associated symptoms	New temporal headache, scalp tenderness, abnormal TAs on palpation, jaw claudication (11 patients) Amaurosis fugax (4 patients)	Pain occasionally noted (3 patients) No amaurosis fugax
Visual acuity	83.3% (10 patients) < 20/200	60% (30patients) > 20/200
Optic disc	Pale > hyperemic edema (11 patients) Cup normal (all 12 patients)	Hyperemic > pale edema (48 patients) Cup small (38 patients)
ESR (mm/h)	> 50 (10 patients)	< 50 (47 patients)
CRP (mg/L)	> 5 (11 patients)	< 5 (48 patients)
Temporal artery biopsy (TAB)	Granulomatous inflammation of the media layer (all 12 patients)	No TAB
Color coded Duplex sonography of the retrobulbar (orbital) vessels	Severe diminished blood flow velocities in the PCAs, especially on the affected side, and high RI in all retrobulbar vessels, in both orbits (all 12 patients)	Blood flow velocities and RI in PCAs were preserved (all 50 patients)
Fluorescein fundus angiography	Disc and choroid filling delay (all 12 patients)	Disc filling delay (all 50 patients)
Treatment	Corticosteroids (all 12 patients)	None proved (all 50 patients)

TAs – temporal arteries; ESR – erithrocyte sedimentation rate; CRP – C-reactive protein; PCAs – posterior ciliary arteries; RI – resistance index.

All 12 A-AION patients presented with GCA. The NA-AION patients presented with: a) systemic associations: 20 (40%) patients with arterial hypertension, 13 (26%) patients with diabetes mellitus, 10 (20%) patients with nocturnal arterial hypotension, 4 (8%) patients with ipsilateral ICA's disease, and 3 (6%) patients with neoplasms, and/or; b). ocular and ONH risk factors: 38 (76%) patients with small cup in the optic disc and 2 (4%) patients optic disc drusen. On the other hand, all 12 A-AION patients presented large cups. Anterior segment examination of both eyes was normal in all 62 patients. Amaurosis fugax was an important early visual symptom in 4 (33.3%) A-AION patients and preceded permanent visual loss. The 8 other A-AION cases developed permanent visual loss without any warning. However, amaurosis fugax was never found in NA-AION patients.

Visual fields

The most common visual field defect in NA-AIONs cases was an inferior nasal sectorial defect found in 32 (64%) patients which was relative or absolute. The next most common visual field defect was relative or absolute inferior altitudinal in 12 (24%) patients.

Color coded duplex Doppler of the retrobulbar (orbital) vessels features

1) Spectral Doppler analysis of the retrobulbar vessels in 12 A-AION patients: in the first week of evolution found an undetectable or severe diminished of blood flow velocities in the PCAs on the affected side (orbit), with an increased RI in all other retrobulbar vessels in both orbits (including the PCAs in the contralateral orbit) (Figure 1A and B), despite the treatment with high-dose corticosteroids was found.

In month one, CDI examinations of orbital blood vessels revealed practically the same aspects.

2) Spectral Doppler analysis of the retrobulbar vessels in 50 NA-AION patients: by contrast, in all NA-AIONs cases, blood flow velocities and RI in PCAs were generally preserved. In the first week of evolution, this analysis revealed only a slight decrease of PSV in PCAs (nasal and temporal) in the affected eye, compared to the unaffected eye and a very slight decrease of PSV in CRA of the affected eye due to papillary edema. In OAs, PSV were variable – normal to decreased, according to ipsilateral ICA's status. In 4 patients severe ICA stenosis (> 70% of vessel diameter) combined with an insufficient Willis polygon led to decreased PSVs in the ipsilateral OAs.

In month 1, CDI examinations of orbital vessels revealed that blood flow normalization was reached. The only exceptions were the 4 cases with ipsilateral severe ICAs stenosis.

Analysis of the data (Table 2) revealed that a threshold value of 0.71 for the IR of the temporal PCAs in the clinically affected eye, provided the best combination of Se (86%), Sp (96%), PPV (88%) and NPV (96%), respectively, for the detection of A-AION. A threshold value of 0.68 for the IR of the nasal PCAs in the clinically affected eye, provided the best combination of Se (86%), Sp (93%), PPV (76%), and NPV (96%), respectively, for the detection of A-AION.

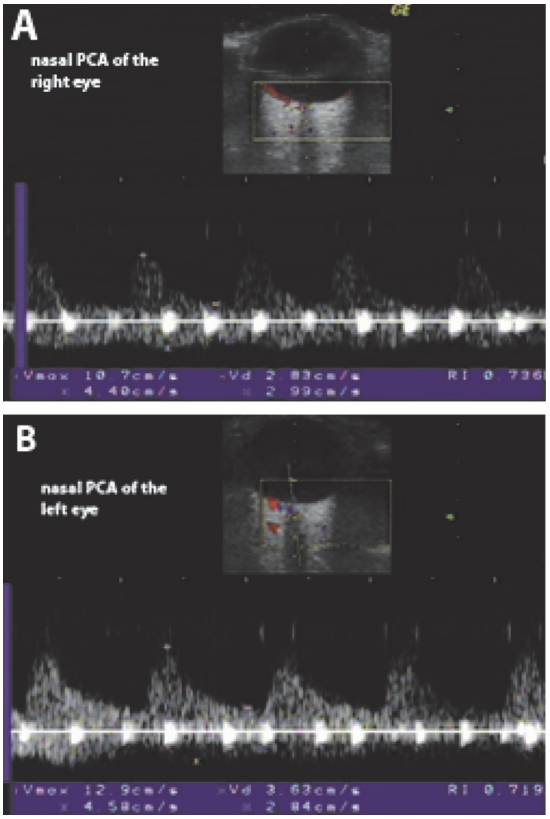

Fig. 1 – Color Doppler imaging (CDI) of the posterior ciliary arteries (PCAs) in arteritic anterior ischemic optic neuropathies (A-AION): decreased blood flow velocities (especially enddiastolic velocity – EDV) in the nasal PCAs: A) of the clinically affected right eye, and B) of the clinically unaffected left eye.

Table 2

The threshold values of resistance index (RI) in the orbital vessels and the corresponding values of sensitivity (Se), specificity (Sn), positive predictive value (PPV) and negative predictive value (NPV)

Arteries	CRA	PCA t	PCA n	OA
Cut- off point	0.67	0.71	0.68	0.81
Se	0.76	0.86	0.86	1
Sp	0.81	0.96	0.93	0.96
PPV	0.51	0.88	0.76	0.89
NPV	0.92	0.96	0.96	1

CRA – central retinal artery; PCAt – temporalu posterior ciliar artery; PCAn – nasal posterior ciliar artery; OA – opthalmic artery.

Extracranial Duplex sonography (EDS)

1) EDS in all 12 A-AION patients: EDS demonstrated segmental inflammation of TAs in 12 patients with A-AION. At the presentation, we identified: a) "dark halo" sign in 11 patients (Figure 2); b) stenoses in 5 cases, and c) acute occlusions in 2 patients. Similar ultrasound patterns were discovered in other branches of the ECAs, including the facial, internal maxilary and lingual arteries. In week 2 and in month 1, the "halo" revealed by TAs ultrasound disappeared, because of the treatment with corticosteroids.

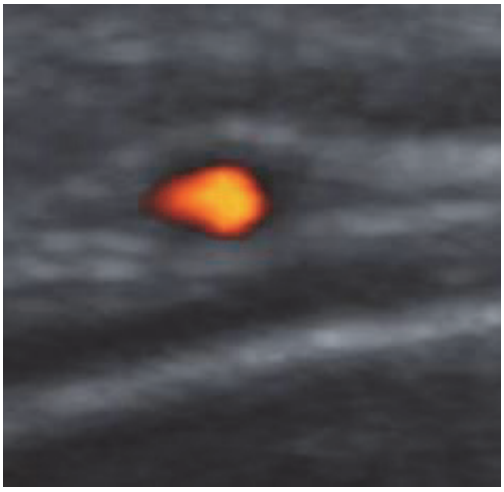

Fig. 2 – Extracranial duplex sonography (EDS) of the temporal ramus of the right temporal artery (TA) – "dark halo" sign.

2) EDS in 50 NA-AION patients: at the presentation we identified 4 cases with ipsilateral severe ICA stenosis and consecutive NA-AION;

CT and CT-A

CT-scanning excluded strokes in all 62 patients. CT-A done in 4 patients confirmed severe ICA stenosis and consecutive ipsilateral NA-AION in their cases.

ECG and TTE

These examinations excluded an eventual cardiac embolic source of NA-AION.

Treatment and evolution of A-AION patients

In all 12 GCA patients with A-AION, the treatment was initiated before TAB with intravenous methyl-prednisolone, 1g/day for 3 consecutive days after the presentation, followed by oral prednisone 60 mg daily for one month. The daily dose was then reduced by 5 mg weekly in the next month of follow-up. All 12 patients with A-AION presented stationary ophthalmologic evolution in 2 months (unilateral visual loss) without classic clinical symptoms of GCA (temporal headache, etc.) and any systemic manifestations (fever, malaise, etc.).

Discussion

In the patients with A-AION due to GCA, transient visual loss caused by optic-nerve or choroidal ischemia (amaurosis fugax) often precedes permanent visual loss by days to weeks (like in 4 of our 12 cases with A-AION). This symptom is unusual in NA-AIONs cases [1, 3–7, 10–14] and we did not come across them in our NA-AION patients.

Biousse and Newman [1] mentioned that the degree of visual loss is often more severe in A-AION than in NA-AION. In one study, 54% of the patients with A-AION had an initial visual loss degree ranging from counting fingers to no light perception, as compared to 26% in the NA-AION group [4]. This result shows that acute, painless, severe permanent loss of vision is extremely suggestive of A-AION, as in 10 out of 12 our cases with A-AION. Different authors noted that once an untreated patient with GCA lost vision in one eye, the risk of GCA – related visual loss in the second eye is highest in the following hours to weeks (in at least 50% of cases) [1, 15–18]; we did not come across with this situation in our A-AION patients because they were treated with high doses of corticosteroids.

We noted that inferior nasal field defect was the most common diagnostic visual field defect in our NA-AION patients, such as it was found reported in literature [3–7, 10].

According to Biousse and Newman [1], NA-AION is manifested as isolated, sudden, painless, monocular vision loss with edema of the optic disc. Ophthalmoscopy indicated that optic disc edema was associated more frequently with hyperemia in our NA-AION patients and with pallor (a chalky white color) in our A-AION patients, like in other studies [3–7, 10–14].

We found a small, crowded ONH with a small physiological cup in 38 of our 50 patients with NA-AION. The small cup-to-disc ratio defines a "disc at risk" [1, 9]. Although this is difficult to observe during the acute phase of NA-AION, when the optic disc is swollen, examination of the clinically normal fellow eye should show a "disc at risk" [1]. According to different authors, the absence of a crowded optic disc in the second eye of a patient with AION should increase the probability of A-AION [1, 9]; we found only large cups in our 12 A-AION patients.

A-AION results from entire PCAs trunk vasculitis and the consecutive ONH infarction. Human autopsy studies of acute A-AION demonstrated ischemic necrosis of the ONH and infiltration of the PCAs by chronic inflammatory cells. In some of the cases reported in these studies, segments of PCAs were occluded by inflammatory thickening and thrombi [3–7]. Severe diminished blood flow velocities in the PCAs, especially on the affected side, and high RI in all retrobulbar vessels in both orbits, represented characteristic signs of the CDI of the orbital vessels in A-AION in our study [10–14, 19–21]. In NA-AION, blood velocities and RI in PCAs were preserved. Similar results were obtained in other studies [10–14, 19–21]. The small number of A-AION cases could influence the calculated values (both the threshold values and the classifier quality). In spite of this, high PPV and NPV values in cases of temporal and nasal PCAs of the clinically affected eye, suggest that color coded duplex Doppler of orbital vessels may be a valid tool in the diagnosis of A-AION [10].

Extremely delayed or absent filling of the choroid, which was identified in all our 12 A-AION fluorescein-angiograms, was suggested in other studies as a fluorescein-angiograms characteristic of A-AION [3–7, 10–14].

In our study, Duplex Doppler of retrobulbar vessels and fluorescein angiogram data supported the histopathological evidence from other studies [3–7] of the involvement of the entire PCA trunk in A-AION (impaired both ONH and choroidal perfusion in these patients) [10–14, 19–21]. In contrast, in NA-AION cases, impaired flow to the ONH is distal to the PCA trunk, usually at the level of the paraoptic branches [10–14, 19–21]. These branches directly supply the ONH with only one-third of the flow from the PCAs (impaired optic disc perfusion, with relatively preserved choroidal perfusion in NA-AION patients) [10–14, 19–21].

While color coded Doppler sonography of orbital blood vessels does not eliminate the need for intravenous fluorescein angiography, it does, however, enhance the precision and reliability of the diagnostic evaluation for these patients, because it accurately, reproducibly and safely assesses the vascular supply of the optic nerve and retina [10].

There were certain cases in our study where the differential diagnosis between A-AION and NA-AION was difficult: a) three patients with NA-AION had high ESR levels due to an associated neoplasm; b) two patients with GCA had a normal ESR; c) one case with GCA without systemic/clinical symptoms, even without a swollen TA (occult GCA) [7].

Biousse and Newman [1] asserted that systemic symptoms of GCA (malaise, fever, temporal headache) may precede visual loss by months; however, about 25% of patients with positive TAB for GCA presented isolated A-AION without any systemic symptoms (so-called occult GCA) [7, 16–18, 22–24].

We believe that when duplex Doppler does not show evidence for A-AION, the patient should not receive high dose of corticosteroids until a TAB is performed, even if the ESR is elevated. On the other hand, patients with clinical evidence of A-AION, who have typical signs on Doppler of retrobulbar vessels, should be treated with corticosteroids before TAB in order to protect the fellow eye from going blind [10, 16–19, 23–25].

Ultrasonography of the TAs in temporal arteritis is very important for the GCA diagnosis [15, 16]. Schmidt et al. [26] and Schmidt [27] asserted that the most specific (almost 100% Sp) and sensitive (73% Se) sign for GCA was a concentric hypoechogenic mural thickening "halo" which was interpreted as vessel wall edema. Other positive findings for GCA are the presence of occlusion and stenoses. We detected these signs in 11 out of 12 our patients in our A-AION group with GCA. The 12th patient presented an occult GCA. Ultrasound investigation of the TAs needs to be performed before corticosteroid treatment or within the first 7 days of treatment because the "halo" revealed by TAs ultrasound disappears within 2 weeks of cortico-therapy, like in our 11 GCA cases with temporal arteritis [7, 22, 26, 27]. Schmidt et al. [26] and Schmidt [27] compared the results of TAs ultrasound examinations with the occurrence of visual ischemic complications in 222 consecutive patients with newly diagnosed, active GCA. However, findings of the TAs ultrasound examinations did not correlate with eye complications. For this reason, color coded duplex sonography of the retrobulbar vessels is of critical importance to identify A-AION [10, 14].

Conclusion

A history of transient visual loss (amaurosis fugax) associated with an acute, painless, and severe visual loss of the involved eye, with concomitant diffuse pale optic disc edema were characteristics of A-AION. On the other hand, none of these symptoms and signs were found in the NA-AION patients. The presence of a disc at risk in the fellow eye in a patient with unilateral AION increased the probability of NA-AION. Color coded duplex sonography of the orbital vessels identified the alterations in orbital blood flow, especially in the PCAs, which coresponded with the clinical features of A-AION, and enabled prompt differentiation between NA-AION and A-AION.

Conflict of interests

The autors declare no conflict of interest.

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Prolonged postoperative ileus after elective colorectal cancer surgery

Prolongirani postoperativni ileus posle elektivne kolorektalne hirurgije zbog karcinoma

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Abstract

Background/Aim. Postoperative ileus is a frequent and frustrating occurence for both, patients and surgeons after abdominal surgery. Besides clinical importance of postoperative ileus, its economic aspect is also important. The aim of this prospective study was to analyze development of prolonged postoperative ileus after elective colorectal surgery for cancer and its impact on early postoperative outcome. **Methods.** This prospective study included all eligible patients, 18 years or older, scheduled for open colorectal resection for cancer from June, 2015 to February, 2016. Patients with metastatic disease, prior hemoirradiation or any resection other then curative were excluded. The study duration was up to 30 days postoperatively. Primary outcome measure was development of prolonged postoperative ileus according to strict definition. The impact of prolonged postoperative ileus on other outcome measures such as postoperative complications, surgical site infections, anastomotic leakage, reoperations, mortality and length of hospital stay were of great interest, too. **Results.** This prospective study included 103 patients, 64 (37.9%) men and 39 (62.1%) women, mean age 66 years. Prolonged postoperative ileus developed in 12 (11.3%) patients. One third of the patients had some type of surgical site infection, while 47.6% had complications. Ten (9.7%) patients required reoperation. Comparing the group of patients with prolonged postoperative ileus with those without, there were no statistically significant differences in rates of surgical site infection and anastomotic leakage. There was statistically significant difference in terms of complications ($\chi^2 = 34.966$; $p < 0.001$), complications grade III ($\chi^2 = 23.43$; $p < 0.001$) and reoperations ($\chi^2 = 15.724$; $p < 0.001$). Patients who developed prolonged postoperative ileus had statistically significant longer postoperative hospital stay ($Z = 2.291$, $p = 0.022$) and longer total length of hospital stay ($Z = 2.377$, $p = 0.015$). According to regression analyzes prolonged postoperative ileus represents a risk factor for reoperations [odds ratio (OR) = 12.286; $p = 0.001$]. **Conclusion.** Prolonged postoperative ileus, although not life-threatening complication effects recovery, increases length of hospital stay and contributes to poor surgical outcome.

Key words:
colorectal neoplasms; postoperative complications; ileus; digestive system surgical procedures; elective surgical procedures; risk factors.

Apstrakt

Uvod/Cilj. Postoperativni ileus je česta i frustrirajuća pojava i za bolesnike i za hirurge posle abdominalnih hirurških intervencija. Osim kliničkog, važan je i ekonomski aspekt postoperativnog ileusa. Cilj rada bila je analiza pojave postoperativnog ileusa posle elektivnih kolorektalnih operacija zbog karcinoma i njegov uticaj na rani postoperativni ishod. **Metode.** Studijom su bili obuhvaćeni bolesnici životnog doba od 18 i više godina, lečeni od juna 2015. do februara 2016. godine, kod kojih je bila planirana kolorektalna resekcija zbog karcinoma. Isključujući kriterijumi bili su metastatska bolest, prethodna hemio- i zračna terapija i nekurativna resekcija. Studija je trajala do 30 dana posle operacije. Pojava prolongiranog postoperativnog ileusa, utvrđenog prema strogoj definiciji, postavljena je kao krajnji cilj, a ispitivan je i njegov uticaj na druge parametre ishoda lečenja kao što su: postoperativne komplikacije, infekcija na mestu operativnog rada, dehiscencija anastomoze, reoperacije, mortalitet i trajanje hospitalizacije. **Rezultati.** Prospektivnom studijom obuhvaćena su 103 bolesnika, 64 (37,9%) muškarca i 39 (62,1%) žena, prosečne starosti 66 godina. Prolongirani postoperativni ileus se javio kod 12 (11,3%) bolesnika. Trećina bolesnika imala je neki tip infekcije na mestu operativnog rada, dok je stopa

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komplikacija iznosila 47,6%. Ukupno 10 (9,7%) bolesnika je reoperisano. Uporedivanjem grupe sa i bez prolongiranog postoperativnog ileusa nisu uočene statistički značajne razlike u stopama hirurških infekcija i dehiscencija anastomoze. Utvrdjena je statistički značajna razlika u pogledu komplikacija (χ^2 = 34.966; $p < 0.001$), komplikacija III stepena (χ^2 = 23.43; $p < 0.001$) i reoperacija (χ^2 = 15.724; $p < 0.001$). Bolesnici koji su razvili prolongirani postoperativni ileus imali su statistički značajno duži period hospitalizacije posle operacije (Z = 2.291, p = 0.022) kao i duže ukupno trajanje hospitalizacije (Z = 2.377, p = 0.015). Prema regresionom modelu prolongirani postoperativni ileus predstavlja faktor rizika za reoperaciju (OR = 12.286; p = 0,001). **Zaključak.** Mada ne predstavlja komplikaciju koja neposredno ugrožava život, prolongirani postoperativni ileus utiče na oporavak, produžava vreme bolničkog lečenja i doprinosi lošem ishodu hirurškog lečenja.

Ključne reči:
kolorektalne neoplazme; postoperativne komplikacije; ileus; hirurgija digestivnog sistema, procedure; hirurgija, elektivna, procedure; faktori rizika.

Introduction

Postoperative ileus was first mentioned by Cannon and Murphy in 1906. It was described as transient postoperative gastrointestinal dysmotility. For a long time considered as inevitable postoperative event, nowadays it brought itself into attention due to the effect on postoperative recovery [1]. If it extends longer than usually expected, it may cause other serious adverse events. Postoperative ileus is multifactorial in origin. Etiology includes neurogenic, inflammatory, hormonal and pharmacologic influences. Neurogenic component is related to pain, while surgical manipulation causes inflammatory response. Hormonal influence on ileus is primarily mediated via corticotropin-releasing hormone as a response to trauma. Opiate based medications are often mentioned factor involving in pathophysiology of postoperative ileus for its inhibitory effect through stimulation of μ-opioid receptors in the bowel [2, 3]. Various terms can be found in literature addressing the same problem, from postoperative paralytic ileus, prolonged ileus, and pathologic ileus [4]. To make reporting and research on the subject more comprehensive, there is a need for clearer definition of the factors that constitute prolonged postoperative ileus (PPOI) with increased awareness and recognition of its impact [5]. More than several parameters were identified as risk factors for development of PPOI such as: advanced age, male gender, blood loss, duration of surgery, previous operation, emergency surgery, opiate use and procedures requiring stoma. Risk factors vary between different studies [6]. Symptoms of postoperative ileus include nausea and vomiting, abdominal distension, lack of bowel sounds, passage of flatus and defecation [4]. Intolerance of oral intake due to nausea and vomiting aggravate catabolism which further compromises recovery [7]. Although not life-threatening PPOI affects recovery, increases hospital length of stay and healthcare resource utilization and costs [2].

The rate of PPOI after abdominal operations is reported to be from 4% to as far as 75% [8]. The cause for such difference in reported rates is believed to be in variations in its definition [9]. Highest rates of PPOI are reported after operations in urology such are radical cystectomies, from 10%–40%, where it accounts for 50%–70% of all complications [10, 11]. PPOI is frequent complication in gynecology, especially following debulking surgery with the incidence from 7.6%–30% [12]. According to the latest meta-analysis incidence of PPOI after colorectal resections is around 10% [13].

Apart from clinical importance of postoperative ileus, its economic aspect is often mentioned. Retrospective analysis of more than 17,000 primary procedures has shown that patients with PPOI have statistically significant higher costs of treatment in comparison to patients without this complication ($25,089 vs. $16,907) [8]. Increased costs are not only related to longer hospital length of stay but also to additional measures taken for diagnostics and management of this complication [14].

The aim of this prospective study was to analyze development of PPOI and its impact on early outcomes after elective colorectal resections for cancer.

Methods

This prospective study included all eligible patients aged 18 years or older scheduled for open colorectal resection for cancer in the period from June, 2015 to February, 2016. The study was approved by the local ethics committee and before the inclusion patients signed informed consent. Patients with metastatic disease, prior chemoradiotherapy or any resection other then curative were excluded from the study. The seventh edition of the Union for International Cancer Control (UICC) classification from 2009 was used for staging colorectal adenocarcinoma. All patients were operated by the same group of surgeons using the same protocol of preoperative and postoperative care. All patients had mechanical bowel preparation. In cases of placement of nasogastric catheter for decompression, the same was removed at the conclusion of the operation. Liquid diet was prescribed to all patients on postoperative day one, followed by solid food as tolerated. None of the patients received opiate based analgesia postoperatively, nor there were epidurals used. The study duration was up to 30 days after the surgery. Primary outcome measure was development of PPOI according to definition proposed by Vather et al. [9], as two or more of the following occurring on or after day 4 postoperatively without prior resolution of postoperative ileus: nausea/vomiting, inability to tolerate an oral diet over the preceding 24 h period, the absence of flatus over the preceding 24 h period, abdominal distension and/or radiological evidence of bowel distension without mechanical obstruction.

The impact of PPOI on other outcome measures such as postoperative complications, surgical site infection (SSI), anastomotic leakage, reoperations, mortality and length of hos-

pital stay were also of great interest. Postoperative complications were graded according to Clavien-Dindo classification [15]. Anastomotic leakage was defined as purulent or fecal discharge from a drain, pelvic abscess on computed tomography (CT) scans or peritonitis evidenced at laparotomy. Analyzed variables were stratified according to the presence or absence of PPOI and presented as percent frequency. Normally distributed data are presented as mean ± standard deviation (SD) and in the case when the normality was not assessed as median ± interquartile range. Student's *t*-test was used to assess statistical significance of parametric continuous data and Mann–Whitney *U* test for nonparametric continuous variables. The comparison of frequency distributions was performed by χ^2 or Fisher`s exact test. The relative risk was evaluated by logistic regression analysis and the results presented as odds ratio (OR) with 95% confidence interval and p-value. Results were considered statistically significant at p value < 0.05. Statistical analysis was performed using SPSS for Windows (Version 20; SPSS, Chicago, IL).

Results

Prospective analysis included 103 patients with colorectal cancer, 64 (37.9%) men and 39 (62.1%) women, mean age 66 ± 10.1 years (range 29–85), without statistically significant difference in age between genders ($p = 0.542$). Baseline characteristics of the patients with colorectal cancer are shown in Table 1.

One quarter of the patients had either low rectal resection with protective ileostomy or right hemicolectomy, 25 (24.3%) and 27 (26.2%) patients, respectively. Superior rectal resection was done in 17 (16.5%) patients, followed by left hemicolectomy in 14 (13.6%), and low rectal resection in 8 (7.8%) patients. In others abdomino-perineal resection, Hartmann' procedure or total colectomy was performed.

Near half of the patients, 49 (47.6%), had some grade of complication, of whom 38 developed complications grade I or II (Table 2). According to grading, these patients experienced complications which did not required any surgical, radiological or endoscopic intervention, was not consisting of organ dysfunction, was not life threatening and did not required ICU treatment. Recorded complications grade I or II were: SSI, postoperative diarrhea, PPOI and postoperative bleeding requiring transfusions. Most common postoperative complication was SSI developed in 37 (26.2%) patients. The rate of anastomotic leakage was 5.4%. PPOI occurred in 12 (11.3%) patients. Association of certain demographic variables such as age and gender, American Society of Anesthesiologists (ASA) grade, duration of surgery, type of anastomosis and presence of stoma, with PPOI is shown in Table 3. None of the analyzed parameters reached statistical significance in regression analysis as a risk factor for development of PPOI. Ten patients (9.7%) required reoperation in the early postoperative period (Table 2). Indications for reoperation were as follows: PPOI in 3 (30%) of patients, small bowel obstruction in 2 (20%) of patients, anastomotic leakage in 4 (40%) of patients and ischemic perforation in one (10%) patient.

Table 1
Clinical and pathological characteristics of patients with colorectal cancers in analyzed cases (according to the UICC classification, 7th edition, 2009)

Characteristics of colorectal cancers	Patients n (%)
Tumor site	
right colon	27 (26.2)
left colon	25 (24.3)
rectum	50 (48.5)
synchronous	1 (1.0)
Stage (TNM classification)	
I	20 (19.4)
IIA	35 (34.0)
IIB	3 (2.9)
IIC	3 (2.9)
IIIA	7 (6.8)
IIIB	25 (24.3)
IIIC	10 (9.7)
T (Tumor)	
1	9 (8.7)
2	17 (16.5)
3	69 (67.0)
4	2 (1.9)
4a	3 (2.9)
4b	3 (2.9)
N (Nodus)	
0	58 (56.3)
1a	12 (11.7)
1b	9 (8.7)
1c	4 (3.9)
2a	11 (10.7)
2b	9 (8.7)

UICC – Union for International Cancer Control;
TNM – tumor, nodus, metastasis.

Total average length of hospital stay was 12.60 ± 6.65 days (range 7–49). Mean duration of treatment in intensive care unit was 1.62 ± 1.43 days. Patients spent in hospital an average of 9.39 ± 6.07 (4–46) days after the operation.

Comparing the group of the patients with PPOI with those without there was no statistically significant difference in SSI and anastomotic leakage. However, there was a statistically significant difference in terms of complications ($\chi^2 = 34.966$; $p < 0.001$). Complications grade III (requiring surgical, endoscopic or radiologic intervention) were seen more often in the patients with PPOI ($\chi^2 = 23.43$; $p < 0.001$). Also, reoperations were more often done in the patients with PPOI ($\chi^2 = 15.724$; $p < 0.001$) (Table 2). The patients who developed PPOI had statistically significant longer postoperative hospital stay ($Z = 2.291$; $p = 0.022$), which substantially contributed to statistically significant longer total length of hospital stay ($Z = 2.377$; $p = 0.015$). The length of the treatment in the intensive care unit did not differ between groups ($Z = 1.662$; $p = 0.096$) (Table 4).

Logistic regression analysis was used to evaluate PPOI as a risk factor for complications, reoperations and mortality. According to analysis PPOI is not a risk factor for the development of other complications (OR = 1.197; $p = 0.773$), nor for anastomotic leakage in particular (OR = 2.167; $p = 0.509$) (Table 5). Multinominal logistic regression confirmed

that PPOI had no influence on occurrence of any type of SSI (Table 5). Our results showed that a patient who had PPOI had 12 times more chances to undergo reoperation (OR = 12.286; $p = 0.001$), while PPOI poses no risk of mortality (OR = 0.291; $p = 0.170$) (Table 5).

Table 2

Postoperative complications in patients operated due to colorectal cancer

Complications	Overall	No PPOI	PPOI	χ^2	p
	n (%)	n (%)	n (%)		
SSI					
none	76 (73.8)	69 (75.8)	7 (58.3)		
superficial incisional	13 (12.6)	11 (12.1)	2 (16.7)		
deep incisional	5 (4.9)	4 (4.4)	1 (8.3)		
organ/ space infection	9 (8.7)	7 (7.7)	2 (16.7)	1.932	0.587
Other then PPOI					
no	64 (62.1)	57 (62.6)	7 (58.3)		
yes	39 (37.9)	34 (37.4)	5 (41.7)	0.083	0.773
PPOI					
no	91 (88.7)				
yes	12 (11.3)				
Graded according to Clavien and Dindo					
no	54 (52.4)	54 (59.3)	0 (0.0)		
I	21 (20.4)	16 (17.6)	5 (41.7)		
II	17 (16.5)	15 (16.5)	2 (16.7)		
III	3 (2.9)	0. (0.0)	3 (25.0)		
IV	1 (1.0)	1 (1.1)	0 (0.0)		
V	7 (6.8)	5 (5.5)	2 (16.7)	34.966	< 0.001
Anastomotic leakage					
no	87 (94.6)	78 (95.1)	9 (90.0)		
yes	5 (5.4)	4 (4.9)	1 (10.0)	0.455	0.500
Reoperation					
no	93 (90.3)	86 (94.5)	7 (58.3)		
yes	10 (9.7)	5 (5.5)	5 (41.7)	15.724	< 0.001

PPOI – prolonged postoperative ileus; SSI – surgical site infection.

Table 3

Association between prolonged postoperative ileus (PPOI) and demographic and operative variables

Parameters	Values	OR	95%CI	p
Age (years), mean ± SD	66.00 ± 10.06	0.999	0.941–1.061	0.973
Male gender, n (%)	64 (37.9)	0.361	0.170–1.907	0.361
ASA grade, n (%)				
I*	14 (13.6)			
II	57 (55.3)	0.840	0.155–4.566	0.840
III	30 (29.1)	0.667	0.098–4.520	0.678
IV	2 (1.9)	0.588	0.036–6.658	0.999
Duration of surgery (min), mean ± SD	124.90 ± 39.72	1.013	1.000–1.027	0.051
Anastomosis, n (%)				
ileo-colic*	26 (28.6)			
colo-colonic	14 (15.4)	0.323	0.034–3.083	0.326
colo-rectal	50 (54.9)	0.365	0.089–1.500	0.162
ileo-rectal	1 (1.1)	0.000	0.000	1.000
Stoma	33 (32)	2.370	0.701–8.010	0.156

***referent value. ASA – American Society of Anesthesiologists; SD – standard deviation; OR – odds ratio.**

Table 4

Hospitalization variables in observed groups

Parameters	Length of stay (days), mean ± SD		Z^*	p
	no PPOI	PPOI		
Total	12.39 ± 6.91	14.17 ± 3.99	2.377	0.017
Preoperative stay	3.11 ± 2.34	2.75 ± 1.35	0,105	0.916
Intensive care	1.55 ± 1.41	2.17 ± 1.52	1.662	0.096
Postoperative length of stay	9.24 ± 6.24	10.58 ± 4.60	2.291	0.022

PPOI – prolonged postoperative ileus.

Table 5

Prolonged postoperative ileus (PPOI) as a risk factor for other complications

Complications	OR	95%CI	*p*
All	1.197	0.352–4.071	0.773
Anastomotic leakage	2.167	0.218–21.554	0.509
Reoperation	12.286	2.855–52.873	0.001
Mortality	0.291	0.050–1.699	0.170
SSI			
superficial incisional	0.558	0.102–3.040	0.500
deep incisional	0.406	0.040–4.151	0.447
organ/space infection	0.355	0.062–2.050	0.247

SSI – surgical site infection; OR – odds ratio.

Discussion

The current prospective study was undertaken to evaluate PPOI following elective colorectal resection for cancer and its effect on other complications such as SSI, anastomotic leakage, reoperation, mortality and hospital stay. In order to eliminate factors that might influence gastrointestinal recovery all patients having the same indications for operation were included in the study and were operated using open approach, without epidurals or opiate based analgesia postoperatively, no nasogastric tubes nor use of prokinetics [16]. According to the results in this study, the incidence of PPOI, as defined by Vather et al. [9], after open elective colorectal surgery was 11.3%. Similar rate of 12.7% was reported by Moghadamyeghaneh et al. [17], although studies are hard to compare since they were different in design and definition of prolonged postoperative ileus. Retrospective study by Juarez-Parra et al. [18] used the same definition of PPOI but with reported incidence almost twice higher (22.3%), even though some of the patients were operated by laparoscopic approach. Some authors argue that after laparoscopic surgery faster bowel recovery is to be expected because there is less surgical trauma, reduction in release operative stress hormones, less postoperative pain with the reduction in need for analgetics. Additional factor that might have the influence is intraabdominal humidity, since laparotomy induces evaporation from the bowel surface and exposes the intestine to a nonphysiological environment. Chen et al. [19], confirmed a statistically significant lower rate of postoperative vomiting after laparoscopic colectomy in comparison to open group and significant differences related to diet tolerance (2.1 vs. 3.2 days), length of ileus (3.5 vs. 5.3 days), and length of hospitalization (6.6 vs. 8.1 days). In their study, cancer was the indication for surgery in only 13% of patients [19], which could have been influenced on the extent of resection, thus effecting a level of operative truma. In a large retrospective analysis from 2015 on 32,392 elective colectomies, PPOI was reported in 14% of cases. This study also included indications other than cancer, like diverticular disease or volvulus. A higher PPOI rate was noted especially in these cases. The highest difference in rates of PPOI was also found between open and laparoscopic approach, where for every type of procedure open approach was associated with > 10% higher rates of ileus compared to laparoscopic approach [20]. In a prospective study in which only laparoscopic colectomies for benign and malignant colorectal disorders were analyzed, the rate of PPOI was reported to be 10.2% [21], just slightly lower than the rate reported in this study which included only open procedures. Limitation in use of opiates in this study protocol for postoperative pain management could be the reason for similar rates of PPOI. Different design of studies in terms of indications for surgery, definition of PPOI, approach, postoperative care pathway, use of opiate based analgesia, makes results difficult to interpret.

According to latest meta-analysis the rate of PPOI after colorectal surgery is around 10%, with lower incidence after laparoscopic colonic resections, but with variation in incidence depending on the definition used [13]. Although minimal invasive surgery is evolving parallel with enhanced recovery protocols, PPOI is still important postoperative event [21].

Various risk factors for development of PPOI are identified in studies, also due to diversity in definitions of PPOI used as well due to different study designs. For example, according to retrospective analysis from Millan et al. [22], male sex, COPD and ileostomy were independed factors associated with higher risk of this complication. On the other hand Kronberg et al. [21] showed that age, preoperative albumin level, previous abdominal surgery and chronic preoperative use of narcotics were associated with PPOI. Moghadamyeghaneh et al. [17] found that advanced age, duration of surgery and ileocolic anastomosis was associated with PPOI. According to our results, neither age, gender, ASA grade, duration of surgery, type of anastomosis and creation of stoma reached statistical significance in regression analysis. Identification of other potential risk factors that could be related to PPOI was beyond the scope of this paper.

Our study showed that patients with PPOI hadstatistically significant difference in grade of complications, especially grade III (complications requiring surgical, radiological or endoscopic intervention). Five out of 12 patients, (41.6%), with PPOI in our study, suffered from additional complications. Retrospective study on more than 32,000 colectomies also showed that patients with PPOI were more likely to suffer from another postoperative complications compared to patients without ileus (50% vs. 21%, respectively) [20]. In this study, almost 60% of patients with ileus suffered from additional adverse outcome. We did not find statistically significant difference between rates of SSI and anastomotic leakage amongst the patients with and without PPOI. According to some authors PPOI is significantly asso-

ciated with intra abdominal infections (p = 0.01) and anastomotic leakage (p = 0.01) [17]. The reason could be the fact that in their study ileus was defined as no return of bowel function within 7 days from operation, which is important since PPOI could develop secondary to other complication, and this underling complications must be ruled out. It is of cardinal importance to differentiate PPOI from anastomotic leakage or intraabdominal sepsis, as PPOI could represent one of their clinical features. Out of 5 patients reoperated from group with PPOI in our study, one had unrecognized anastomotic leakage. Postoperative fever in conjunction with tachycardia, hypotension, or raised inflammatory markers suggested a source of sepsis, and was to prompt investigation to exclude intra-abdominal infection [23]. In a retrospective study with laparoscopic colectomies no difference in rates of postoperative abdominal abscess in the patients with and without PPOI was observed (1.6% vs. 2.4% p = 0.53). The same study reports that deep vein thrombosis (DVT) in the postoperative period was more frequently seen in patients with PPOI (7.1% vs. 1.1%; p = 0.026) which authors found difficult to interpret, since it is hard to know what was the cause and what was the effect; it was assumed that it was related to delayed activation [21].

Our results show that patient with PPOI has 12 times more chance to be reoperated in comparison to the patient without this complication (OR = 12.286; p = 0.001), which is much higher than previously reported [17, 20]. We presume that this was mostly due to our fear from another precipitating complications since in 3 patients operated from the group of PPOI, no underlying cause was found.

Distinguishing other postoperative complications from PPOI is of paramount importance since symptoms of PPOI are similar to, for example, postoperative small-bowel obstruction or anastomotic leakage or intraperitoneal bleeding. Many of these complications require immediate intervention since they are life-threatening conditions [4].

None of the patients was readmitted for PPOI during the study period, although PPOI was often mentioned as a reason for rehospitalization [8, 17, 20]. Our study also failed to identify PPOI as a risk factor for mortality (OR = 0.291; p = 0.170). According to most studies [16, 24–26], the patients with PPOI have significantly higher mortality rate. According to a large study by Tevis et al. [20], ileus alone did not increase mortality, but with every additional complication, mortality was rising dramatically to as high as 28%, like in cases with seven different complications. Increase in mortality also varied among different complications. For example ileus accompanied by SSI did not have an impact on mortality while the highest impact on mortality was seen in combination of ileus with pulmonary complications, which increased the death rate from 2% to 22% [20].

Longer hospital stay of the patients with PPOI was confirmed in many studies [8, 17, 20–25] including our. In a large study from the US which included 17,876 patients with colectomy [8] not only that patients with PPOI had prolonged hospitalization but they were less likely to be discharged home after surgery (67.2% vs. 77.4%; $p < 0.001$) and more likely to be discharged to another institution (7.7% vs. 4.9%; $p < 0.001$) or to home health care (21.7% vs. 15.0%; $p < 0.001$). Prolonged hospitalization in PPOI may potentiate hospital-acquired infections [21].

Conclusion

PPOI is a concern for colorectal surgeons and patients even in the era of minimally invasive surgery and after implementation of enhanced recovery protocols. PPOI in addition to economic burden has an impact on other adverse events such as other complications, reoperations, even mortality according to some authors. In every case of PPOI is important to carrfully rule out possible complications. Although not life-threatening complication and by that sometimes underestimated, it affect recovery, increases hospital length of stay and contributes to poor surgical outcome.

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Discoloration of resin based composites in natural juices and energy drinks

Prebojavanje kompozita prirodnim sokovima i energetskim pićima

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Abstract

Background/Aim. Discoloration of dental restorations makes them aesthetically unacceptable and is a frequent reason for replacement of composite restorations. The aim of this study was to evaluate changes of color and fluorescence of resin-based composites (RBCs) exposed to natural juices and energy drinks. **Methods.** Microhybrid composite Gradia Direct™ Extra Bleach White disc-shaped specimens (n = 35) were immersed in three different natural juices and four different energy drinks. Absorption spectra of natural juices and energy drinks, diffuse reflection and fluorescence of composite samples were measured prior and after seven- day immersion by spectrophotometer Thermo Evolution 600 and spectrofluorometer Fluorolog-3-221. Composite's color was calculated from diffuse reflection spectra and expressed in CIELAB color space (*Commission International de l'Eclairage*). **Results.** All natural juices and energy drinks induced color change of resin based composites, but to the different extent. Only aronia and carrot juices induced total color change considerably higher than clinically acceptable threshold, 9.3 and 6.2, respectively. All energy drinks and aronia juice induced notable decrease in fluorescence; the highest change of 28% was evidenced in the case of aronia juice. **Conclusion.** Change of color and fluorescence will appear differently with various solutions due to different chemical composition and concentration of colorant species in different beverages. Solutions with higher optical absorption induced higher total color change. Discoloration of composites in aronia and carrot juices is similar to those earlier reported for red wine, tea and coffee.

Key words:
dental materials; composite resins; materials testing; color; energy drinks; fruit and vegetable juices; fluorescence.

Apstrakt

Uvod/Cilj. Diskoloracija zubnih nadoknada čini ih estetski neprihvatljivim i često je razlog za njihovu zamenu. Cilj ove studije bio je da se ispita promena boje i fluorescencije kompozita nakon izlaganja prirodnim sokovima i energetskim pićima. **Metode.** Uzorci mikrohibridnog kompozita Gradia Direct™ (n = 35) potopljeni su u tri različita prirodna soka i četiri različita energetska pića. Apsorpcioni spektri prirodnih sokova i energetskih pića, difuzni refleksioni spektri i fluorescencija kompozitnih uzoraka izmereni su pre i nakon sedmodnevnog potapanja na spektrofotometru Thermo Evolution 600 i spektrofluorometru Fluorolog-3-221. Iz difuznih refleksionih spektara izračunata je boja kompozita i izražena u CIELAB sistemu (*Commission International de l'Eclairage*). **Rezultati.** Svi prirodni sokovi i energetska pića doveli su do promene boje kompozita, ali u različitom obimu. Ukupna promena boje je bila značajno veća od klinički prihvatljivog praga samo kod sokova od aronije i šargarepe (ΔE = 9.3 i 6.2, redom). Sva energetska pića i sok od aronije izazvali su primetno smanjenje fluorescencije; najveća promena od 28% zabeležena je u slučaju soka od aronije. **Zaključak.** Promene boje i fluorescencije razlikuju se u različitim rastvorima zbog različitog hemijskog sastava i koncentracije prebojavajućih supstanci u različitim pićima. Rastvori sa većom optičkom apsorpcijom pokazali su veću ukupnu promenu u boji. Prebojavanje kompozita u sokovima od aronije i šargarepe slično je već ranije zabeleženom u slučaju crvenog vina, čaja i kafe.

Ključne reči:
stomatološki materijali; smole, kompozitne; materijali, testiranje; boje; energetski napici; sokovi od voća i povrća; fluorescencija.

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Introduction

Resin-based composites (RBCs) should mimic the aesthetic characteristics of natural teeth and possess a color stability throughout the functional lifetime of the restoration. However, RBCs are prone to discoloration when exposed to saliva, food and beverages, and different stains in the oral environment. Discoloration of dental restorations makes them aesthetically unacceptable and is a frequent reason for replacement of composite restorations, with 16.9% of incidence – coming second after secondary caries [1]. Regularly consumed food and beverages may affect color stability of teeth and RBCs and in recent times many literature reports have addressed on their stain-causing effects and problems [2–5].

In the majority of reports changes in the color of restorations after storage in different food and beverages have been assessed by the total color change (ΔE*) of CIELAB color system coordinates (*Commission International de l'Eclairage*). So far, red wine, tea and coffee were demonstrated as frequently consumed beverages which may cause a significant discoloration of teeth and restorations [6–10]. However, literature data on the deterioration of fluorescence is scarce [11], even though the contribution of fluorescence to the visual appearance of teeth and restorations should not be neglected. Also, limited data were available regarding the biochemical constituents of food and beverages responsible for stain-causing effects [12] despite the fact that the proper knowledge of biochemistry behind the staining may aid and improve the effectiveness of stain removal.

Natural juices and energy drinks are gaining increased attention of customers in the last years; recent data (reviews and meta-analyses) indicate a current trend of increased consumption of fruit and vegetable juices and energy drinks [13]. Though many scientific studies analyzed the influence of these beverages on overall health, less work was put in the investigation of their effects on the color stability of dental restorations [14–17]. Thus, the aim of this study was to thoroughly investigate changes in the optical properties of resin composites exposed to some popular natural juices and energy drinks by evaluating changes both in their color and fluorescence as well as to identify colorant species responsible for observed effects.

Herein, we analyzed *in vitro* staining effects of tree natural juices (beet, carrot and aronia) and four energy drinks (Guarana Kick®, Red Bull®, Energi·s® and Burn®) on color and fluorescence of microhybrid commercial composite. Biochemical constituents of the beverages which are responsible for staining were recognized on the basis of optical absorption and reflection measurements.

The null hypotheses tested were: 1) there are no differences in color among the RBC samples stained in energy drinks and natural juices and non-stained samples; 2) there are no differences in fluorescence among the RBC samples stained in energy drinks and natural juices and non-stained; and 3) immersion of composites in different-type energy drinks and natural juices produce similar effects on the optical properties of composites.

Methods

Specimen preparation and staining procedure

Disc shaped specimens of Gradia Direct™ (GC Corp. Tokyo, Japan) extra bleach white composite (n = 40) were prepared in silicon molds, 2 mm thick and 13 mm in diameter. The molds were placed on a glass slab, filled with composite material and gently pressed with a glass slide to extrude excess material. Polymerization was performed for 20 s with a polywave LED light-curing unit (bluephase G2, Ivoclar Vivadent, Schaan, Lichtenstein) with light intensity of 1100 mW/cm^2. The distance between the light source and the specimen was standardized by the use of 1 mm glass slide. After polymerization, the samples were removed from the mold and polished under wet conditions with a series of Super-Snap Buff disks (medium, soft, super soft) and Super-Snap SuperBuff disks(Shofu Dent Cor, San Marco, Japan) and stored in distilled water at 37 °C for 24 h. Specimens were divided in equal groups and immersed in following fresh natural juices: beet juice (Rote-Bete-Saft®, SchneeKoppe, Germany), carrot juice (Mohrensaft®, SchneeKoppe, Germany), aronia (Aronia®, Aroniada-Agro, Bulgaria) and energy drinks: Guarana Kick® (Knjaz Miloš, Serbia), Red Bull® (Red Bull, Austria), Energi·s® [Frutti, Serbia (Sinalco International, Germany)], Burn® (Coca Cola HBC, Hungary), as shown in Table 1.

Table 1

Staining solutions used in this study

Product	Composition	Manufacturer
Rote-Bete-Saft®	Beet juice (99%), lemon juice (1%), antioxidant, ascorbic acid	SchneeKoppe, Germany
Mohrensaft®	Carrot juice (99%), lemon juice (1%), vitamin A	SchneeKoppe, Germany
Aronia®	100% pressed aronia berries juice (no sugar, no additives, no preservative)	ARONIADA-AGRO, Bulgaria
Guarana Kick®	Caffeine (max. 32 mg/100 mL), water, sugar, CO_2 (min.5 g/L), citric acid, taurine	KNJAZ MILOŠ, Serbia
Red Bull®	Caffeine (max.32 mg/100 mL), taurine (400 mg/100 mL), citric acid, CO_2, water, sugar, sodium carbonate, magnesium carbonate, colors (caramel, riboflavin), vitamins	Red Bull, Austria
Energi·s®	Caffeine, taurine (400 mg/100 mL), citric acid, CO_2 (min. 4g/L), water, sugar, preservative (E211 max. 150 mg/L), inozitol (19.5 mg/100 mL), colors (E150c, E101), vitamins	FRUTTI, Serbia (Sinalco International, Germany)
Burn®	Caffeine (max.32 mg/100 mL), taurine (4000 mg/L), citric acid, CO_2 (min. 2g/L), water, sugar, preservative: sodium benzoate ipotassium sorbate, colors (E150d), inositol (max. 200 mg/L), vitamins, guarana extract, ascorbic acid	Coca Cola HBC, Hungary

Storage time was seven days at 37 °C to simulate the mouth environment. All solutions were renewed daily to prevent bacterial contamination. After that specimens were rinsed with tap water and blotted dried with a tissue paper before measurements.

Diffuse reflection measurements

Spectrophotometer Thermo Evolution 600 (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an integrated sphere (Labsphere RSA-PE-19) was used for diffuse reflection measurements in the 220–900 nm range with 1 nm step.

Fluorescence measurements

Fluorolog-3 Model FL3-221 spectrofluorometer (Horiba JobinYvon) was used for obtaining excitation-emission matrices (EEMs) of the samples utilizing a 450-W Xenon lamp as the excitation source and R928 photomultiplier tube as a detector in the front-face configuration. Excitation range was from 270 nm to 550 nm and the emission range 300 nm and 650 nm, with 5 nm and 1 nm step, respectively. Excitation and emission slits were set at 3 nm with acquisition time set to 0.07 s. Fluorescence was measured before and after seven-day immersion in staining solutions.

Digital imaging

Digital images were acquired with Canon digital camera EOS 1200D and Intel QX3 Computer Microscope before and after specimen staining.

Data analysis

All color testing were carried out according to the CIE-LAB color system defined by *Commission International de l' Eclairage* (CIE) which uses the three dimensionless colorimetric measurements (L^*, a^* and b^*):

$$\Delta L^* = L^*_{sample} - L^*_{reference},$$

$$\Delta a^* = a^*_{sample} - a^*_{reference},$$

$$\Delta b^* = b^*_{sample} - b^*_{reference.}$$

CIE $L^*a^*b^*$ color coordinates were calculated from diffuse reflection measurements, relative to standard illuminant (D65), against a white background (barium sulfate). The total color difference (ΔE^*) and chroma (ΔC^*) for each disk sample was calculated using the following equation [18]:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

$$C_{ab}^* = (a^{*2} + b^{*2})^{1/2}$$

$$\Delta C^* = C^*_{ab,\ sample} - C^*_{ab,\ reference}.$$

Total fluorescence emission (TF) was calculated as a volume under the fluorescence intensity surface of the excitation-emission plane:

$$TF = \sum_{\lambda EX = 270\ nm}^{550\ nm} \sum_{\lambda EM = 300\ nm}^{650\ nm} I(\lambda_{EX}, \lambda_{EM})$$

Differences in fluorescence were quantified as percentage of TF change compared to TF of the reference sample by using the following equation:

$$\Delta TF\ (\%) = \frac{TFsample}{TFreference} \times 100$$

Results

Figure 1 present absorption spectra in the 220–900 nm spectral range of staining solutions used in this study. All solutions display strong absorption in the UV spectral range (< 400 nm). Energy drinks showed well-resolved absorption peak at ~280 nm (Figure 1a). In the visible spectral range fresh natural juices showed moderate absorption (Figure 1b) while energy drinks showed quite low (see inset in the Figure 1b). Comparing overall absorption, among fresh natural juices aronia solution showed the strongest absorption and beet juice the lowest. Among energy drinks, Burn® had the highest absorption. Distilled water was used as a reference and showed no absorption.

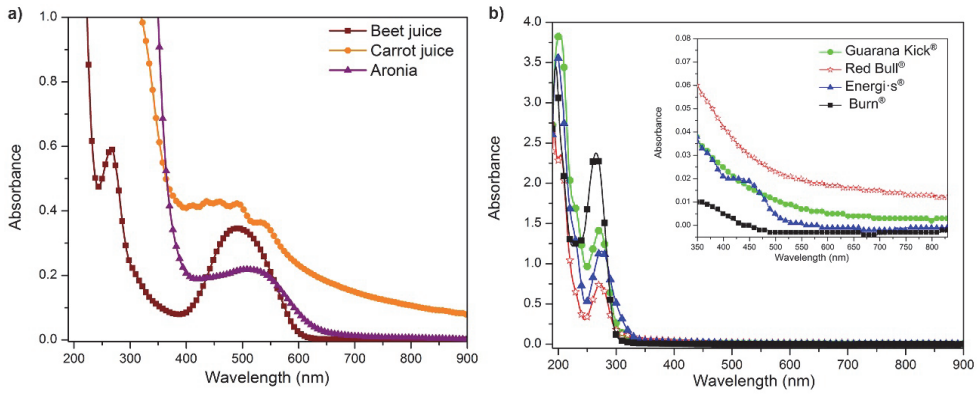

Fig 1. – Absorption spectra of: a) fresh natural juices – beet juice (brown line), carrot juice (orange line) and aronia juice (purple line); b) energy drinks – Guarana Kick® (green line), Red Bull® (red line), Energi·s® (blue line) and Burn® (black line).

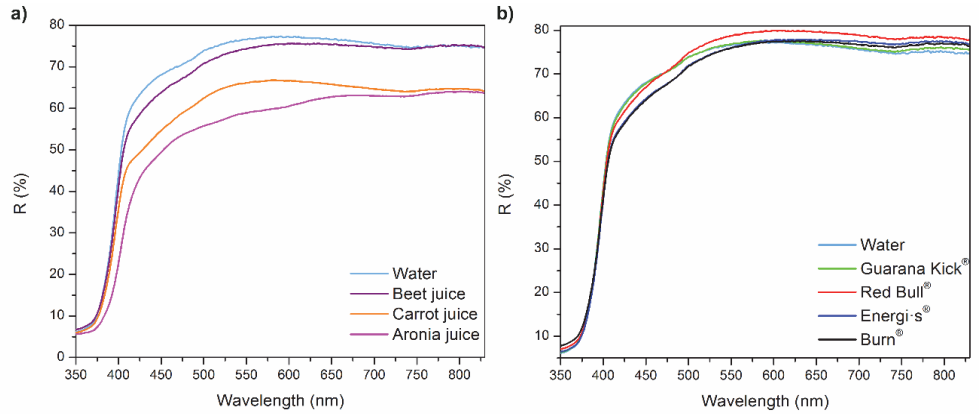

Fig 2. – Diffuse reflection spectra of: a) fresh natural juices – water (reference, blue line), beet juice (purple line), carrot juice (orange line) and aronia juice (pink line); b) different energy drinks – water (reference, blue line), Guarana Kick® (green line), Red Bull® (red line), Energi·s® (dark blue line), Burn® (black line).

Table 2

Mean values of *Commission International de l'Eclairage* (CIELAB) color coordinates for resin composites after seven-day staining in natural juices and energy drinks; total color change (ΔE^*) and chroma change (ΔC^*)

Product	L^*	a^*	b^*	ΔE^*	ΔL^*	ΔC^*
Water	89.9	-1.9	6.7	-	-	-
Beet juice	88.8	-1.9	8.4	2.1	1.1	1.7
Carrot juice	84.7	-3.1	9.5	6.1	5.2	3.1
Aronia juice	81.1	-1.3	9.2	9.1	8.8	2.3
Guarana	89.9	-2.1	7.1	0.4	0	0.4
Red Bull	90.8	-2.2	8.9	2.4	-0.9	2.2
Energi·s	89.6	-1.8	9.2	2.6	0.3	2.5
Burn	89.5	-1.9	9.3	2.7	0.4	2.5

Diffuse reflection spectra of the composite samples were measured in 350–850 nm spectral region before and after staining. Spectra of the samples stained in natural juices are displayed in Figure 2a and spectra of the samples stained in energy drinks are given in Figure 2b. In both cases, spectra were obtained by averaging data obtained from measurements on all samples from each group; spectrum of samples immersed in distilled water is presented as a reference.

Figure 2a shows a considerable decrease of reflection of the samples stained by natural juices when compared to reflection of the reference samples; the largest decrease was observed in samples exposed to aronia juice, then in these exposed to carrot juice and the smallest, but still of significant magnitude, in the samples stained with beet juice. Changes of reflection were considerably lower in the samples stained in energy drinks; the largest one was found in the samples exposed to Burn® and the smallest exposed to Guarana Kick®.

Color coordinates (in Lab color system) were calculated from diffuse reflection spectra and are given in the Table 2 along with the values of total color change (ΔE^*) and change of chroma (ΔC^*) calculated with respect to the reference samples. Staining with natural juices lowered the lightness (L^*) and altered color coordinates (a^* and b^*) of composites. The total color change was therefore comprised of the change in lightness and change in chroma and was the largest ($\Delta E^* = 9.3$) in aronia juice. Staining in energy drinks slightly changed the color coordinates, but did not change the lightness. The most pronounced color changes of $\Delta E^* = 2.8$ were seen in a case of Burn®.

Changes of fluorescence of the resin composites after staining in natural juices and energy drinks were perceived in fluorescence excitation-emission matrices (EEM's) which are composed of series of emission spectra measured for different excitation energies. Contour plots (projection of emission intensity into excitation-emission plain) of the fluorescent EEM spectra recorded with the samples stained in natural juices and energy drinks are presented in Figure 3. For all samples, two strong excitation bands can be observed; the first from 270 nm to 340 nm and the second from 360 nm to 470 nm. Both excitations produced emissions in the 350–550 nm spectral region, with the most intense blue emission around 450 nm.

Staining-induced changes in fluorescence of composites were quantified as a relative difference of the total fluorescence of the stained sample over the fluorescence of the reference sample (Table 3). Among staining with natural juices, only composite exposed to aronia juice showed significant decrease in fluorescence (28%). On the other hand, staining in all types of energy drinks led to the large decrease of fluorescence; the largest value of 25% was observed with Red Bull®.

Table 3

Decrease of total fluorescence of resin composites after seven-day exposure to natural juices and energy drinks

Product	Decrease of fluorescence (%)
Aronia juice	28
Beet juice	~ 0
Carrot juice	~ 0
Red Bull®	25
Guarana Kick®	20
Burn®	14
Energi·s®	13

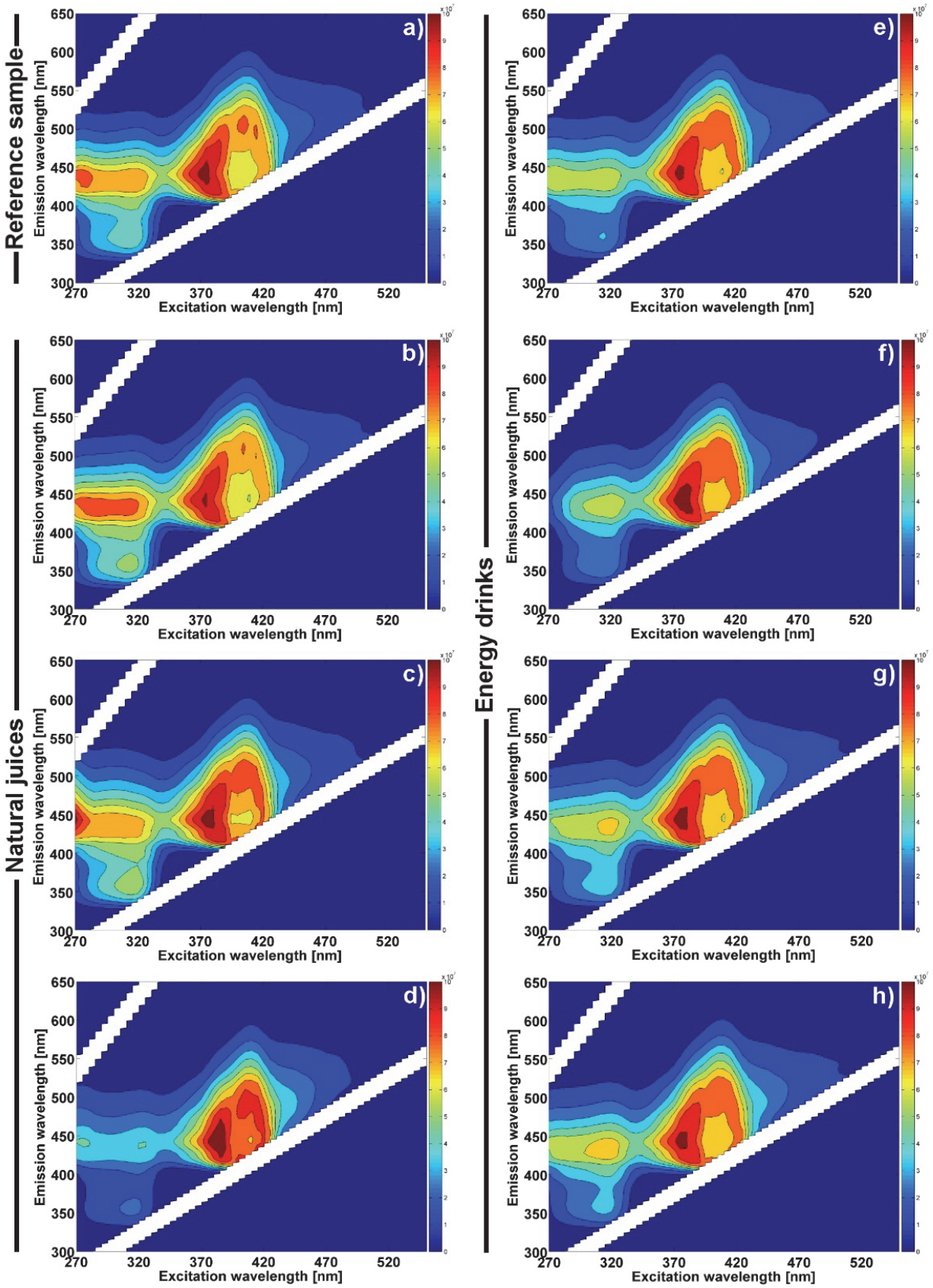

Fig 3. – Fluorescence excitation-emission matrices (EEM) spectra of specimens after 7-days staining in natural juices and energy drinks, with immersion in distilled water as a reference: sample immersed in a) distilled water, b) beet juice, c) carrot juice, d) aronia juice, e) Guarana Kick®, f) Red Bull®, g) Energy®, h) Burn®.

Changes in the appearance of resin composites (color and fluorescence) after staining is illustrated in Figure 4. Images were recorded by digital camera and optical microscope (60× magnification) under daylight and under UV illumination.

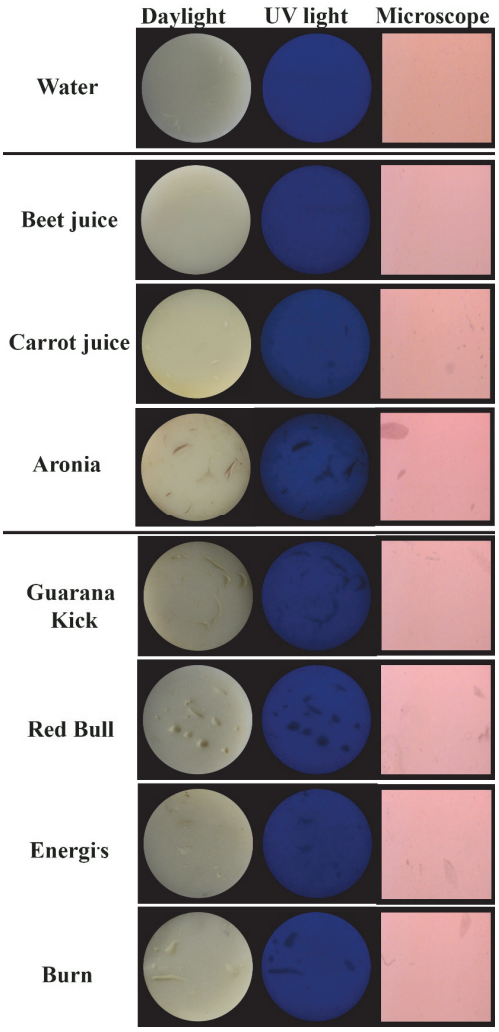

Fig 4. – Images of samples immersed in distilled water, fresh natural juices and different energy drinks for 7 days, recorded by digital camera and optical microscope under different illumination [daylight and ultraviolet (UV)].

Discussion

Results show that staining of composites is more intense in solutions that have higher absorption in the visible spectral range; therefore, the third hypothesis was rejected. Natural juices have larger absorption than energy drinks (Figure 1), and, as a consequence, reflection of the samples exposed to natural juices was lower than reflection of the samples exposed to energy drinks (Figure 2). Having in mind that color coordinates are calculated from the diffuse reflection spectra, the total change of color and change of chroma showed the same effect (Table 2). One should note that the total color change larger than 2.7 (clinically acceptable threshold [19]) was observed on the samples stained by aronia juice (9.3) and carrot juice (6.2). The degree of discoloration is comparable to those recently assessed for staining of the same resin composite in tea, coffee and red wine 12. Total color changes of the samples stained by Guarana® (0.5), beet juice (2.2) and Red Bull® (2.3) were below clinically acceptable threshold while the values for Energie·s® (2.7) and Burn® (2.8) were just on the threshold value and would exceed it if the staining time was longer. Based on these results, the first hypothesis could not be rejected or confirmed since different staining solutions produced different effects. One should also note that in the case of staining in energy drinks total change of color is mainly due to the change in chroma (no changes in the lightness), while staining in natural juices significantly reduced lightness of the samples and moderately altered chroma.

Aronia juice was the only tested juice in this research which caused a decrease in the fluorescent response of the composite samples; this decrease of 28% was the highest among all tested solutions in this study and similar to ones found in several types of beer [20]. Regarding energy drinks, Red Bull® and Guarana Kick® showed considerable decrease in fluorescence, much higher than Burn® and Energi·s®. Therefore, the second hypothesis was not confirmed nor rejected. In all cases shapes of fluorescence spectra were not changed and only intensity of the fluorescence was affected.

The changes of color and fluorescence of RBCs after seven-days immersion in natural juices and energy drinks are of such intensity that can be easily proved by microscope images obtained by 60 times magnification power. The loss of white appearance of the composite samples is illustrated on digital camera images taken under daylight illumination (Figure 3).

Having in mind matching results of absorption and diffuse reflection measurements (Figures 1 and 2), it is possible to state that changes in color and fluorescence of the resin composites upon exposure to natural juices and energy drinks was a consequence of adsorption and absorption of colorant species. Chemical composition and concentration of colorant species are different in various beverages; therefore, discoloration and change of fluorescence will appear differently with different staining solutions as evidenced from results presented in Tables 2 and 3. Main colorant constituents of carrot juice are carotenoids (lycopene and β-carotene) which have characteristic absorption maximum in 400–500 nm spectral range and retinol (vitamin A) which absorbs around 330 nm [21]. First absorption band of aronia juice is typical for polyphenolic (flavonoids) compounds that absorb at about 330 nm [22], while the other peak (400–600 nm) is due to the presence of anthocyan [23]. Regarding beet juice, the peak at 270 nm originates from proteins (tryptophan and thyrosine). The absorption of proteins was also present in two other juices, but protein absorption peaks were of high intensity to be clearly resolved without considerable dilution of juices. Peak at a 470–550 nm in absorption spectrum of beet juice corresponds to a group of betalains pigments [24, 25] and is an overlapped absorption of: 1) betaxanthins (yellow pigments) which have a characteristic absorption maximum at 260 and 474 nm, 2) betanin – type betacyanins (red-violet pigments) with a characteristic absorption at 538 nm [26].

Energy drinks – Guarana Kick®, Red Bull®, Energi·s®, Burn® showed strong absorption in the 190–350 nm spectral

range (Figure 1b). The difference in the absorption of tested energy drinks comes from the difference in the concentration of actual energizers (caffeine, taurine and vitamins B). The UV absorption spectrum of caffeine exhibits a pair of absorption bands peaking at 205 nm and 273 nm with a characteristic shoulder between them [27, 28]. Strong yellowish color change with Energi·s® may be caused by the presence of riboflavin (E101) which absorbs at 450 nm [29].

Conclusion

Within the limitations of this *in vitro* study, it can be concluded that after seven-day immersion in natural juices and energy drinks RBCs change color and fluorescence. Magnitudes of color and fluorescence changes depend on the concentration and chemical composition of colorant species in natural juices and energy drinks. Strong absorbing aronia and carrot juices induce total color change considerably higher from clinically acceptable threshold. All energy drinks and aronia juice induce notable decrease in RBC fluorescence. This study identified biochemical compounds responsible for RBC staining in natural juices and energy drinks which should clarify staining mechanisms and improve the effectiveness of stain removal.

Conflict of interest

The authors do not have any financial interest in the companies whose materials were included in this study.

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Exposure to potential drug-antimicrobial agent interactions in primary health care

Izloženost potencijalnim lek-antimikrobni agens interakcijama u primarnoj zdravstvenoj zaštiti

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Abstract

Background/Aim. Drug-drug interactions involving antimicrobials present important and often unrecognized complications of pharmacotherapy which can be prevented. The aim of the present study was to identify the frequency and type of potential drug-antimicrobial agent interactions among outpatients and to define recommendations for their management. **Methods.** Cross-sectional prescription database study was conducted. The analysis randomly included 823 patients who visited Health Center Novi Sad over 1-month period (November 1–30, 2011) and had prescribed ≥ 2 drugs where at least one drug was antimicrobial agent for systemic use. All interacting drug combinations involving antimicrobials were identified according to Drug Interaction Facts. Additionally, based on the compendium, potential interactions were classified into categories: pharmacological mechanisms, potential clinical outcomes and management advice. **Results.** Overall, 88 potential clinically significant drug-antimicrobial agent interactions were identified among 69 (8.4%) exposed outpatients [the mean age 61.7 years (SD ± 15.4); the mean number of prescribed drugs 7.5 (SD ± 2.9); 56.5% females]. The most common identified potential interacting pairs were benzodiazepines undergoing oxidative metabolism and clarithromycin or erythromycin, and aminophylline and ciprofloxacin. In 83.0% of all cases underlying mechanism was pharmacokinetic involving primary inhibition of metabolic pathways mediated by CYP3A4 and CYP1A2 isoenzymes. Excessive sedation (22.7%), cardiotoxicity (20.5%), miscellaneous aminophylline adverse effects (13.6%), and bleeding (10.2%) were the most frequently implicated potential clinical outcomes. Risk for adverse interactions could be managed by close monitoring of simultaneous administration of drugs (37.5%), different risk-modifyng strategies (31.8%), and avoiding combinations (30.7%). **Conclusion.** Among outpatients, there was common potential for clinically significant interactions involving antimicrobials. Information based on the results of the present study could be integrated in existing computerized physician order entry system in the Health Center as a form of clinical support.

Key words:
drug therapy; anti-bacterial agents; drug interactions; outpatients; adverse drug reaction reporting systems; pharmacovigilance.

Apstrakt

Uvod/Cilj. Interakcije antimikrobnih lekova predstavljaju važne i često neprepoznate komplikacije farmakoterapije koje mogu biti prevenirane. Cilj prezentovane studije bio je da se identifikuje učestalost i tip potencijalnih interakcija antimikrobnih lekova kod ambulantnih bolesnika, i da se definišu preporuke za kontrolu istih. **Metode.** Sprovedena je studija preseka koristeći bazu podataka o propisanoj terapiji. U analizu je randomizacijom uključeno 823 bolesnika (propisana ≥ 2 leka a najmanje jedan lek bio je antimikrobni agens za sistemsku upotrebu) koji su posetili Dom zdravlja Novi Sad tokom jednomesečnog perioda (1–30. novembar 2011). Sve interakcije antimikrobnih lekova su identifikovane saglasno *Drug Interaction Facts*. Dodatno, bazirano na kompedijumu, potencijalne interakcije su klasifikovane u kategorije: farmakološki mehanizmi, potencijalni klinički ishodi, i preporuke za kontrolu. **Rezultati.** Ukupno, 88 potencijalnih, klinički značajnih lek-antimikrobni agens interakcija identifikovano je kod 69 (8,4%) izloženih bolesnika [prosečna starost 61,7

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godina (SD ± 15,4); prosečan broj propisanih lekova 7,5 (SD ± 2,9); 56,5% su bile žene]. Najzastupljeniji potencijalni interakcijski parovi bili su benzodiazepini koji se metabolizuju oksidacijom i klaritromicin ili eritromicin kao i aminofilin i ciprofloksacin. U 83% svih slučajeva u osnovi je bio farmakokinetski mehanizam interakcija uključujući primarno inhibiciju metaboličkih puteva posredovanu izoenzimima CYP3A4 i CYP1A2. Izražena sedacija (22,7%), kardiotoksičnost (20,5%), različite neželjene reakcije na aminofilin (13,6%), krvarenje (10,2%) bili su najčešće implicirani potencijalni klinički ishodi. Rizik za neželjene interakcije mogao je biti kontrolisan pažljivim monitoringom uporedne upotrebe lekova (37,5%), različitim strategijama za modifikaciju rizika (31,8%), i izbegavanjem kombinacija (30,7%). **Zaključak.** Kod ambulantnih bolesnika postojao je značajan potencijal za klinički važne interakcije antimikrobnih lekova. Informacije bazirane na rezultatima istraživanja mogle bi biti integrisane u postojeći sistem za elektronsko propisivanje kao vid kliničke podrške.

Ključne reči:
lečenje lekovima; antibiotici; lekovi, interakcije; ambulantno lečenje; lekovi, neželjeno dejstvo, sistemi za izveštavanje; farmakovigilanca.

Introduction

It is well known that adverse drug interactions (ADIs) involving antiinfective agents can be complication of pharmacotherapy. Thus, according to the World Health Organization Global Individual Case Safety Report (WHO Global ICSR) database, during the past 20 years, among the 15 most frequently reported adverse drug interacting combinations, 4 included antimicrobials [1]. Molden and Andersson [2] described two men with rhabdomyolysis, who received simvastatin 80 mg/day and who were hospitalized after the completion of short-term treatment with macrolide antibiotics (clarithromycin and erythromycin). Flockhart et al. [3] reported on the case of a 27-year-old man who experienced a prolonged QT interval and sudden cardiac death two days after coadministration of pimozide and clarithromycin. Additionally, reports on fatal *torsades de pointes* induced by terfenadine during its coadministration with ketoconazole or erythromycin contributed to the withdrawal of terfenadine from the United States market [4]. Also, antimicrobials can lead to a reduction or loss of therapeutic efficacy of concomitantly used drugs. Thus, ketoconazole affects formation of clopidogrel active metabolite causing reduced inhibition of platelet aggregation [5]. Also, bioavailability of tetracyclines and quinolones can be significantly reduced in presence of aluminium, magnesium or calcium-containing antacids [6, 7].

Besides safety aspect, interactions are important because they are often avoidable or preventable adverse drug events (ADEs). Thus, Juurlink et al. [8] estimated that at least 3.3% of hospital admissions due to hypoglycemia was caused by concomitant using of glibenclamide and cotrimoxazole, so as at least 2.3% of hospitalizations because of digoxin toxicity during its coadministration with clarithromycin could be prevented. The basis for the prevention of ADIs is possession of knowledge or possibility to predict situations when simultaneous administration of drugs presents risk for drug-mediated toxicity or therapeutic failure.

In literature a large number of interactions of antimicrobial drugs are listed and several reviews describe the ones which are clinically relevant [9, 10]. More specific, Spriet et al. [11] gave overview of significant CYP450-mediated interactions involving antiinfective agents and drugs frequently received in the Intensive Care Unit (ICU) and Becker [12, 13] described adverse interactions of antibiotics commonly used in dental practice while Tey et al. [14] reported on drug interactions with often prescribed antimicrobials in dermatological practice. However, differences in morbidity structure or complexity of healthcare contribute to specificity of study findings [15]. Hence, as intention to improve the safety of pharmacotherapy in the Health Center, the primary aim of this study was to identify the frequency and type of clinically significant potential drug-antimicrobial agent interactions among outpatients and to define recommendations for their control subsequently based on these local reports.

Methods

The Ethics Committee of the Health Center Novi Sad (HCNS), Novi Sad, Serbia approved the protocol of the present study.

Study design and data collection

The prevalence and type of potential drug-antimicrobial agent interactions among outpatients at the HCNS were analyzed in the cross-sectional, single-center study. HCNS provides primary health care for population of approximately 340,000 people living in Novi Sad, the administrative seat of the northern Serbian province Vojvodina. Medical care is offered to outpatients within 45 Basic Health Units (BHUs) involving health promotion and education, prevention and early management of health problems as well as curative care. The study was carried out using data from all BHUs.

HCNS possesses a health information system certified by the European Institute for Health Records. Computerized medical record contains all relevant facts about patient and his/her therapy. Hence, study data were obtained from the electronic prescription database and their collection was done automatically by the computer server administrator. Data collection was described in detail by Nikolic et al [16]. In brief, there was no access as well as direct manipulation of the healthcare data by researchers, and strict registration routines and access controls support the security and accuracy of information involved in the electronic dataset. Prescription records referred to all reimbursed drugs by National Health Service, according to the List of drugs prescribed and dis-

pensed under the mandatory health insurance scheme, and database did not include information on *over-the-counter* (OTC) drugs. For the purpose of the study, the following data were selected from medical documentation: year of birth and patient's sex, prescribed drugs and date of their prescribing, dose regimen, quantity (number of prescribed packs) and route of the administration. Drugs were coded according to Anatomical Therapeutic Chemical (ATC) classification system as recommended by the WHO [17].

Medical records of drug users in the HCNS during one-month observed period (November 1–30, 2011) were recruited to the study if patients had been prescribed two or more than two drugs where at least one of medicines was antibacterial for systemic use. Two researchers (BN and DR) were responsible for determination of subjects eligible for inclusion in the study. For each outpatient was assumed that using of medicines started at the same day when the medicine was prescribed and the duration of therapy for each medicine was calculated in days by multiplying a daily dose by the number of daily doses contained in the prescribed packs. Potential for the drug-antiinfective agent interactions was studied when the exposure period for two medicines overlapped. Overlapping was defined as the presence of at least a day of co-prescription of two medicines. This definition is consistent with previous studies using administrative claims databases, evaluating the exposure of patients to potential drug-drug interactions (DDIs) rather than clinically manifest DDIs and their relative severity [18, 19]. Furthermore, monitoring of one-day overlap in therapy is beneficial in the cases when clinical effects are evident within 24 hours of administration of the interacting drugs (e.g. diazepam and clarithromycin, ciprofloxacin and iron salts) and when immediate action is necessary to avoid the effects of the interaction [20].

Interacting combinations not involving antibacterial agents were not considered in the study.

Identification and analysis of potential drug-antimicrobial agent interactions

Potential drug-antimicrobial agent interactions were identified and classified according to the *Drug Interaction Facts* (DIFs) [20]. In the compendium, based on the Editorial Group's assessment of interaction severity (the magnitude of the effect of a drug interaction) and documentation (the quality and clinical relevance of the primary literature supporting the occurrence of an interaction), significance rating was assigned by number 1 through 5 to each interaction monograph. In the current study, interactions ranked as 1 and 2 were considered as potentially harmful and therefore clinically relevant. According to the compendium, these interactions have a reasonable probability of occurrence (proven to occur in well-controlled studies; or, very likely but not proven clinically; or may occur, they are some good data, but more studies are needed); their effects are potentially life-threatening or capable to cause permanent damage (significance rating 1); or, may cause a deterioration in patient's clinical status, hence additional treatment, hospitalization, or an extended hospital stay may be necessary (significance rating 2) [20]. For each subject exposed to overlapping prescriptions, all pairs of drug combinations were analysed for interacting potential by two independent researchers (BN and DR). In the case of disagreement among assessors, evaluation of potential drug-antiinfective agent interaction was discussed until consensus view was achieved. The assessment of interrater agreement (determined before a consensus was reached) indicated acceptable consistency among observational ratings (kappa, 0.76; 95% confidence interval – 0.50 to 1.00).

Additionally, the drug-antimicrobial agent interactions were classified in "pharmacological mechanisms", "potential clinical outcomes" and "management advice" categories. The DIFs provide textual information about these parameters for each interaction. The compendium text was converted into aforementioned categories by three researchers (BN, JP, MB). Differences in classification were resolved by discussion. Interrater agreement (based on the estimation before a consensus was reached) was substantial for "pharmacological mechanisms" (kappa, 0.82; 95% confidence interval – 0.73 to 0.91), "potential clinical outcomes" (kappa, 0.95; 95% confidence interval – 0.91 to 0.99) as well as "management advice" (kappa, 0.76; 95% confidence interval – 0.66 to 0.86).

Statistical analysis

Descriptive statistics was used to describe patient characteristics. The mean and standard deviation were calculated for age and number of prescribed drugs, while proportion was calculated for sex. The selected sample for analysis was divided into two different groups, thus subjects with ≥ 1 potential drug-antimicrobial agent interaction were in the exposed group and those without potential drug-antimicrobial agent interaction were in the unexposed group. Intergroup differences in the continuous variables, age and number of drugs, were assessed applying nonparametric Mann-Whitney U test because they failed to show a normal distribution. A categorial variable, sex, was compared using χ^2 test of independence. Parameters of potential interactions (pIs) ("pharmacological mechanisms","potential clinical outcomes", and "management advice") were evaluated by absolute and relative frequencies. For all of tests, p value < 0.05 was considered as statistically significant. Data were analyzed using Statistical Package for the Social Sciences (SPSS) 20.0 software.

Sample size calculation was based on assumption on 10% exposure to pIs (variable derived from a small pilot study conducted within our population). Standard tabular values of 95% confidence limit factors for estimate of a Poisson-distributed variable were used to assist in carrying out this computation [21]. Thus, 800 outpatients (95% confidence interval, 384 to 1472) were needed for study to be confident. Additionally, calculated size was increased by 3% to account for potential losses.

Results

During the study period medication records for 823 patients were analysed, the mean age of subjects was 50.8 years (SD ± 23.3) ranged from 1 to 94 years, 520 (63.2%) were

females, and the average number of prescribed drugs was 4.7 (SD ± 2.6). Overall, 88 clinically significant potential drug-antiinfective agent interactions were identified among 69 (8.4%) outpatients. Exposed subjects were significantly older ($p < 0.01$) and they had more complex therapeutic regimen ($p < 0.01$), while risk for occurrence of pIs was not in line with patient sex ($p = 0.285$), (Table 1). The average number of interactions involving antibacterials per exposed patients was 1.3 (ranged 1–5), and 56 subjects had 1, and 13 subjects ≥ 2 pIs.

Potential drug-antimicrobial interactions

In total, 31 different interacting combinations were identified, the most common pIs were benzodiazepines undergoing oxidation and clarithromycin or erythromycin and aminophylline and ciprofloxacin (Table 2). The proportion of pIs involving antimicrobials was 44.3% for macrolides, 33.0% for quinolones, 9.1% for azole antifungals, 5.7% for aminoglycosides, 4.5% for penicillins, 3.4% for cephalosporins, and 2.3% for tetracyclines.

Pharmacological mechanisms

The reported mechanisms for pIs were classified as pharmacodynamic (11.4%), pharmacokinetic (83.0%), a combination of both types (2.3%) and unknown (3.4%). Pharmacodynamic pIs were in line with potentiation of pharmacological effects while pharmacokinetic pIs were associated primarily with inhibition of metabolic pathways mediated by CYP3A4 and CYP1A2 isoenzymes (Table 3).

Potential clinical outcomes

In 89.8% of cases there was an increased risk for ADEs including excessive sedation (22.7%), cardiotoxicity (20.5%), miscellaneous adverse effects of aminophylline (13.6%), bleeding risk (10.2%), miscellaneous adverse effects of corticosteroids (8.0%), etc. (Table 4). The potential for decreased effectiveness of antiinfective agents was reported in the 12.5% of cases (Table 4).

Table 1

Patient general characteristics according to exposure to potential drug-antimicrobial agent interactions

Characteristics	Exposed (n = 69)	Unexposed (n = 754)	*p* value
Age (years), median (IQR)	67.0 (19.0)	56.0 (33.0)	< 0.001
Female, n (%)	39 (56.5)	481 (63.8)	0.285
Number of prescribed drugs, median (IQR)	7.0 (4.0)	4.0 (3.0)	< 0.001

IQR – interquartile range; *p* value < 0.05 was considered as statistically significant.

Table 2

The most common potential drug-antimicrobial agent interactions

Drug combination	pIs, n (%)
BZs (diazepam, alprazolam)/clarithromycin or erythromycin	17 (19.3)
Aminophylline/ciprofloxacin	12 (13.6)
CCBs (verapamil, diltiazem)/ clarithromycin	7 (8.0)
Digoxin/clarithromycin or azithromycin	5 (5.7)
Iron salts/ciprofloxacin or norfloxacin or levofloxacin	5 (5.7)
Antiarrhythmic agents (amiodarone, sotalol)/levofloxacin	5 (5.7)
Methylprednisolone/clarithromycin	4 (4.5)

pIs – potential interactions; BZs – benzodiazepines; CCBs – calcium channel blockers.

Table 3

Overview of pharmacological mechanisms for identified drug combinations

Overall mechanism	Mechanisms	pIs, n (%)
Pharmacodynamic	Additive pharmacological effect	10 (11.4)
Pharmacokinetic	Drug absorption[a]	7 (8.0)
	Drug metabolism[b]	55 (62.5)
	Drug excretion[c]	8 (9.1)
	Other[d]	3 (3.4)
	Pharmacokinetic (total)	73 (83.0)
Pharmacodynamic/pharmacokinetic		2 (2.3)
Unknown		3 (3.4)

pIs – potential interactions; [a]Drug absorption: chelation (6 pIs), high gastric pH (1 pIs); [b]Drug metabolism: CYP3A4 inhibition (36 pIs), CYP3A4 induction (1 pIs), CYP1A2 inhibition (17 pIs), CYP2C9 inhibition (1 pIs); [c]Drug excretion: P-glycoprotein (Pgp) inhibition (5 pIs), glomerular filtration reduction (2 pIs), competition for organic anion transporter (1 pIs); [d]Drug absorption/drug metabolism combination:Pgp/CYP3A4 inhibition (3 pIs).

Table 4

Potential clinical outcomes for drug-antimicrobial agent interactions

Overall risk	Risks	pIs, n (%)
Increased risk for ADEs	Bleeding risk	9 (10.2)
	Cardiotoxicity	18 (20.5)
	Excessive sedation	20 (22.7)
	Corticosteroids adverse effects	7 (8.0)
	Aminophylline adverse effects	12 (13.6)
	ABs adverse effects	3 (3.4)
	Antipsychotics adverse effects	3 (3.4)
	Other	7 (8.0)
	Increased risk for ADEs (total)	79 (89.8)
Risk for decreased effectiveness	Failure of ABs effectiveness	11 (12.5)

pIs – potential interactions; ADEs – adverse drug events; ABs – antimicrobials; * Percentages do not add up to 100% because one pI could have multiple clinical outcomes.

Table 5

Advised management strategies for drug-antimicrobial agent interactions

Overall recommendation	Recommendations	pIs, n (%)
Monitoring	Clinical monitoring of toxicity	22 (25.0)
	Monitoring of physiological markers[a]	11 (12.5)
	Monitoring (total)	33 (37.5)
Adjust dose as needed		33 (37.5)
Avoid combination		27 (30.7)
Risk-modifying strategy	Separate administration	6 (6.8)
	Therapeutic alternative	20 (22.7)
	Supplements	2 (2.3)
	Risk-modifying strategy (total)	28 (31.8)
Contraindicated combination		1 (1.1)

pIs – potential interactions; [a]Monitoring of physiological markers: serum creatinine (2 pIs), coagulation parameters (9 pIs); *Percentages do not add up to 100% because one pI could have multiple management advice.

Management advice

To control the ADI risk, common recommendation was monitoring of simultaneous administration of drugs (37.5%) and in that case advice also included dose adjustment as needed (37.5%). Additionally, frequent advice were to avoid combination (30.7%) as well as different risk-modifying strategies (31.8%), and as a part of latter, significant proportion related to the choice of therapeutic alternative (22.7%) (Table 5).

Discussion

In the study, of the 823 patients included, 69 (8.4%) were exposed to a risk for the clinically significant ADIs involving antimicrobial agents. In the literature there is a lack of reports about frequency of these type of pIs. One study was conducted in the Netherlands among home-dwelling patients aged ≥ 75 years who used ≥ 4 drugs and the prevalence of pIs involving antiinfectives for systemic use was 14.3% [22]. Lower prevalence of pIs in our study could be explained by general characteristics of study population, given that outpatients in the HCNS were younger (50.8 *vs* 81 years in the Dutch study) and had less number of prescribed drugs on average (4.7 *vs* 6.8 medicines, respectively). According to the results of previous studies, both variables contribute to a greater risk for exposure to pIs [23–25]. Further comparison is difficult with regard that the primary aim of the Dutch study was to determine the nature, volume and clinical relevance of prescription-related points of attention in the main ATC groups and there were no more information in line with prescriptions of anti-infectives for systemic use.

In the present study, the proportion of potential benzodiazepine and macrolide interactions was the most frequent (17 cases), thus co-administration of diazepam and clarithromycin, alprazolam and clarithromycin, and diazepam and erythromycin represented an increased risk for excessive sedation. Reis' et al. [26] study showed that excessive sedation was ADE which was most frequently related to clinical manifestations of DDIs in the ICU, and among others, it was caused by administration of the interacting pair midazolam and clarithromycin. Benzodiazepines metabolized by oxidation were recognized as substrates of CYP3A4 isoenzyme, and macrolide antibiotics can inhibit their metabolism [20]. However, Yeates et al. [27] reported that azithromycin did not affect midazolam metabolism. Hence, to prevent the risk, it is necessary to caution patients about over-sedation and to reduce the benzodiazepine dose as needed, or, to consider the use of benzodiazepines metabolized by conjugation (e.g.

lorazepam), which are unlikely to interact, or, to take into consideration azithromycin as therapeutic alternative for erythromycin and clarithromycin. To facilitate health professionals detection of pharmacokinetic interactions as well as interventions for reducing adverse events, numerous information about CYP450 substrates, inhibitors and inductors could be implemented in CYP450-based software [28].

In the study, among commonly reported pIs, there was the interaction between aminophylline and ciprofloxacin (12 cases). The inhibitory effects of quinolones on aminophylline metabolism were mediated by CYP1A2 isoenzyme [20]. But, among quinolones there were significant differences in pharmacokinetic features. Thus, enoxacin was the most potent inhibitor of theophylline metabolism (reduced clearance by more than 50%), pipemidic acid, ciprofloxacin and pefloxacin reduced theophylline clearance to a smaller extent (approximately 20% to 30%), norfloxacin, ofloxacin and nalidixic acid had minimal effects [29]. Finally, there was no pharmacokinetic interaction between orally administered levofloxacin and intravenously administered theophylline [30]. When theophylline toxicity was studied in a 19-year period concomitant drug and/or substance exposure was positive in 87.8% of patients admitted to the Department of Emergency Medicine, and antimicrobials were among commonly co-administered medicines [31]. The choice of therapeutic alternative without or with a limited potential for interaction with theophylline as well as monitoring its plasma concentration and clinical response can prevent adverse effects. However, considering an intermittent contact and an infrequent communication between clinicians and patients in primary health settings, it is very important to advise patients to report unexplained abdominal pain, nausea, vomiting, tachycardia, palpitations, headache or insomnia.

In the current study, antimicrobial drugs (benzylpenicillin, ceftriaxone, clarithromycin, ciprofloxacin, and fluconazole) had potential for interactions with warfarin (9 cases; 10.2% of all pIs) increasing the risk of bleeding. Thus, co-administration of specified antibiotics or oral azole antifungals and warfarin were considered as indicator of a high risk when prescribed in primary care patients because of the consistency in article reportings about clinically significant bleeding [32]. Macrolides, quinolones and metronidazole were defined as interacting antibiotics. Furthermore, according to the data on spontaneous reported ADEs to the WHO Global ICSR database decreased prothrombin level, increased International Normalized Ratio (INR), and haematuria, there were commonly noted adverse events during administration of interacting combinations involving warfarin and antimicrobials [1]. There were several pharmacodynamic and pharmacokinetic factors which may potentiate warfarin's effect. Thus, beta-lactams modifying gut flora reduced endogenous vitamin K production, additionally penicillins induced inhibition of adenosine diphosphate-mediated platelet aggregation [20, 33]. Fluconazole was identified as an inhibitor of CYP2C9 isoenzyme which mediated in oxidative biotransformation of S-enantiomer of warfarin [20, 34]. The R-enantiomer of warfarin was metabolised by CYP1A2 and CYP3A4 and quinolones (ciprofloxacin, enoxacin, norfloxacin) inhibited CYP1A2 while macrolides (clarithromycin, erythromycin) and azoles (fluconazole, itraconazole, ketoconazole, miconazole) inhibited CYP3A4 activity [20]. Hence, if combined using of interacting drugs cannot be avoided, it is necessary to monitor anticoagulant activity more frequently when starting or discontinuing antiinfective agent, and to adjust the warfarin dose accordingly. The reported fact should be taken into account. One of the most common reasons for preventable drug related hospital admissions was overdosing of oral anticoagulants due to the lack of the INR monitoring at patients known to be hard to control or following introduction of an antibiotic [35].

In the HCNS, increased risk for cardiotoxicity was among the most prevalent potential clinical outcomes (20.5% of all cases), adverse interacting combinations were digoxin and clarithromycin, digoxin and azithromycin, amiodarone and levofloxacin, sotalol and levofloxacin, verapamil and clarithromycin, and diltiazem and clarithromycin. Just, interacting drug pair digoxin and clarithromycin was the second most frequently reported combination with adverse effects to the WHO Collaborating Center for International Drug Monitoring, the study covered reports from January 1990 to February 2010 [1]. To control the ADIs, if possible, the administration of these drug pairs should be avoided or patients should be monitored more frequently and guidance about possible adverse effects should be provided. Generally, in ambulatory care, cardiovascular events are among the most frequent type of ADEs and among the most preventable or ameliorable events, 18% and 18% (the denominator is the total number of patients taking the medications), respectively [36].

Besides increased risk for the ADEs among outpatients, there was a risk of decreased effectiveness of antibiotics, mainly because of potential for the formation insoluble chelates of quinolones and iron salts as well as tetracyclines and calcium salts. Iron reduced the mean bioavailability of ciprofloxacin 64% in 12 healthy men [37]. Similarly, a study of 8 volunteers demonstrated 55% of a decrease in urinary excretion of norfloxacin 400 mg taken with ferrous sulfate 300 mg, suggesting a reduction in norfloxacin bioavailability [38]. Tetracycline absorption may be decreased by more than 90% by its chelation with calcium salts [6]. Hence, separate administration of these agents is recommended as long as possible, at least 2 hours.

The present study has some limitations. Using outpatient prescription database which did not include information on OTC product prescriptions (e.g. iron salts, zinc salts, antacids, ibuprofen) could contribute to underestimating the prevalence of pIs. Additionally, in relation to the methodology, one source of drug interaction checking was used, and it could lead to a less sensitive identification of drug pairs minimizing the possibility for detection DDIs. For example, combination of the ACE inhibitors or angiotensin receptor blockers and co-trimoxazole was not listed in the compendia and therefore it was not considered in the present study, but it elevates a risk for hospitalization in older adults [39]. On the other hand, the lack of information about compliance could lead to the overestimation of the prevalence of pIs. In addition, information on physician advice for the ADIs control was not captured. For example, the risk for exposure could

be avoided in case when it was recommended to separate administration of iron salts and fluoroquinolones.

Authors did not focus on clinically manifested drug-antimicrobial interactions. It would be interesting for further research to consider common percentage of outpatients exposed to pIs involving antiinfective agents. Additionally, in the present study frequently reported interacting combinations (benzodiazepines and clarithromycin, digoxin and clarithromycin, aminophylline and ciprofloxacin, calcium channel blockers and clarithromycin, warfarin and antimicrobials) were listed in recent literature as risk factors associated with pharmacotherapy problems. Considerable frequency of pIs as well as strong epidemiological evidence about risk co-prescription of antimicrobials pointed out the importance of interactions with this drug class using for short-term intercurrent diseases.

In spite of its limitations, our study discussed the prevalence and type of potential drug-antimicrobial agent interactions in primary medical care which could cause a deterioration in a patient's clinical status. For assessment of interacting combinations, the parameters as quality of evidence, rating of clinical significance, pharmacological mechanisms, clinical outcomes and management strategies were considered. By evaluation of these features for each potential interaction, we got the set of information which could be the base for taking measures to their prevention and consequently reduction of harming the patient.

Conclusion

The current study showed that among outpatients there was a common potential for clinically significant interactions involving antimicrobials. Antiinfective agents could contribute to overdosing of co-administered drugs frequently used in primary health care (benzodiazepines, calcium channel blockers, digoxin, corticosteroids, aminophylline), primarily by inhibition of their metabolic pathways mediated by CYP3A4 and CYP1A2 isoenzymes. On the other hand, the efficacy of certain antibiotics (quinolones, tetracyclines) could be compromised. From a clinical perspective, there are opportunities to improve primary care prescribing associated with drug-antimicrobial interactions related to close monitoring of simultaneous administration of drugs, different risk-modifying strategies and avoiding hazardous combinations. Information based on the results of the present study could be integrated into existing computerized physician order entry system in the Health Center as a form of clinical support.

Acknowledgments

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The impact of accreditation on health care quality in hospitals

Uticaj akreditacije na kvalitet zdravstvene zaštite u bolničkim ustanovama

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Abstract

Background/Aim. Accreditation is considered to be the oldest and most widespread mechanism of independent external evaluation of health care quality and is implemented in over 70 countries worldwide. Despite numerous studies in this field, there is still no solid evidence about its impact on health care quality and patient safety. The goal of this paper was to investigate if the accreditation process has an effect on the difference in values of health care quality indicators. **Methods.** The study was conducted in two tertiary level health care hospitals, one accredited, the other non-accredited. Values of seven quality indicators in the period before, during and immediately after the completion of accreditation (from 2007–2015), which measure health care quality, patient safety, the efficiency and productivity of the institution, were compared. **Results.** Of the seven monitored quality indicators, a positive effect of the accreditation process can be attributed to a shorter length of waiting for the first scheduled health check at the institution, shorter length of waiting for the first scheduled surgical check, lower rate of patients with decubitus as well as a decrease of the rate of hospital days per patient with acute myocardial infarction. No effect of accreditation was found on the mortality rate, mortality rate within the first 48 hours of hospitalization, and the average rate of hospital days per patient at the level of the institution. **Conclusion.** The process of accreditation undoubtedly intensifies activities that contribute to improving health care quality, which results in better health outcomes. Additional research in this field and new evidence about the relationship between accreditation and quality upgrading in health care institutions are required because this could motivate their managers to decide more easily to enter into this process and implement it, despite the additional efforts and financial investments associated with accreditation.

Key words:
accreditation; quality assurance, health care; health status indicators; quality improvement.

Apstrakt

Uvod/Cilj. Akreditacija se smatra najstarijim i najrasprostranjenijim nezavisnim mehanizmom spoljašnjeg ocenjivanja kvaliteta zdravstvene zaštite i sprovodi se u preko 70 zemalja širom sveta. Uprkos velikom broju istraživanja u ovoj oblasti i dalje nema čvrstih dokaza o njenom uticaju na kvalitet zdravstvene zaštite i bezbednost bolesnika. Cilj ovog rada je da se istraži da li proces akreditacije utiče na razliku u vrednostima pokazatelja kvaliteta zdravstvene zaštite. **Metode.** Istraživanje je sprovedeno u dve bolnice tercijernog nivoa zdravstvene zaštite, od kojih je jedna akreditovana, a druga nije. Poređene su vrednosti sedam pokazatelja kvaliteta u periodu pre, za vreme i neposredno posle završene akreditacije (od 2007–2015 godine), koji mere kvalitet zdravstvene zaštite, bezbednost bolesnika, efikasnost i produktivnost ustanove. **Rezultati.** Od sedam praćenih pokazatelja kvaliteta, pozitivnom uticaju procesa akreditacije se može pripisati kraće čekanja na zakazan prvi pregled za nivo ustanove, kraće čekanje na prvi pregled kod hirurga, niža stopa bolesnika sa dekubitusima, kao i pad dužine bolničkog lečenja bolesnika sa akutnim infarktom miokarda. Nije nađen uticaj akreditacije na stopu mortaliteta, procenat umrlih u prvih 48h hospitalizacije i prosečnu dužinu bolničkog lečenja za nivo ustanove. **Zaključak.** Proces akreditacije nesumnjivo intenzivira aktivnosti koje doprinose unapređenju kvaliteta zdravstvene zaštite, što rezultira i boljim zdravstvenim ishodima. Potrebna su dodatna istraživanja u ovoj oblasti i novi dokazi o povezanosti akreditacije sa unapređenjem kvaliteta u zdravstvenim institucijama, jer bi to moglo motivisati njihove menadžere da se lakše odluče za ulazak u ovaj proces i njegovu realizaciju uprkos dodatnom trudu i finansijskim ulaganjima povezanih sa akreditacijom.

Ključne reči:
akreditacija; zdravstvena zaštita, obezbeđenje kvaliteta; zdravstveno stanje, indikatori; kvalitet, unapređenje.

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Introduction

Accreditation is considered to be the oldest and most widespread mechanism of independent external evaluation of health care quality and is implemented in over 70 countries worldwide [1, 2]. The very fact that it is present to such a degree, according to some authors, is a tangible signal that it is important for increasing the quality and safety of health care [3]. Given that the process of accreditation requires quite a considerable engagement of employees and the investment of significant financial funds [4], its purposefulness is still a topic that is often discussed among all stakeholders involved in this process [5].

Because of the limitations of studies conducted to date, literature does not offer enough evidence about the effects of accreditation on the quality of health care [6], so that there are conflicting opinions in the professional community, pertaining to its potential contribution to improving quality and the safety of patients.

According to one group of authors, quality improvement is incorporated into the accreditation process via accreditation standards that encourage the institution to achieve quality while accreditation bodies periodically revise standards according to new knowledge [1, 7, 8]. Thus, patients receive the maximum of what science knows and applies. This is a direct contribution to the quality of health care. Authors with this point of view argue that accreditation improves health care outcomes for a wide range of clinical states [9].

On the other hand, there are groups of authors with more scepticism about the impact of accreditation on quality. These doubts result from the fact that there are insufficient numbers of studies that could document the impact of accreditation on quality as well as the mechanism of that impact [10–12].

Some authors believe that the impact of accreditation on quality can be seen immediately after the completion of the process, but that over time this impact ceases [13].

The quality of health care in the Republic of Serbia has been recognized as one of the most important characteristics of the health care system [14], and in line with this, in 2010 a Rulebook on Quality Indicators was adopted. According to this Rulebook, all health care institutions are required to collect data and calculate quality indicators envisaged for them as well as to submit, within the set deadline, a report pertaining to this to district public health institutes and departments. These "quantitative indicators can be used to monitor the quality of care and treatment of patients, but also to support health care activities" [15].

Given that the effects of accreditation can be seen through its capacity to improve the quality of care and patient safety, studies that monitor changes in these segments, before and after the accreditation process as well as the differences that may occur by monitoring the quality of medical treatment in accredited and non-accredited hospitals are of great importance [16].

The aim of this study was to investigate if the process of accreditation contributes to differences in values of quality indicators of work in two hospitals, one accredited and the other non-accredited.

Methods

Study venue and period

The study was conducted in two tertiary level hospitals, one accredited (Institution A) and the other non-accredited (Institution B). Institutions where the study was conducted were selected based on the fact that at the time of enrollment, Institution A was a hospital where in 2014 accreditation of all organizational segments, established statutorily and from the aspect of systematization, was fully implemented, while Institution B was chosen because during the entire period of monitoring it had still not started the accreditation process. The study was approved by institution directors as well as by ethical committees of the institutions where it was conducted.

Monitored data were indexed values according to quality indicators presented annually for the 2007–2015 period.

Study design

The study was designed as a quasi-experimental research of the "Difference-in-Difference" (DiD) between two groups of chronologically arranged data and included the following activities: assessment of the difference of quality indicators between institutions for the entire monitored period (institution factor); assessment of the trend of variation for values of selected quality indicators, for the 2007–2015 period (year factor); assessment of the direct impact of activities related to accreditation on quality indicators between hospitals (year × institution factor interaction).

Quality indicators

Seven quality indicators were monitored: total hospital mortality rate (%), mortality rate within the first 48 hours of hospitalization (%), average length of waiting for the first scheduled health check at the institution (days), average length of waiting for the first scheduled surgical check (days), average rate of hospital days per patient, rate of patients with decubitus (%), and rate of hospital days per patient with acute myocardial infarction (AMI).

Data sources were annual reports on performance indicators for quality and productivity (measures) of the monitored hospitals. Data were obtained from the competent city Institute for Health Care.

Statistical methods

The assessment of difference of chronologically ordered data by quality indicators between the two institutions was done using the so-called Difference-in-Difference (DiD) method. Basically, the DiD method is the use of multiple regression with three predictors: time; intervention and intervention × time interaction. The accepted minimum level of significance was 0.05. Statistical analysis was performed using the statistical package IBM SPSS Statistics 20, New York, USA.

Results

According to the data as on 31 December 2015, the Institution A employed 294 doctors and 866 nurses and technicians. During 2015, a total of 247,636 specialist examinations were done, while the number of registered patients was 25,930. The Institution B employed 258 doctors and 700 nurses and technicians and during this period 187,825 specialist examinations were done for 21,109 registered patients.

DiD analysis showed that compared to the Institution B, the Institution A had a significantly lower total mortality rate for the entire period covered by the investigation while the Institution B had a significantly lower mortality rate within the first 48 hours of hospitalization (Table 1). Average values of these quality indicators demonstrated their common significant decline from 2007 to 2015.

DiD analysis detected a significant impact of the accreditation process reflected in significant influence of the institution × year interaction on the length of waiting for the first scheduled health check at the institution (Table 2) as well as the length of waiting for the first scheduled surgical check (Figure 1). In the 2010–2015 period, the Institution A recorded a significant decrease, while the Institution B recorded a significant increase of the length of waiting for the first scheduled health check at the institution. Taking into account the average value for the entire study period, the Institution A had a significantly shorter length of waiting for the first health check compared to the Institution B. On the other hand, since 2012 the Institution A has had a significantly steeper decline of the trend of the length of waiting for the first scheduled surgical check compared to the Institution B.

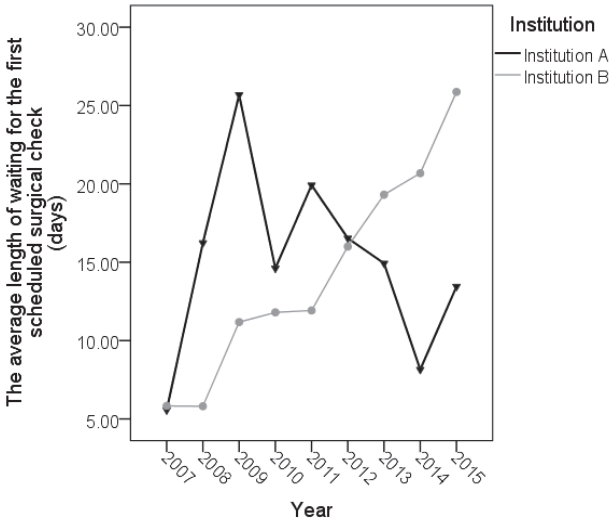

Fig. 1 – The average length of waiting for the first scheduled surgical check per Institution and per year.
Institution A – accredited
Institution B – non-accredited.

The results showed a significant impact of the institution factor on the average rate of hospital days per patient whereas no significant impact of the year factor and the year × institution factor was found. The Institution A had a significantly lower average rate of hospital days per patient compared to the Institution B.

Table 1

The mortality rate within the first 48 hours of hospitalization (DiD analysis)

DiD Model Predictors	Unstandardized coefficients		Standardized coefficients	T	*p*	95% Confidence interval for B	
	B	SE	Beta			lower bound	upper bound
Constant	52.622	3.968		13.262	0.000	44.112	61.132
Year	-1.568	0.705	-0.718	-2.224	0.043	-3.080	-0.056
Health institution	-13.970	2.509	-1.239	-5.567	0.000	-19.352	-8.588
Health institution x Year	0.789	0.446	0.670	1.770	0.098	-0.167	1.746

DiD – Difference in Difference; SE – standard error.

Table 2

The average length of waiting for the first scheduled health check at the Institution per Institution and per year (DiD analysis)

DiD Model Predictors	Unstandardized coefficients		Standardized coefficients	T	*p*	95% confidence interval for B	
	B	SE	Beta			lower bound	upper bound
Constant	55.623	5.268		10.558	0.000	44.323	66.923
Year	-7.091	0.936	-2.779	-7.574	0.000	-9.099	-5.083
Health institution	-20.012	3.332	-1.519	-6.006	0.000	-27.159	-12.866
Health institution x Year	3.939	0.592	2.862	6.652	0.000	2.669	5.209

DiD – Difference in Difference; SE – standard error.

In addition, the DiD analysis showed that the impact of the accreditation process (institution × year interaction) was also detected for the rate of patients with decubitus (Figure 2), and rate of hospital days per patient with AMI (Figure 3), where the Institution A had significantly lower both values compared to the Institution B. Since 2008, the Institution A has recorded a significantly steeper decline of the rate of hospital days per patient with AMI compared to the Institution B. The reduction of the rate of patients with decubitus in the Institution A is particularly evident in 2013 while, in that same year, the Institution B showed an increase in the rate of patients with decubitus.

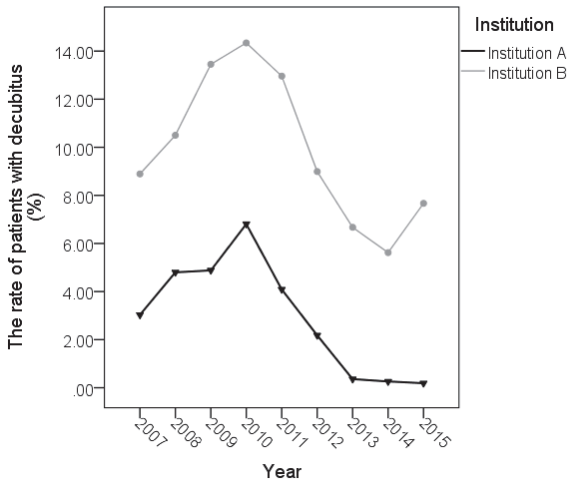

Fig. 2 – The rate of patients with decubitus per Institution and per year.
Institution A – accredited
Institution B – non-accredited.

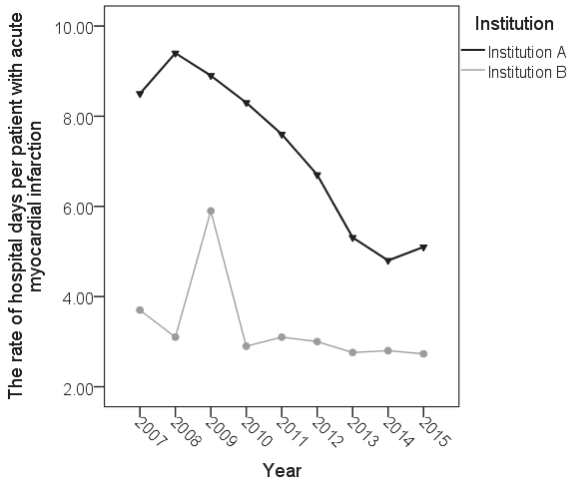

Fig. 3 – The rate of hospital days *per* patient with acute myocardial infarction per Institution and per year.
Institution A – accredited
Institution B – non-accredited.

Discussion

The essential idea of accreditation is to contribute to the creation of continuous quality improvement in health care to include the patient and his family as partners in the treatment process and to improve employee satisfaction by improving work safety and efficiency. However, in addition, accreditation should also lead to quality improvement, reflecting in improvement of specific indicators and to use of achieved improvements, in negotiations with stakeholders as an argument that would encourage them to make new financial investments in the institution [17].

Research conducted in South Africa, showed that the very participation of hospitals in accreditation programs improves harmonization of their standards with accreditation standards while this is not the case with non-accredited hospitals. On the other hand, results obtained in the same study do not suggest that accreditation had contributed to improving the value of quality indicators [18].

According to our study, although during the monitored period the accredited hospital had a lower mortality rate compared to the non-accredited hospital, this fact did not reflect the impact of activities associated with accreditation. However, it can be said that factors that contribute to a lower mortality rate were present in the Institution A years before preparation for accreditation began and that institutions with lower mortality might possibly decide to undergo accreditation sooner, comparing to the ones where the total mortality rate is high. Given the fact that no significant variation in the values of this indicator was found during the entire monitored period, necessary steps should be undertaken at the level of the health care system which would contribute to reducing mortality in hospitals. In addition, the current accreditation standards should be revised, so that in the future, the impact of harmonization of hospital standards with them would result in the reduction of the total mortality rate.

The fact that in percentages the mortality rate within the first 48 hours of hospitalization, in both hospitals, during the monitored period decreased continuously, speaks in favor of the presence of positive interventions at the level of the health care system. It is very interesting that in the Institution A, in the year immediately following the accreditation, there was an increase of this indicator. This increase could be explained by more precise reporting due to the introduction of a new information system at the institutional level at the beginning of 2015. Given that our results do not show the impact of activities related to accreditation on the value of this indicator, this is another argument that speaks in favor of the need to revise the accreditation standards. In addition to the fact that it is an imperative for the standards to be adequate for a particular medical institution and to be defined in accordance with potential specificities of the health services that this institution provides, it is also necessary to continuously modify and adjust them to the technological progress in the health care system[19, 20].

Our results showed that during the 2010–2015 period the accredited hospital recorded a significant decrease of the average length of waiting for the first scheduled health check while the non-accredited hospital recorded a significant increase. This positive development in the Institution A is the result of organizational changes that occurred in 2010, which included the introduction of work in two shifts in the outpatient department every day. On the other hand, our analysis

showed a significant impact of activities related to accreditation on the value of this indicator. It is interesting to note that in 2015 the length of waiting for the first scheduled health check at the institution at the accredited hospital was less than 11 days, while at the non-accredited hospital, the length of waiting for the first health check was about 26 days. The situation is similar with the average length of waiting for the first surgical check, which is the result of activities related to accreditation. Before 2013, the length of waiting for the first surgical check was longer in the Institution A than in the Institution B, while in 2015 the length of waiting in the accredited hospital was half the length of waiting time in the non-accredited hospital (26 days).

However, although the results showed that during the monitored period the Institution A had a significantly lower average rate of hospital days compared to the Institution B, this cannot be attributed to the impact of accreditation but to the fact that the reduction of the rate of hospital days per patient follows the reduction of mortality rate in the hospital, generated in intensive care units among the critically ill [21, 22].

During the 2010–2015 period, in the Institution A the value for the rate of the patients with decubitus continuously decreased, but it was from 2013 (the year in which the decision was made to enter the accreditation process) that its value fell below 1%, becoming the lowest in the year after accreditation. Notwithstanding the continued downward trend of this indicator value during the monitored period, results of our research show that there is a direct impact of activities related to accreditation on the decline of the rate of patients with decubitus. It has already been confirmed that certain accreditation standards related to the prevention of decubitus significantly influenced the decrease of the rate of patients with decubitus [20].

On the other hand, despite the fact that the rate of hospital days per patient with AMI was significantly lower in the Institution B compared to the Institution A, the accredited hospital has recorded a significantly steeper downward trend of this indicator value, especially since 2013. According to the results of our research this is the effect of the significant impact of activities related to accreditation.

Results of our study confirm that it is important for the accreditation agencies to continuously revise their programs and standards and to introduce new metods [17], because, in addition to resulting in improving the work of the agencies themselves, this could serve as an incentive for a new research in this area and for collecting new evidence about the relationship between accreditation and the improvement of the quality of the medical procedures in institutions. Greenfield et al. [19] stated that very critical issues related to the process of accreditation were the reliability of self-assessors in accredited programs, the criteria for selection of new assessors for the accreditation process as well as the quality of their training [23]. There is also uncertainty about the level of transparency that the accreditation agencies might be ready to demonstrate when it comes to presenting results. They allege that it is a challenge for the accreditation agencies to publish their research protocols and their either positive or negative results in literature which are subjected to a review [1].

Limitations of this study result from a relatively small number of monitored indicators, the fact that they were observed in only two health care institutions as well as the short period of monitoring of their values after implemented accreditation. Taking into account that the research done, included the application of the "quasi-experiment" design and that the possibility of randomization was excluded, it is clear that the institutions which were the subject of the research, can differ in a number of characteristics. Having in mind that these "baseline" characteristics can have a significant impact on the accessed values of the researched indicators, it is possible that the observed differences between the health institutions could be attributed to those other factors and not to the process of accreditation itself. Such bias corresponds more to confounding rather than to the bias of choice, and could be called „confounding by hospital-specific baseline characteristics".

Conclusion

Results of our study confirm that the process of accreditation leads to a significant improvement of individual quality indicators, both during the phase when the management of the institution makes the decision on accreditation, i.e., the preparation for accreditation, and during the accreditation process, as well as immediately after the completion of accreditation.

Given the fact that a considerable number of studies have been conducted about the impact of accreditation on the quality of health institutions and that despite this fact there is not enough evidence that would confirm this influence with certainty, the question about the sensitivity and discriminatory value of certain quality indicators in measuring the effects of accreditation, arises. Thus, a new research which would focus on measuring the benefits of accreditation for a health care institution, could give a new meaning to this and for some health care institutions financially and organizationally a very challenging process.

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The effect of the aqueous and methanol fennel stem extracts (*Foeniculum vulgare Miller*) on isolated rat ileum contractility

Uticaj vodenog i metanolnog ekstrakta stabla morača (*Foeniculum vulgare Miller*) na kontraktilnost izolovanog tankog creva pacova

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Abstract

Background/Aim. The fennel *(Foeniculum vulgare* Miller, Apiaceae) has a long history of use as traditional herb medicine due to its carminative properties. The study was aimed to investigate the effects of aqueous and methanol fennel stem extracts on intestinal activity. **Methods.** Relaxant activity of aqueous and methanol fennel stem extracts was evaluated *in vitro* in three experimental models: spontaneous contraction, acetylcholine and potassium chloride (KCl)-induced contraction of an isolated rat ileum. The composition of aqueous and methanol fennel stem extracts was qualitatively analyzed using the high performance liquid chromatographic (HPLC) analysis. **Results.** In the presence of an aqueous fennel stem extract at a concentration of 3 mg/mL, the inhibition of the spontaneous contractions of isolated rat ileum was 35.05% ± 3.57%. In presence of a methanol fennel stem extract at the same concentration, the maximum reduction of the spontaneous contractions was 48.91% ± 6.31%. The extracts in a concentration-dependent manner significantly inhibited the acetylcholine and KCl induced contractions of the isolated rat ileum ($p < 0.01$). The following components were identified in fennel methanol stem extract: 3-caffeoylquinic acid, chlorogenic acid, 4-caffeoylquinic acid, 1,3-dicaffeoylquinic acid, rutin, miquelianin, quercetin heterosides, 1,5-dicaffeoylquinic acid, 1,4-dicaffeoylquinic acid, apigenin and rosmarinic acid. In an aqueous extract, their presence is found in trace amounts. **Conclusion.** The results of this study showed that the aqueous and methanol fennel stem extracts have spasmolytic effects on the intestinal smooth muscle and may be used for the control of intestinal motility.

Key words:
ileum; rats; foeniculum; acetylcholine; potassium chloride; treatment outcome.

Apstrakt

Uvod/Cilj. Morač (*Foeniculum vulgare* Miller, Apiaceae) se od davnina koristi u tradicionalnoj medicini kao karminativ. Ova studija je imala za cilj da istraži efekte vodenog i metanolnog ekstrakta stabla morača na aktivnost tankog creva. **Metode.** Relaksantna aktivnost vodenog i metanolnog ekstrakta morača ispitivana je *in vitro* pomoću tri eksperimentalna modela na izolovanom ileumu pacova: spontana aktivost, acetilholinom i kalijum-holridom (KCl)-indukovane kontrakcije. Kvalitativni sastav vodenog i metanolnog ekstrakta morača određivan je pomoću tečne hormotografije visokih performansi (HPLC). **Rezultati.** U prisustvu vodenog ekstrakta stabla morača u koncentraciji od 3 mg/mL maksimalna inhibicija spontanih kontrakcija izolovanog ileuma pacova je iznosila 35.05% ± 3.57%, dok je u prisustvu metanolnog ekstrakta stabla morača iste koncentracije maksimalna redukcija spontanih kontrakcija iznosila 48.91% ± 6.31%. Ekstrakti stabla morača proporcionalno primenjenoj koncentraciji značajno su inhibisali kontrakcije ileuma stimulisane acetilholinom i kalijum-hloridom ($p < 0.01$). U metanolnom ekstraktu morača utvrđeno je prisustvo 3-kafeoilhine kiseline, hlorogenske kiseline, 4-kafeoilhine kiseline, 1,3-dikafeoilhine kiseline, rutina, mikvelianina, kvercetin heterozida, 1,5-dikafeoilhine kiseline, 1,4-dikafeoilhine kiseline, apigenina i rozmarinske kiseline. U vodenom ekstraktu njihovo prisustvo je nađeno u tragovima. **Zaključak.** Rezultati ove studije potvrđuju da vodeni i metanolni ekstrakti stabla morača deluju spazmolitički na glatke mišiće tankog creva i mogu se koristiti za kontrolu intestinalne motorike.

Ključne reči:
ileum; pacovi; foeniculum; acetilholin; kalijum hlorid; lečenje, ishod.

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Introduction

Functional bowel disorders include a group of several functional gastrointestinal syndromes: irritable bowel syndrome (IBS), functional constipation, diarrhea, bloating, and abdominal pain. The symptoms are manifested in the middle or lower gastrointestinal tract [1]. Many drugs used in the treatment of these disorders exhibit adverse effects during long-term use. Therefore, there is an increased use of natural herbal medicines and teas for the treatment of these disorders, especially the herbs of the Apiaceae family [2].

Fennel (*Foeniculum vulgare* Miller) is a member of the Apiaceae family and has been traditionally used for a long time, both as a medicinal plant and as a spice.

Since prehistoric times, fennel fruit has been used in China, India and Pakistan to treat many conditions such as digestive disorders and for the improvement of vision problems. Also, the fennel is effective in treatment of headache, flu and as a brain tonic. This plant has been used as a household remedies for digestive tract spasms release and for the bloating treatment [3].

Digestive disorders like meteorism, colic, stomach ache are successfully treated with the fennel in herbal medicine [4, 5]. It has also been used in the cosmetic formulations, the perfume industry, as well as in liqueur preparation to improve the taste [6–11]. Literature data has reported that the seeds of this plant regulate the menstrual cycle in women, reduce menopausal symptoms and increase libido [12].

Fennel demonstrates *in vitro* and *in vivo* studies antispasmodic, anti-inflammatory, analgesic and diuretic effects. A review of the literature showed antioxidant, antimicrobial, gastroprotective and hepatoprotective activity of the fennel extracts and essential oil [13]. Also, the ingestion of essential oil induced hypoglycaemic effect in diabetic rats [14]. Pradhan et al. [15] showed that the methanol extract of *F. vulgare* induced cytoprotective and anti-tumour activities in cultured cells.

It has been previously reported that in experiments with isolated uterus, the essential oil of *F. vulgare* reduced the intensity and frequency of oxytocin and prostaglandin E2 induced contractions [16].

Till now, there is no report about the effects of the aqueous and methanol fennel stem extracts on the contractility of intestinal smooth muscles. The present study was designed to examine the potential effects of the aqueous and methanol fennel stem extracts on the contractile responses of an isolated rat ileum.

Methods

Chemicals

All reagents and solvents in this study were of analytical and HPLC grade. Acetylcholine chloride, papaverine, atropine, 3-caffeoylquinic acid, chlorogenic acid, 4-caffeoylquinic acid, 1,3-dicaffeoylquinic acid, rutin, 1,5-dicaffeoylquinic acid, miquelianin, rosmarinic acid and apigenin were purchased from Sigma-Aldrich (Sigma-Aldrich Co., St Louis, MO). 1,4-dicaffeoylquinic acid was obtained from Chem Faces (Wuhan, PRC). All chemicals were dissolved in distilled water for each experimental protocol. The composition of Tyrode's solution (in mM) was: NaCl (136), KCl (2.7), $CaCl_2$ (1.8), $NaHCO_3$ (12), NaH_2PO_4 (0.3), $MgCl_2$ (1.8) and glucose (5.6).

Plant material

The stems of fennel were harvested in the region of Niš during August 2012. The plant material was taxonomically identified by assistant professor Bojan Zlatković from the Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš. The fennel stems were open-air dried in the shade.

Extraction procedure

Dried and pulverized stems (200 g) were extracted for 30 minutes in an ultrasonic bath with 250 mL of distilled water and absolute methanol. Separate parts of material were extracted with water and methanol. After the filtration, the extracts were concentrated in a rotary evaporator at a reduced pressure (40 ºC) till a constant weight was achieved. By extraction, from 100 g of dry stem, 8.72 g (8.72% of yield) of dry methanol extracts and 2.5 g (2.5% of yield) of dry water extracts were obtained. The extracts were stored at -20 ^{o}C. For experimental purposes the fresh dilutions were prepared on the day of experiments. The dry residues were solubilized in the distilled water in order to obtain 10% solution used for the experiments. All concentrations were presented as final bath concentrations.

Animals

All of the experimental procedures with animals were in accordance with the European Council Directive of September 22nd, Directive 2010/63/EU and were also approved by the Ethic Committee Medical Faculty University of Niš (number 12-2466-3). Fifteen male Wistar albino rats (200–250 g) were used from the Animal Research Center of Faculty of Medicine, University of Niš, Serbia. The animals were maintained at standard environmental laboratory conditions, fed with standard pellet, and had free access to food and water.

Experimental protocol

Animals were anesthetized by ether and the abdominal cavity of rat was opened. The segments of 2 cm long distal parts of the ileum were dissected out and mounted in an organ bath (10 mL) containing the Tyrode's solution (37 ºC, pH 7.4, aerated with mixture of 5% carbon dioxide in oxygen) between two stainless steel hooks with continuous air-bubbling. The change of intestinal activity was recorded using system TSZ-04-E; Spell Iso (Experimetria Ltd). The areas under the curves were evaluated [16]. The aqueous and methanol fennel stem extracts and control chemicals were

added directly to the organ bath. Six segments of isolated ileum were used in each experiment.

The first part of experiments was related to spontaneous rhythmic rat ileum contractions. After the stabilization period the ileum was treated with the aqueous and methanol fennel stem extracts (0.01, 0.03, 0.1, 0.3, 1 and 3 mg/mL). Papaverine (0.01, 0.03, 0.1, 0.3, 1 and 3 μg/mL) was used as a positive control. The relaxant effect of the aqueous and methanol fennel stem extracts was expressed as a percentage of the control contractility without extract.

In the second part of the experiment, the ileum contractions were stimulated by increasing concentrations of acetylcholine (5–1500 nM). Increasing concentrations of acetylcholine were added to the organ bath cumulatively until obtaining the maximum contractile response curve. The contractions were registered in the presence of the aqueous and methanol fennel stem extracts (1–3 mg/mL). Atropine (140 nM) was used as a positive control.

In the third part of the experiment the ileum contractions were induced by KCl solution (80 mM). Then the aqueous and methanol fennel stem extracts were cumulatively added to the tissue bath (0.01–3 mg/mL).

The relaxation of the intestinal preparation in the presence of extracts or antagonist was expressed as a percentage of the maximum contractile response induced by acetylcholine and KCl. After each part of the experiment the tissues were flushed with the fresh Tyrode's solution and left to adapt for 10 minutes.

HPLC analysis

The high performance liquid chromatographic (HPLC) analysis of the aqueous and methanol fennel stem extracts was performed on an Agilent 1200 HPLC system (Agilent Technologies, Palo Alto, Calif., U.S.A.) with a diode array detector (DAD). The analytical column was Purospher STAR RP-18e (150 × 4.6 mm) with particle size of 5 μm, manufactured by Merck, Darmstadt, Germany. Injected volume was 10 μL and flow rate was 0.7 mL/min. The eluent system consisted of 0.1% water-trifluoroacetic acid solution (A) and acetonitrile (B) with linear gradient: 0–3 min 5%–5% B, 3-32 min 5%–28% B, 32–44 min 25%–50% B, 44–52 min 50%–80% B, 52–54 min 80%–90% B, 54–59 min 90%–5% B, and 59–60 min 5% B. The column temperature was maintained at 30 ºC. The compounds were identified on the basis of UV-VIS signal response in comparison to standards. The extracts were dissolved in methanol, HPLC grade, to a concentration of 10 mg/mL. The measurement was performed on 280, 330 and 360 nm wavelength.

Statistical analysis

All values were expressed as mean ± standard deviation. Statistical analysis of the differences between two means was assessed using Student's *t*-test. Halt maximal effective concentration (EC_{50}) was established by regression analysis. The significance level considered in all tests was $p < 0.05$.

Results

Rat ileum suspended in the Tyrode's solution showed spontaneous contractile activities which were significantly reduced in the presence of the aqueous and methanol fennel stem extracts (Figure 1). The maximal inhibitory effect of the aqueous and methanol fennel stem extracts was achieved with 3 mg/mL concentration. In the presence of the aqueous fennel stem extract at a concentration of 3 mg/mL the spontaneous contractions of isolated rat ileum were reduced. The inhibition was 35.05% ± 3.57% ($p < 0.05$). The EC_{50} value for the aqueous fennel stem extract induced relaxation was 19.53 ± 2.68 mg/mL. In the presence of the methanol fennel stem extract at a concentration of 3 mg/mL the spontaneous contractions of the isolated rat ileum was significantly reduced (48.91% ± 6.31%, $p < 0.01$). The EC_{50} value for the methanol fennel stem extract induced relaxation was 5.14 ± 0.42 mg/mL.

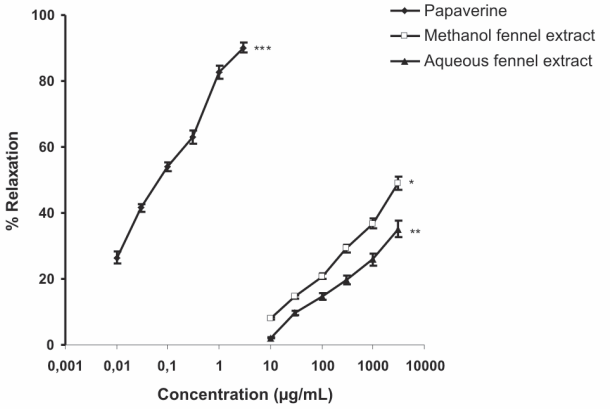

Fig. 1 – Inhibitory effects of the aqueous and methanol stem extracts and papaverine on spontaneous contractions in isolated rat ileum. Each point represents the mean value of at least six experiments and the vertical bars the standard error of the mean (*$p < 0.001$, **$p < 0.01$, *$p < 0.05$).**

It was found that the difference between the relaxant effect of the aqueous and methanol fennel stem extracts was statistically significant ($p < 0.05$). Papaverine was used as a positive control and significantly reduced the intestinal basal tonus in a concentration-dependent manner ($p < 0.001$). The relaxant effects of the aqueous and methanol fennel stem extract on spontaneous rat ileum contractions were terminated after flushing the preparation.

Acetylcholine (5–1500 nM) induced a concentration dependent contraction of the rat ileum. The aqueous fennel stem extract (1–3 mg/mL) caused a significant reduction in contractile response which was registered as a depression of the cumulative concentration response curve for acetylcholine ($p < 0.05$).

The aqueous extract caused a reduction of acetylcholine induced contraction to 80.35% ± 10.22% at a concentration of 3 mg/mL (Figure 2). The methanol fennel stem extract (1–3 mg/mL) significantly and dose-dependently reduced the rat ileum contractions induced by acetylcholine ($p < 0.01$) (Figure 3). The methanol extract caused a reduction of acethyl-

choline induced contraction to 74.89% ± 8.36% at a concentration of 3 mg/mL. Atropine, the non-selective blocker of muscarinic receptors, produced a significant inhibition of contractions caused by acetylcholine ($p < 0.001$). The inhibitory effects of the aqueous and methanol fennel stem extracts on acetylcholine induced rat ileum contractions were terminated after removing the extracts from the organ baths.

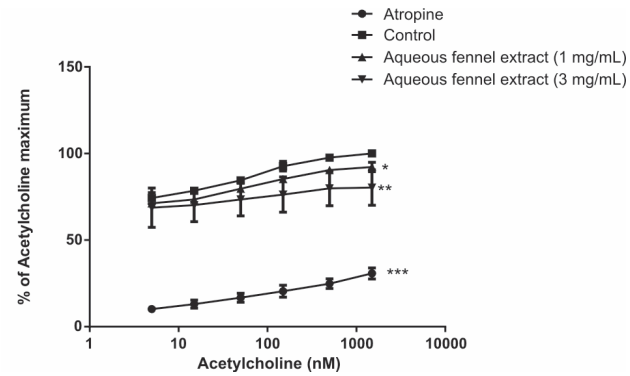

Fig. 2 – Inhibitory effects of the aqueous fennel stem extract and atropine on ileum contraction induced by acetylcholine. Each value is expressed as means ± standard error (n = 6), (*$p < 0.001$, **$p < 0.01$, *$p < 0.05$).**

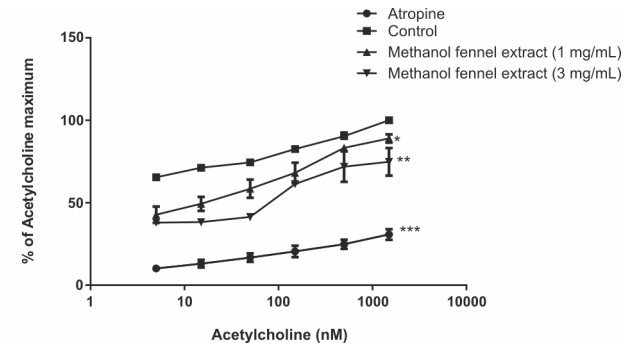

Fig. 3 – Inhibitory effects of the methanol fennel stem extract and atropine on ileum contraction induced by acetylcholine. Each value is expressed as means ± standard error (n = 6) (*p < 0.001, **p < 0.01, *p < 0.05).**

High concentration of potassium ions (80 mM) induced tonic contractions of the rat ileum. The KCl induced contractions of the isolated rat ileum were significantly reduced in the presence of the aqueous and methanol fennel stem extracts (Figure 4). The maximal inhibitory effect of the aqueous and methanol fennel stem extracts was achieved with a concentration of 3 mg/mL. The aqueous fennel stem extract caused a mean contractile response of 88.83% ± 1.52% (at a concentration of 3 mg/mL) ($p < 0.05$). The methanol fennel stem extract caused a mean contractile response of 72.97% ± 3.87% (at a concentration of 3 mg/mL) ($p < 0.01$).

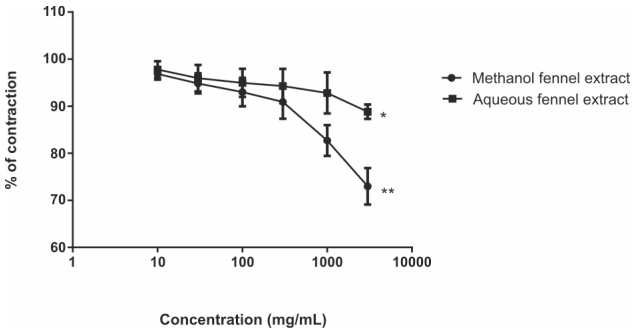

Fig. 4 – Inhibitory effects of the aqueous and methanol fennel stem extracts on ileum contraction induced by potassium chloride (KCl). Each value is expressed as means ± standard error (n = 6), ($p < 0.01$, *$p < 0.05$).**

The HPLC fingerprints of the aqueous and methanol fennel stem extracts were shown in Figure 5. The components of 3-caffeoylquinic acid, chlorogenic acid, 4-caffeoylquinic acid, 1,3-dicaffeoylquinic acid, rutin, miquelianin, quercetin heterosides, 1,5-dicaffeoylquinic acid, 1,4-dicaffeoylquinic acid, apigenin and rosmarinic acid were identified in the fennel methanol stem extract.

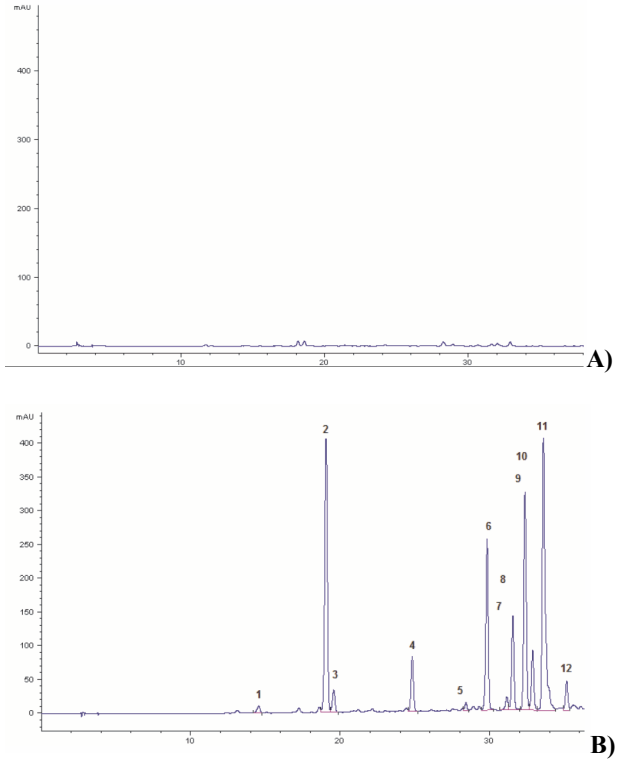

Fig. 5 – Chromatogram from aqueous (A) and methanol (B) fennel stem extracts. Peak identification: (1) 3-caffeoylquinic acid, (2) chlorogenic acid, (3) 4-caffeoylquinic acid, (4) 1.3-dicaffeoylquinic acid, (5) rutin, (6) miquelianin, (7.8) quercetin heterosides, (9) 1.5 dicaffeoylquinic acid, (10) 1.4 dicaffeoylquinic acid, (11) apigenin and (12) rosmarinic acid.

Discussion

Our study demonstrates that the aqueous and methanol fennel stem extracts express spasmolytic effects on rat ileum contractions. The aqueous and methanol extracts induced inhibition of spontaneous contractions of the isolated rat ileum in a concentration-dependent manner. The methanol fennel stem extract is more potent than the aqueous one.

To evaluate possible mechanisms for the spasmolytic activity, isolated rat ileum contractions were induced in two different ways (by application acetylcholine and KCl). Acetylcholine is the main excitatory neurotransmitter in the visceral smooth muscle which induced contractions of the ileum in a concentration-dependent manner. Contractions of the small intestine stimulated by acetylcholine were realized through two different mechanisms related to muscarinic receptors. Activation of non-selective cation channels in the plasma membrane causes membrane depolarization and influx of calcium ions through voltage-dependent calcium channels. Another mechanism of the activation of contraction occurs due to the release of intracellular calcium [17, 18]. The interactions of acetylcholine with muscarinic receptors in the intestinal smooth muscle induce a G protein-mediated signal transduction that increases cytosolic calcium ion concentration, depolarization and the contractions of the intestinal smooth muscle [19, 20].

In our study, it was found that the aqueous and methanol fennel stem extracts caused a significant reduction in contractile response which is registered as a depression of the cumulative concentration response curve for acetylcholine. Moreover, the methanol fennel stem extract had higher effect than the aqueous extract. Inhibitory effects of the aqueous and methanol fennel stem extracts on the acetylcholine induced contraction were less potent than those ones caused by atropine, a muscarinic acetylcholine receptor antagonist. These results indicated that the antispasmodic effect of fennel stem extracts was probably mediated through the blockade of muscarinic receptors. After flushing the contractility of the isolated ileum restored to baseline, meaning that the blockade of muscarinic receptors are reversible.

The contractile activity in the smooth muscle is dependent on the intracellular Ca^{2+}concentration. High concentration of KCl (80 mM) induced tonic contractions of the isolated rat ileum. Activation of the voltage dependent Ca^{2+} channels and influx of extracellular Ca^{2+} [21, 22] was used as a method of depolarization. The aqueous and methanol fennel stem extracts relaxed the tonic contraction of the isolated rat ileum induced by KCl. The methanol fennel stem extracts were more potent than the aqueous extract. According to the literature, plant extracts may cause inhibition of high K^+-induced contractions and act as a blocker of calcium influx [23].

Our results regarding the relaxant activity of the fennel stem extracts are similar to the results obtained by Boskabady et al. [24], who reported that the extracts and essential oil of *F. vulgare* expressed relaxant effect on isolated guinea pig trachea.

Fennel is used in traditional medicine for the treatment of infantile colic. This traditional usage might be related to *in vitro* relaxant activity of fennel. The treatment of infantile colic with *F. vulgare* seed essential oil may improve symptoms in babies [25, 26].

The HPLC analysis of the fennel stem extracts showed that this plant contained 3-caffeoylquinic acid, chlorogenic acid, 4-caffeoylquinic acid, 1,3-dicaffeoylquinic acid, rutin, miquelianin, quercetin heterosides, 1,5-dicaffeoylquinic acid, 1,4-dicaffeoylquinic acid, apigenin and rosmarinic acid. Higher content of these compounds were found in the methanol fennel stem extract than in the aqueous extract.The methanol extract was observed to be more potent than the aqueous extract in inhibiting the rat ileum contraction. The compounds that were analyzed in the aqueous extract were found in traces. It cannot be confirmed that they are responsible for its effects, but sometimes the compounds in small quantities can give effects. It is possible that other unidentified substances may act as smooth muscle relaxants.

The results of this study are in agreement with a study of Salami et al. [27]. They found high variation in composition of methanol extract in 23 fennel samples. The major flavonoid were quercetin, apigenin and rutin and phenolic compounds were chlorogenic, caffeic and 1,5-dicaffeoylquinic acid. It was reported that rutin induced an antispasmodic effect in the isolated ileum of the guinea-pig [28]. The rosmarinic acid was documented to possess an antinociceptive property [29]. Also, a methanol extract of the fennel seeds contains rosmarinic acid, chlorogenic acids, quercetin and apigenin [30]. Our results showed that these compounds are found in the methanol fennel stem extract.

Conclusion

Our research shows that the aqueous and methanol fennel stem extracts (*Foeniculum vulgare* Miller) inhibit spontaneous, acetylcholine and KCl induced ileum contractions. The data we obtained suggest the relaxant activity of the aqueous and methanol fennel stem extracts and may, at least partially, account for the traditional use of fennel for stomach disorders. Further research is needed to clarify whether these extracts exert their effects due to the direct action on smooth muscle or due to the effects on enteric nervous system.

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Minor neurological dysfunction in children aged 5 to 7

Minimalne neurološke disfunkcije kod dece uzrasta od 5 do 7 godina

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Abstract

Background/Aim. Assessment of minor neurological dysfunction (MND) provides information about a child's neurological condition which helps to identify the vulnerability of the child to the development of motor impairment, difficulties in learning or behavioral disorders. The aim of this study was to determine differences in the prevalence of MND in children from the general population with respect to age (5 and 6 years old) and sex. **Methods.** The examination was carried out in a preschool institution in the city of Novi Sad, Serbia. The total sample included 120 children divided into two groups according to age: 60 children aged 5 (group A) and 60 children aged 6 years (group B). The children were recruited at three randomly selected kindergartens and approximately equal sex representation, randomly selected as well. The testing was done by the Touwen's test, modified by Hadders-Algra. The results were classified into three groups: the absence of MND, presence of simple MND (presence of one or two domains of dysfunction) and the presence of complex MND (presence of at least three domains of dysfunction). **Results.** Sixty-seven children out of 120 (55.8%) had a normal neurological condition, while 53 (44.2%) showed MND [49 (40.8%) simple, and 4 (3.4%) complex]. MND occurred more frequently in the youngest age group than in the older children (57% vs. 32%; $p = 0.01$). MND was also more frequent in boys than in girls, but this difference was not statistically significant. **Conclusion.** Our results show the importance of testing children at preschool age in order to detect potential neurological vulnerability and timely begin with the appropriate therapy.

Key words:
neurological manifestations; risk; child; child, preschool; age factors; sex factors.

Apstrakt

Uvod/Cilj. Procena minimalnih neuroloških disfunkcija (MND) pruža informacije o neurološkom stanju deteta, štp pomaže u identifikovanju vulnerabilnosti deteta ka razvoju motornih slabosti, teškoća u učenju ili poremećaja ponašanja. Cilj rada bio je utvrđivanje razlike u učestalosti MND kod dece iz opšte populacije u odnosu na uzrast (5 i 6 godina) i pol. **Metode.** Ispitivanje je urađeno u predškolskoj ustanovi na teritoriji grada Novi Sad. Ukupan uzorak je obuhvatio 120. dece koja su bila podeljena u dve grupe u odnosu na uzrast – 60. dece starosti 5 godina (grupa A) i 60. dece starosti 6 godina (grupa B). Metodom slučajnog izbora izabrana su tri vrtića, a deca su nasumično odabrana, sa približno jednakom zastupljenošću polova. Testiranje je urađeno Touwen-ovim testom modifikovanim od strane Hadders-Algre. Rezultati su grupisani u tri grupe: odsustvo, prisustvo jednostavnih (prisustvo jedne ili dve oblasti disfunkcije) i prisustvo kompleksnih MND (prisustvo najmanje tri oblasti disfunkcije). **Rezultati.** Šezdesetsedam (55,8%) od 120. dece imalo je normalne neurološke nalaze, dok je 53. (44,2%) pokazalo prisustvo MND, 49. (40.8%) jednostavne, a 4 (3.4%) kompleksne. U mlađoj uzrasnoj grupi MND su se češće javljale nego u starijoj (57% vs. 32%; $p = 0.01$). Takođe, MND su se češće javljale kod dečaka u odnosu na devojčice, ali razlika nije bila statistički značajna. **Zaključak.** Naši rezultati pokazuju značaj testiranja dece u predškolskom uzrastu radi otkrivanja eventualnih odstupanja i blagovremenog započinjanja adekvatne terapije.

Ključne reči:
neurološke manifestacije; rizik; deca; deca, predškolska; životno doba, faktor; pol, faktor.

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Introduction

Ontogenetic changes in human CNS cause various manifestations of spontaneous behavior and provoked reactions and reflexes, depending on age. As a result, during the development of children, neurological examination technique, the obtained responses and their interpretation change with the age. The period of the most intensive postnatal development is the first year of life, but the changes in the clinical manifestations of neurological semiology are also present at preschool age and school age until adolescence [1]. The classical neurological examination does not provide a valid assessment of the neurological status during the development of children. Some neurological signs are specific to childhood. Associated movements are characteristics of childhood as well as more noticeable choreiform movements of the upper extremities or minor deviation of gait [2]. Touwen's neurological examination allows registering minor deviations in neurological condition, which may be relevant to clinical work: if there is a suspicion of neurological disease at the initial stage; in children with already diagnosed diseases such as cerebral palsy, where a more detailed insight into the neurological status would make a more adequate treatment possible; to gain insight into the state of children with difficulties in learning, attention and coordination of movements [2].

In all children, and especially children with behavioral and cognitive problems during development, the percentage of children with minor neurological dysfunction (MND) is increasing [3]. There are also differences in prevalence of MND in different generations which were examined at the same age [4]. Determining the prevalence of MND in children of different age and different generations is essential because it provides a starting point for further research relating to vulnerable groups. It is very important to test children for the presence of MND at preschool age in order to start the early treatment in time and thus reduce behavioral and learning problems at school age [5, 6].

It was noticed that in 9-year-old girls with complex MNDs and dysfunction in the domain of posture and muscle tone, there was an increased risk of developing behavioral disorders, while this did not occur in boys of the same age. In contrast, it was observed that if simple MNDs occurred in boys, there was an increased risk of nonspecific behavioral disorders, suggesting that psychological factors play an important role in boys, in contrast to girls [7].

The aim of this study was to determine the differences in prevalence of MND in children from the general population with respect to sex and age (5 and 6 years). The assumption was that MND is more common in younger children and boys.

Methods

The examination was carried out in a preschool institution in the city of Novi Sad, Serbia. The study was approved by the Ethic Committee of the Faculty of Medicine in Novi Sad on 24th October 2013. Before the examination, the children's parents or guardians gave their informed consent for the participation of their children in the study. The total sample included 120 children divided into two groups according to the age; 60 children aged 5 (group A) and 60 children aged 6 years (group B). The children were recruited at three randomly selected kindergartens and randomly selected approximately equal sex representation. The testing was done according to the Touwen's test modified by Hadders-Algra [2]. The test results were grouped into eight domains and the presence of dysfunction was determined for each of them. These eight domains are: 1) posture and muscle tone, 2) reflexes, 3) involuntary movements, 4) coordination and balance, 5) fine manipulative skills, 6) associated movements, 7) senses, 8) cranial nerve functions.

The results were classified into three groups: normal neurological condition (absence of MND), presence of simple MND (presence of one or two domains of dysfunction) and the presence of complex MND (presence of at least three domains of dysfunction).

Data were analysed using SPSS for Windows (version 15). The results for each categorical variable are presented in frequency and percentage values. For determining the prevalence of MND relative to age and sex, we used χ^2 test. For this analysis, we accepted the level of statistical significance according to the more stringent criteria ($p \leq 0.01$).

Results

Table 1 shows the basic characteristics of groups A and B.

The prevalence of MND according to the children's sex was tested by the χ^2 test of independence. After the statistical analysis, it was evident that the MND occured more frequently in boys, however, not at the statistically significant level (Table 2).

Table 1

Baseline characteristics of the children

Characteristics	Group	
	A (n = 60)	B (n = 60)
Males, n (%)	33 (55)	31 (51.7)
Females, n (%)	27 (45)	29 (48.3)
Height (cm), mean ± SD	115.72 ± 5.11	122.08 ± 5.7
Weight (kg), mean ± SD	20.47 ± 2.73	23.05 ± 3.97
Head circumference (cm), mean ± SD	50.93 ± 1.52	51.42 ± 1.48
Age, median (range)	5y 6m (5y-5y11m)	6y 7m (6y1m-6y11m)

A – children aged 5; B – children aged 6; y – years; m – months; SD – standard deviation.

Table 2

Minor neurological dysfunction (MND) in children relation to age and sex

Groups	N	S-MND	C-MND	χ^2	*p*
A (n = 60)					
boys, n (%)	13 (21.7)	17 (28.3)	3 (5.0)	0.176	0.675
girls, n (%)	13 (21.7)	14 (23.3)	0 (0)		
B (n = 60)					
boys, n (%)	18 (30.0)	12 (20.0)	1 (1.7)	2.22	0.136
girls, n (%)	23 (38,3)	6 (10,0)	0 (0)		

A – children aged 5; B – children aged 6; N – neurologically normal; S-MND – simple MND; C-MND – complex MND.

Table 3

Prevalence of various forms of minor neurological dysfunction (MND) in the groups A and B

MND forms	Group A (n = 60) n (%)	Group B (n = 60) n (%)	χ^2	*p*
Severity				
healthy	26 (43.3)	41 (68.3)		
simple	31 (51.7)	18 (30.0)	**6.6**	**0.01**
complex	3 (5.0)	1 (1.7)		
Type				
mild dysfunction in posture and muscle tone	1 (1.7)	0 (0)	NA	NA
mild abnormal reflexes	0 (0)	0 (0)	NA	NA
mild dyskinesia	0 (0)	0 (0)	NA	NA
mild coordination problems	27 (45.0)	11 (18.3)	**8.7**	**0.003**
mild fine manipulative disability	11 (18.3)	5 (8.3)	1.8	0.178
excess of associated movements	11 (18.3)	5 (8.3)	0.2	0.618
mild sensory dysfunction	0 (0)	0 (0)	NA	NA
mild cranial nerve dysfunction	0 (0)	0 (0)	NA	NA

A – children aged 5; B – children aged 6; NA – not available.

In the total sample of participants (120 children), 67 (55.8%) of them had normal neurological results, 49 (40.8%) showed simple MND while 4 (3.4%) showed complex MND. It was found, when the values of MND prevalence of the groups were compared, that there existed a statistical significance (Table 3). According to further analysis of the particular domains, in the domain of coordination and balance, MND occured more frequently in children from the group A at a statistically significant level. However, in other domains, there was no statistical significance observed.

When the sample of children with simple MNDs from the group A (31 children) was analyzed, it showed that 10 of them had a deviation in two domains, and in the group B, only 3 out of 18. The children from both groups with complex MNDs (4 children from the group A and one child from the group B) showed differences in the domains of coordination and balance, fine motor skills and associated movements.

Looking at the entire sample of participants (n = 120, *df* = 1) and comparing the values for boys and girls in the domain of fine motor skills, it was found, at a statistically significant level, that deviations occur in boys more frequently ($\chi^2 = 7.15$; $p = 0.008$). When the values for boys and girls in other domains were compared, there was no statistical significance observed.

Discussion

Neurological condition with respect to MND helps to identify the vulnerability of a child to development of motor impairment, difficulties in learning or behavioral disorders [2]. For this reason, it is extremely important to test children at preschool age, so that we identify the children who are potentially at a risk, begin the adequate therapy in time and reduce the aforementioned problems. Our study showed a high prevalence of MND in a sample of preschool children. MNDs were diagnosed in 44.2% (53 of 120) children who were tested, 40.8% of those who showed the presence of simple MNDs, and 3.4% of those with complex MNDs. Studies of other authors showed the prevalence of 24%–27% in children 6–11 years of age [4, 8]. A higher prevalence of MND in our study could be explained by the age of the respondents as the deviations are more common in younger children, as indicated in studies by other authors [9]. A research conducted in Holland, also showed a significantly higher prevalence of MND in relation to the general population. Namely, when children aged 4 were tested, complex MNDs were present in as many as 25% of children, and such a high prevalence was probably caused by subfertility of the parents as one of the risk factors [10]. Our study showed that there was a statistically significant difference in the prevalence of MNDs with respect to age; the children from the younger group showed a higher prevalence of MND (56.7%)

compared to the children from the older group (31.7%). The prevalence of MND in the general population depends both on the age of children and on the possible risk factors (prematurely born neonates, low birth weight, the use of corticosteroid therapy after birth, artificial nutrition of infants, autism and dyslexia) [6, 11–13]. There were some research about the impact of artificial insemination on the presence of MND in children born in this way compared to naturally conceived children, but they showed that *in vitro* fertilization did not affect the prevalence of MND [14, 15].

The domains of dysfunction with the greatest clinical significance are the domains of fine motor skills and coordination problems and, therefore, it is not surprising that they are most frequently associated with motor disorders, learning disabilities and mental disorders [2, 4, 16]. In our sample, neurological dysfunctions were most frequently present in the domain of coordination (31.7%), associated movements (15.8%) and fine motor skills (13.3%). Deviations in the domain of fine motor skills in the older group were less frequent (8.3%) than in the younger group (18.3%). Similar results were obtained by Stich et al. [9]. In our study, deviations in other domains also prevaled in children of the younger age group, but they were also present in the older group, which can cause difficulties in mastering the school curriculum. Monitoring preschool children is necessary in order to assess and identify MNDs, especially in the older group. This would make it possible to intervene before school in the domain with a more significant deviation, and the ultimate goal is mastering the school curriculum more easily. Also, it is important to test children aged 5 years, because in our country, the oldest groups at the age of 6 years already start preparing for school, focusing on the development of graphomotorics. This would allow early intervention and help specific children prepare for school.

The conducted study showed that MND was more frequent in boys than in girls. Thirty-three children out of 53 (62.3%) children diagnosed with dysfunction were male. Similar results were obtained by Kikkert et al. [7], who examined children aged 9 years, and showed that the deviation occurred more frequently in boys (61%). Researches of other authors also showed a greater prevalence of MND in boys [6]. The explanation of this phenomenon could be sought in the possible differences in development of nervous systems in boys and girls. Numerous studies point to the differences in the structure [17, 18] and functioning of the male and female brain and the cause may be in the differences in the sex hormones as well as genetic differences, namely the presence of Y chromosome in male population carrying certain genes that the genome of a female individual does not have [17, 19, 20]. In the domain of the fine motor skills, there is a statistically significant difference in prevalence with respect to sex – deviations are more common in boys.

The limitations of this study are: longitudinal monitoring of the sample was not done. A possible effect of other factors such as premature birth, gestational age, birth weight, Apgar score, intracranial hemorrhage, etc., were not tested. We did not analyse neuroimaging findings during this study. Also, future research should examine the prevalence of MND regarding social factors, the period of attending kindergarten, as well as sports activities.

Conclusion

Minor neurological dysfunctions are more common in younger children and boys, and the most frequent domains of deviation are coordination and balance, fine motor skills and associated movements. We recommend to test children at the preschool age in order to identify possible deviations and begin with an adequate therapy in time.

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GENERAL REVIEW

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The central nervous system is not immunoprivileged: inflammation and epileptogenesis

Centralni nervni sistem nije imunoprivilegovan: inflamacija i epileptogeneza

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Ključne reči:
nervni sistem, centralni; epilepsija; zapaljenje; stres, oksidativni.

Introduction

Epilepsy is a chronic neurological disorder characterized by seizures which are the result of excitation/inhibition disbalance of neurotransmitters in the central nervous system (CNS) [1]. The main substrate in the occurrence of seizures is a transient, sudden, paroxysmal and hypersynchronous activity of the brain neurons, behaviorally manifested by rapid and repetitive skeletal muscle contractions and relaxations [2]. A process of gradual epilepsy development in previously healthy brain is known as epileptogenesis [3].

Approximately 0.5%–1% (about 65 million) of adults and 0.5% of children suffer from epilepsy in the world, while 2 out of 25 people in the general world population had at least one seizure in their lifetime [4]. These facts reflect the impact of epilepsy on the social, economic and emotional aspects of life of these patients as well as treatment costs of this neurological disorder with multiple sociomedical consequences. It is estimated that 6 million people suffer from epilepsy in Europe and 50 thousand people in Serbia nowadays. More than 65% of patients experienced a first seizure attack in childhood (the highest incidence is in the first years of life). Finally, there is another important fact: about 40% of patients with epilepsy are resistant to current antiepileptic therapy [5].

The central nervous suptur (CNS) homeostasis is dependent on the balance of two opposite processes in the brain, e.g., excitation and inhibition. Thus, even minimal disequilibrium between these two processes (i.e., increase of the excitation, decrease of the inhibition, or both) will lead to hyperexcitability and will cause seizures [6]. Molecular pathways of epileptogenesis are still an enigma, and it is consequently difficult to establish the precise classification of epileptic disorders and prevent seizures completely (despite significant progress in the new antiepileptic drugs development).

Epilepsy, as a chronic disorder, is characterized by spontaneous and recurrent seizures caused by certain pathogenic processes that disrupt neuronal and glial cells structure and/or function. Factors that cause epilepsy and provoke individual, isolated convulsions are numerous and very diverse. The most common among them are: fever in children, tricyclic antidepressants, theophylline, drugs intoxications and drugs abuse, acute neurological disorders and infections (meningitis, encephalitis, stroke, head injury, brain abscess, etc.), alcoholism, metabolic disorders (hypo/hyperglycemia, hypocalcaemia) [7], derivative of phencyclidine-metaphit [8], scabicides-lindane [9, 10], sleep disturbances [11–13], hyperhomocysteinemia [14], visual stimuli [15], stress [16], the menstrual cycle [17], a specific diet regimes [18, 19], vascular abnormalities, stroke sequelae and subarachnoid hemorrhage [20]. Experimental models of epilepsy are significant for resolving the mechanisms of epileptogenesis and play very important role in new antiepileptic drugs development. Extensive review of all models of epilepsy goes beyond 50 entries [21].

Some antibiotics, like imipenem/cilastatin [22, 23] and penicillin [24] induce seizures which is also a paradoxical phenomenon clearly demonstrated in experimental and clinical studies.

Inflammation, engaging the immune cells, the molecular mediators and blood vessels, is a complex biological, protective response to the pathogenic factors [25]. In conditions of excessive activity and/or organism immunity collapse, in-

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flammation seems to be potentially harmful and unuseful. Neuroinflammation could be caused by numerous factors and is characterized by activation of glial cells, known as the CNS resident immune cells [26]. Blood-brain barrier (BBB) is built up of astrocytes and endothelial cells and acts as an important isolator of the CNS. Therefore, the CNS is also partially isolated from the peripheral inflammatory cells [27]. Peripheral inflammation is an inflammatory process which occurs outside the CNS and is characterized by the activation of macrophages (peripheral immune cells) [28]. When the BBB is disrupted or its function compromised, peripheral immune cells could migrate into the CNS and induce neuroinflmamation [29].

Inflammatory processes and epileptogenesis

Numerous epidemiological studies have demonstrated that patients with epilepsy are more prone to different inflammatory disorders, i.e., inflammatory diseases are frequent comorbidities in epilepsy [30].

Numerous clinical and experimental studies have shown that inflammation disrupts the excitation/inhibition balance within the CNS and initiates the process of epileptogenesis. However, unclear relationship exists among the convulsions, inflammation and epilepsy [31–36]. These three factors generate positive feedback loop (*circulus vitiosus*) in which one process is facilitated by another one (Figure 1). Head injuries, tumors and infections of the CNS are characterized by neuroinflammation in its pathological basis. On the other hand, neuroinflammation could be a result of these pathological processes. Also, it is important to notice that seizure could be followed by inflammatory response in the entire CNS [37]. The CNS is not imunoprivileged site in the human body.

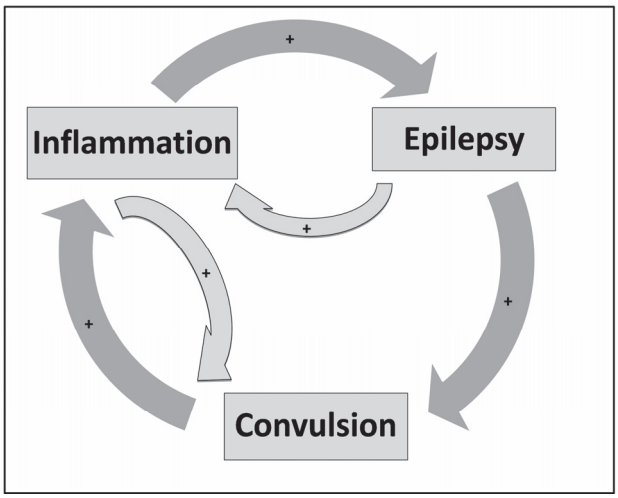

Fig. 1 – The relationship between epilepsy, convulsions and inflammation.
Neuroinflammation and peripheral inflammation could be a etiopathogenic substrate of epileptogenesis and development of epilepsy as a chronic neurological disorder accompanied by convulsive seizures. On the other hand, inflammation could also destroy the excitation/inhibition balance in the central nervous system (CNS) and provoke only isolated convulsions. On the other hand, isolated convulsive seizure could provoke neuroinflammation. Thus, positive feedback loop exists among these factors.

The process of epileptogenesis should not be viewed only through the prism of neural networks disarrangement. Namely, the pathogenesis of epilepsy involves also disarray of non-neural structures such as glial cells, endothelial cells in the CNS, the peripheral leukocytes and BBB injury [38]. Also, the role of extracellular matrix (ECM), as a very important brain substrate consisting of various molecules [e.g., hyaluronan, chondroitin sulfate proteoglycans (CSPGs), glycoprotein tenascin-R and other] derived from both neurons and glial cells [39] has to be addressed in these considerations, having in mind a critical role of cell-ECM interactions. Namely, there is a bidirectional relationship between epileptogenesis and ECM as well as evidences showing that biophysical and biochemical ECM properties modulate immune cell behavior including inflammatory cell migration. Excessive neuronal activity-mediated remodeling of ECM and mutations in ECM molecules (like leucine-rich, glioma-inactivated gene (LGI1), hyaluronan, tissue plasminogen activator (tPA) etc.) and extracellular proteinases [i.e. matrix metalloproteinase-9 (MMP-9)] are potent triggers for epileptogenesis by still unclear mechanisms which include disbalances in GABAergic and glutamatergic activity, mossy fiber sprouting, granule cell dispersion and gliosis (further details could be find in recent focused reviews [39–41]). During inflammation, ECM is altered by several cytokines upregulated at sites of inflammation [like tumor necrosis factor (TNF) and interferon gamma (IFNγ)] as well as by the secretion and/or activation of proteases, like MMPs [42]. On the other hand, neuroinflammation leads to degradation of ECM into immunoreactive fragments (mainly hyaluronan, sulfated proteoglycans and newly expressed tenascin-C), which are considered as damage-associated molecular patterns (DAMPs) capable to activate inflammatory cells via pattern recognition receptors (PRRs) [43]. Hence, progression of neuroinflammation, in turn, leads to further degradation of ECM by positive feedback loop. Therefore, ECM protects neuronal cell rearrangement and excitatory network formation by its nonpermissive function, and any change in neural ECM induced by inflammation, brings a receptive environment for epileptogenesis.

The immune response is proven to be responsible for the initiation and propagation of epilepsy [37, 44, 45]. Neuroinflammation increases the excitability of neurons because it changes the permeability of neurilemmal ion channels, affects the release and reuptake of neurotransmitters and the permeability of the BBB. All these factors decrease the threshold for the occurrence of epileptic discharges [46–48].

Oxidative stress and neuroinflammation: unresolved relationship

Neuroinflammation occurs by activation of glial cells (astrocytes, ependymal and endothelial cells in the first row) with the increased production of proinflammatory cytokines [like interleukin-1 β (IL-1β); tumor necrosis factor-α (TNF-α); prostaglandins, etc.] at the same time. It is also followed by an increased activity of cyclooxygenase-2 (COX-2) [37]. IL-1 receptor /toll-like receptor (IL-1R/TLR) and COX-2-medi-

ated signaling pathways act on neurons via N-methyl-D-aspartate receptor (NMDA), principally via the NMDA receptor subtype 2B (NR2B). It is also followed up by glial dysfunction. All these events may result in increased expression of adhesion molecules on the BBB and dysfunctions of the barrier itself. In this case, serum with albumins as well as activated peripheral leukocytes can "leak" in the brain. This is regarded as a trigger of the process of epileptogenesis and increased concentrations of the excitatory neurotransmitter glutamate and ion channels modulation [1, 38].

Oxidative stress, preponderance of production over the degradation of reactive oxygen species (ROS), manifests its numerous deleterious effects throughout the human body [49, 50] as well as in the brain. Actually, elevated levels of lipid peroxidation, like oxidative stress markers and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase which produces ROS, accompanied with low levels of antioxidants [51, 52] were proved in the blood and the surgically removed brain tissue of patients with epilepsy. Moreover, decreased ability of the brain to defense against oxidative stress led to increased severity of experimentally induced seizures by pentylenetetrazole and kainic acid [53]. We have shown that lipid peroxidation is involved in the etiopathogenesis of the experimental model of seizures caused by lindane [54]. The brain damage, as a consequence of oxidative stress during seizures, was reduced by treatment with antioxidants such as vitamin E [55] and vitamin C [56]. In addition, we have shown that reinforcing the antioxidant capacity of the organism by physical activity reduces the animal susceptibility for development of experimental epileptic activity induced by homocysteine [57].

Although it can be clearly concluded that pathogenesis of epilepsy encompasses both neuroinflammation and oxidative stress, the mutual relationships between these two factors are largely unclear. In addition, it is unclear whether neuroinflammation leads to the generation of epileptic discharges in the brain exclusively via oxidative stress. It is also unclear whether neuroinflammation is a result of the oxidative stress initiated by some third factor (Figure 2).

The role of peripheral inflammation

Peripheral inflammation is associated with a fever, reduction of locomotor activity as well as the excitation/inhibition imbalance in the CNS [58]. It has been concluded that peripheral inflammation could exacerbate seizures and many other neurological disorders [1, 59].

It has been shown that peripheral inflammation decreases the threshold for the occurrence of epileptic seizures in experimental models of peripheral inflammation evoked by administration of bacterial lipopolysaccharide (LPS) [60], a suspension of degraded bacteria, into the peritoneal cavity [61, 62] as well as in experimental models of inflammatory bowel disease [63, 64] and arthritis [63].

Although previous studies did not elucidate exactly how the peripheral inflammation affects the brain, the participation of various cytokines [63] endogenous opioids [60, 64], prostaglandins [60] as well as the signaling pathways mediated by nitric oxide (NO) in this process could be assumed with certainty [60, 62]. According to our results, neurotransmission mediated by NO is an important mechanism in the initiation and propagation of epileptogenesis [9, 65]. Moreover, the activation of the brain glial cells, which are equivalent to the periphery inflammatory cells, increases the production of cytokines in the CNS. Thus, these cells are considered to be an important element of neuroinflammation [35, 36].

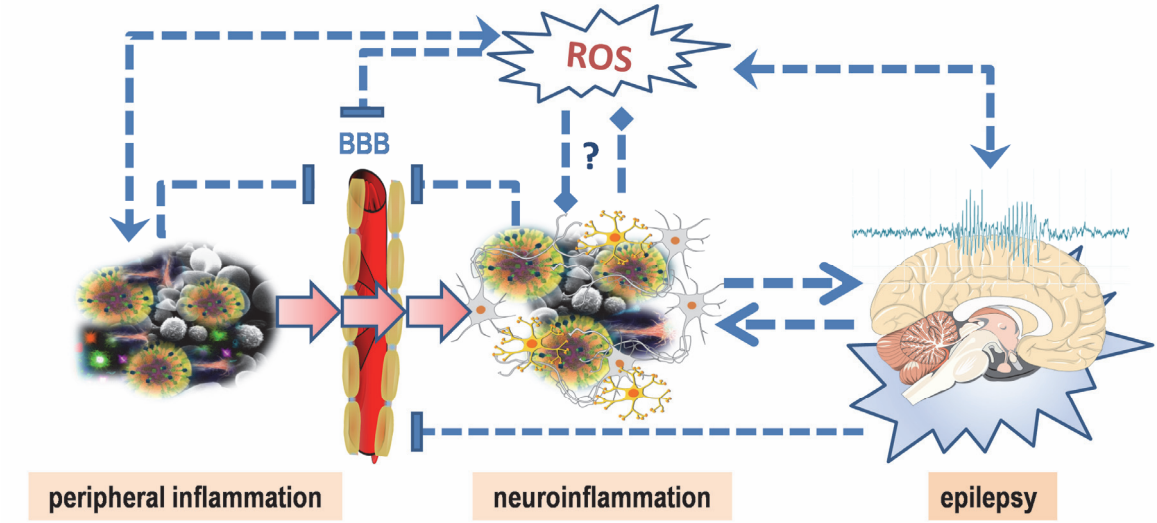

Fig. 2 – The relationship between epilepsy, peripheral inflammation, neuroinflammation and oxidative stress. Peripheral inflammation, after generalization and extension via disrupted blood-brain barrier (BBB) to the central nervous system (CNS) causes neuroinflammation which results in development of epilepsy. Epilepsy followed by convulsions compromised competency of the BBB and enhances neuroinflammatory and oxidative stress response. Oxidative stress [presented as excessive reactive oxygen species (ROS)] and neuroinflammation participate together in the etiopathogenesis of seizures. However, their interrelationship is not clear (denoted with interrogation mark). Oxidative stress, induced by any pathogen may result in development of neuroinflammation, but, on the other hand, neuroinflammation by itself may result in oxidative stress. Arrows denote plus effect, while (I) denote negative effect.

Peripheral inflammation is accompanied by an increased production of proinflammatory cytokines, like IL-1β, IL-6 and TNF-α, which may contribute to the activation of glial cells and induction of neuroinflammation [66–69]. On the other hand, the role of COX-2 was emphasized in the process of neuroinflammation and oxidative stress, both acting to reduce the seizure threshold [70, 71]. The concentration and activity of the COX-2 enzyme sre markedly increased during seizures [72], especially in the hippocampus [73] which has essential role in the epilepsy generalization. Indeed, Ho et al. [70] showed reduction of seizure number in the experimental model of peripheral inflammation accompanied by neuroinflammation and oxidative stress in the hippocampus upon intracerebral administration of a COX-2 inhibitor and ROS scavenger. Also Eun et al. [74] potentiated hyperthermia-induced seizures by administration of LPS, which is a further proof that peripheral inflammation can potentiate seizures.

Conclusion

Many neurological conditions and disorders, in the first raw inflammatory diseases of the CNS (meningitis, encephalitis), CNS injuries, stroke, etc., are associated with inflammation. On the other hand, clinical course of these disorders frequently includes seizures. Inflammation is a common factor that disrupts homeostasis in the CNS neural networks; it acts proepileptogenically and causes seizures.

Taking into account all the above-mentioned facts, it is clear that neuroinflammation, together with its specific relation to ROS and changes in neural ECM, is interconnected with epileptogenesis, being its cause, or a consequence. Neuroinflammation reduces the threshold for initiation of the CNS hyperexcitability and disrupts the competency of the BBB.

It is reasonable to assume that therapeutic treatment of peripheral inflammation alongside with neuroinflammation can provide beneficial effects for patients with epilepsy by reducing the seizure number. Thus, this is a promising alley of future therapeutic modalities in epileptology.

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CASE REPORTS

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Optic nerve head recovery following the intraocular pressure – lowering surgery in the eye with early juvenile glaucoma – nine-year follow-up

Oporavak vidnog živca posle filtracione antiglaukomne operacije u oku sa ranim juvenilnim glaukomom tokom perioda praćenja od devet godina

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Abstract

Introduction. Congenital uveal ectropion (CEU) is a rare, non-progressive condition often accompanied with eyelid ptosis, anterior insertion of the iris, disgenesis of the iridocorneal angle and glaucoma. **Case report.** We present a case of a seven-year-old girl with a congential unilateral uveal ectropion and a secondary glaucoma which had daily variations from 13 up to 50 mm Hg. The patient had no other abnormalities of the iris or underlying systemic diseases. Introduced local anti-glaucomatous therapy initially normalized intraocular pressure (IOP), but failed to provide long term normalisation. Trabeculectomy normalized the IOP which resulted in the reduction of the cup/disc ratio and restitution of neuroretinal rim. The rim area increased to 1.716 mm^2 (0.958 mm^2 preoperative) rim volume, was 0.666 mm^3 (0.195 mm^3 preoperative) while cup-disc (C/D) ratio decreased to 0.330 (0.626 preoperative) as well as linear C/D=0.574 (0.791 preoperative). Neuroretinal rim (NR) was preoperatively preserved in the Ti segment, damaged in T, Ts, N, Ns segments, and borderline in the Ni segment. Postoperatively, neuroretinal rim was preserved in all segments. **Conclusion.** In the presented case trabeculectomy induced recovery of the nerve tissue of the optic nerve head which was confirmed by Haidelberg Retina Tomograph II (HRT II). The treatment results have been maintained during the follow-up period of nine years without topical or systemic antiglaucomatous therapy. Although CEU is a non-progressive and benign eye disease, associated glaucoma can cause severe optic nerve damage if not detected early and treated properly. As can be seen in the presented case, an adequate treatment can prevent and even reverse optic disc neuropathy.

Key words:
ectropion; uvea; congenital abnormalities; glaucoma; trabeculectomy; treatment outcome; child.

Apstrakt

Uvod. Kongenitalni ektropijum uvee je retko, neprogresivno stanje često praćeno ptozom, prednjom insercijom dužice, disgenezom irido-kornealnog ugla i glaukomom. **Prikaz bolesnika.** U radu je prikazana sedmogodišnja devojčica sa kongenitalnim ektropijumom uvee i sekundarnim glaukomom sa dnevnim skokovima pritiska do 50 mmHg. Bolesnica nije imala druge abnormalnosti dužice niti pridružene sistemske bolesti. Uvedena lokalna antiglaukomna terapija iako je dovela do početnog pada, nije dovela i do trajne normalizacije očnog pritiska (IOP). Filtraciona antiglaukomna operacija je dovela do trajne normalizacije IOP što je rezultovalo smanjenjem ekskavacije optičkog diska i oporavkom neuroretinalnog oboda. Površina oboda se uvećala na 1,716 mm^2 sa preoperativnih 0,958 mm^2, zapremnina na 0,666 mm^3 sa preoperativnih 0,195 mm^3, dok se ekskavacija smanjila sa 0,791 na 0,574. Neuroretinalni obod je preoperativno bio sačuvan samo u donjem temporalnom segment. Postoperativno, neuroretinalni segment je bio normalan u svim segmentima. **Zaključak.** Kod prikazane bolesnice trabekulektomija je dovela do oporavka optičkog nerva što je potvrđeno uz pomoć Haidelber Retina Tomograph II (HRT II) softvera. Tokom devetogodišnjeg perioda praćenja bolesnice nije koristila antiglaukomnu terapiju, pritisak je bio normalan, bez oštećenja optičkog nerva. Iako je kongenitalni ektropijum uvee neprogresivno i benigno oboljenje, pridruženi glaukom može da izazove ozbiljno

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oštećenje očnog nerva ukoliko se ne otkrije i ne leči na vreme. Kao što može da se zaključi iz ovog prikaz bolesnika adekvatanim tretmanom može se sprečiti nastanak oštećenja, a čak i dovesti do oporavka vidnog živca.

Ključne reči:
ektropijum; uvea; anomalije; glaukom; trabekulektomija; lečenje, ishod; deca.

Introduction

Congenital ectropion uveae (CEU) is a rare, nonprogressive condition insufficiently mentioned in the ophthalmologic literature.

Etiologically, uveal ectropion can be classified into two groups: acquired and congenital.

CEU is characterised by the ectropionated pigment epithelium layer on the front surface of the iris from the pupillary ruff, anterior insertion of the iris, dysgenesis of the iridocorneal angle and glaucoma.

Some studies have also found CEU joined with congenital anomalies and hereditary diseases.

Case report

In April 2007 a 6-year-old girl was referred to the Institute for Eye Diseases of Clinical Center of Serbia by a pediatric ophthalmologist for clinical examination due to an elevated intraocular pressure (IOP) in her right eye. Mother reported that about 3–4 ago, she started noticing wider right pupil, enlargement of the right eye and slightly lowered upper eyelid. The patient also had eye tenderness and pain with occasional and moderate redness and "bulging".

Best corrected visual acuity (correction -1.25 Dsph = -1.75 Dcyl ax 14 degrees) on her right eye was 20/20. Her left eye visual acuity was 20/20. IOP measured by Goldmann Applanation Tonometer was 36 mmHg on the right, and 18 mmHg on her left eye. Horizontal corneal diameters were 11.5 mm on the right and 10.5 mm on the left eye. Ptosis of the right upper eyelid was evident as well as anisocoria with wider and slowly responsive right pupil. Slit lamp examination showed 2–3 mm wide zone of the uveal pigmented layer ectropion on the anterior iris surface which affected 360 degrees of the pupil margin. There were no signs of the newly formed blood vessels, tumors or other congenital anomalies (Figure 1).

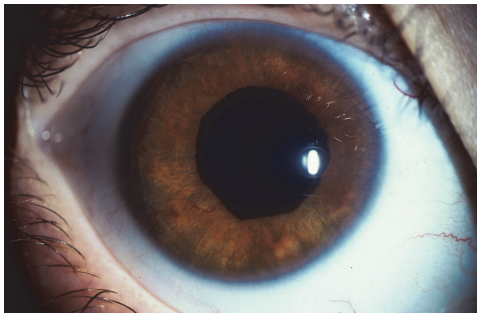


Fig. 1 – Slit lamp: Congenital uveal ectropion.

Gonioscopic findings on her right eye show a wide anterior chamber angle with anterior insertion of the iris of medium pigmentation – *aplasio ligamentum pectinatum* (Figure 2). Left eye gonioscopy showed open, free and moderately pigmented iridocorneal angle.

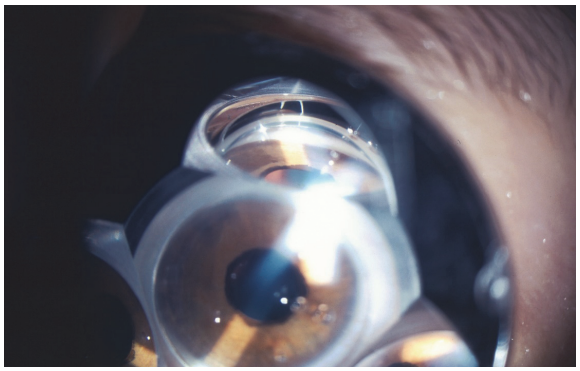


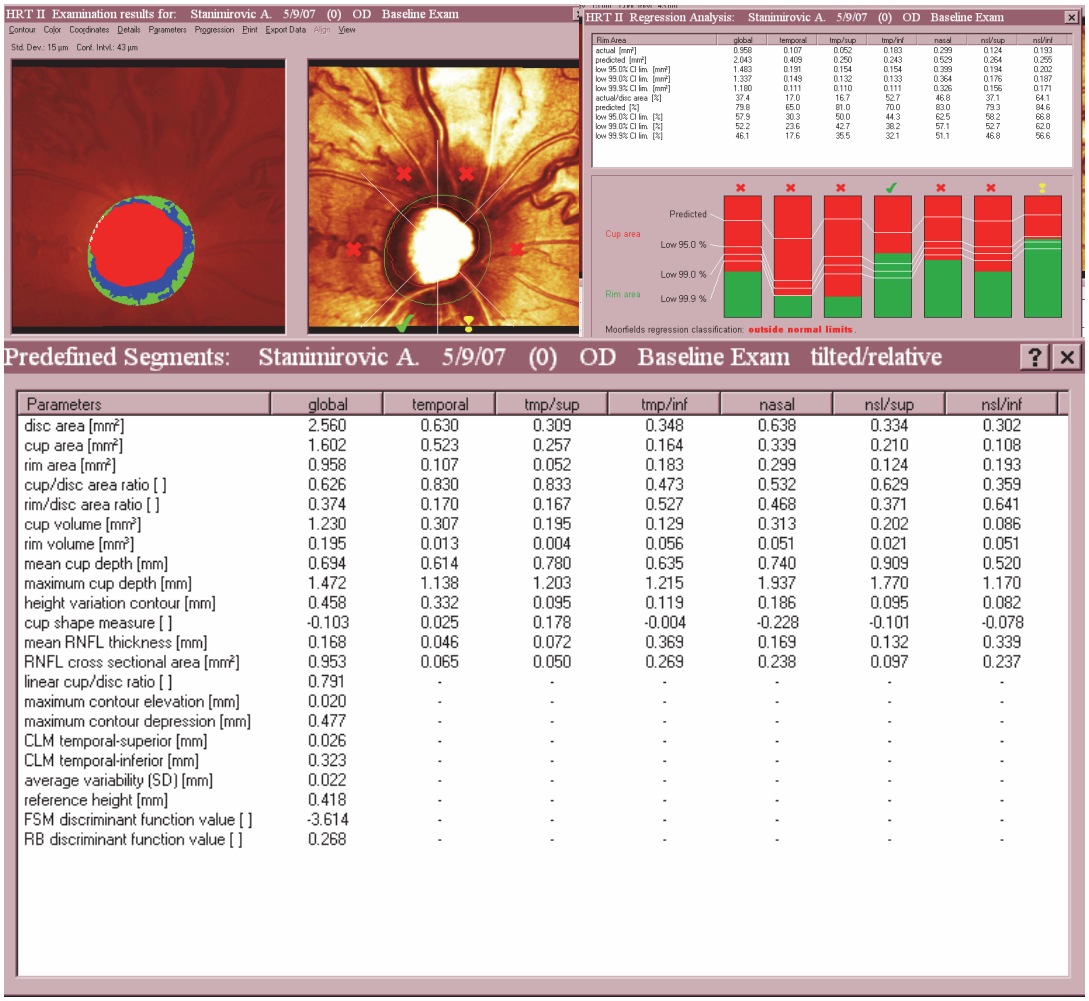
Fig. 2 – Gonioscopic findings of the congenital uveal ectropion.

Red reflex was present in both eyes, without defects. Fundus examination showed excavation of the optic head with cup/disc (C/D) ratio 0.7/I, while left was C/D 0.2/I.

Ultrasound biomicroscopy (UBM) confirmed the results of the gonioscopy (Figure 3).

Fig. 3 – Ultrasound biomicroscopy (UBM) of the affected eye: trabecular iris angle 45.64º, angle opening distance 0.65 mm at 250 µm and 1.09 mm at 500 µm, trabecular-ciliary process distance 0.788 mm, iris-ciliary process distance 0.240 mm, anterior chamber depth 3.12 mm and iris thickness 0.322 mm.

Humphrey computerized perimetry was performed, but the results were invalid due to poor cooperation. As shown on the Figure 4 neuroretinal rim (NR) was damaged in nearly all segments. Disc area was 2.560 mm^2, rim area 0.958 mm^2 and rim volume = 0.195 mm^3, C/D ratio was = 0.626, linear cup C/D = 0.791. Moorfields regression analysis was classified as "outside normal limits". HRT II of the left eye showed no abnormalities.



Parameters	global	temporal	tmp/sup	tmp/inf	nasal	nsl/sup	nsl/inf
disc area [mm²]	2.560	0.630	0.309	0.348	0.638	0.334	0.302
cup area [mm²]	1.602	0.523	0.257	0.164	0.339	0.210	0.108
rim area [mm²]	0.958	0.107	0.052	0.183	0.299	0.124	0.193
cup/disc area ratio [ ]	0.626	0.830	0.833	0.473	0.532	0.629	0.359
rim/disc area ratio [ ]	0.374	0.170	0.167	0.527	0.468	0.371	0.641
cup volume [mm³]	1.230	0.307	0.195	0.129	0.313	0.202	0.086
rim volume [mm³]	0.195	0.013	0.004	0.056	0.051	0.021	0.051
mean cup depth [mm]	0.694	0.614	0.780	0.635	0.740	0.909	0.520
maximum cup depth [mm]	1.472	1.138	1.203	1.215	1.937	1.770	1.170
height variation contour [mm]	0.458	0.332	0.095	0.119	0.186	0.095	0.082
cup shape measure [ ]	-0.103	0.025	0.178	-0.004	-0.228	-0.101	-0.078
mean RNFL thickness [mm]	0.168	0.046	0.072	0.369	0.169	0.132	0.339
RNFL cross sectional area [mm²]	0.953	0.065	0.050	0.269	0.238	0.097	0.237
linear cup/disc ratio [ ]	0.791	-	-	-	-	-	-
maximum contour elevation [mm]	0.020	-	-	-	-	-	-
maximum contour depression [mm]	0.477	-	-	-	-	-	-
CLM temporal-superior [mm]	0.026	-	-	-	-	-	-
CLM temporal-inferior [mm]	0.323	-	-	-	-	-	-
average variability (SD) [mm]	0.022	-	-	-	-	-	-
reference height [mm]	0.418	-	-	-	-	-	-
FSM discriminant function value [ ]	-3.614	-	-	-	-	-	-
RB discriminant function value [ ]	0.268	-	-	-	-	-	-

Fig. 4 – Hajdelberg Retina Tomograph II (HRT) initial report of the right eye (OD), neuroretinal rim (NR) was preserved in the Ti segment; NR was damaged in T, Ts, N, Ns segments, and borderline parameters of NR were found in the Ni segment, disc area = 2.560 mm^2, cup volume 1.230 mm^3, cup area = 1.602 mm^2, mean cup depth = 0.694 mm, max. cup depth = 1.472 mm, rim area 0.958 mm^2 and rim volume = 0.195 mm^3, cup/disc (C/D) ratio = 0.626, linear C/D ratio = 0.791. RNGL – retinal nerve fiber layer; CLM – contour line modulation; FSM – Mikelberg discriminant function; RB – right bank.

Local antiglaucomatous therapy was introduced and during the hospitalisation intraocular pressure normalized. The patient was discharged with the IOP values 16 mmHg in the right eye and 15 mmHg in the left eye without therapy.

On the regular monthly check-ups the right eye IOP varied from 13–30 mmHg in the morning, reaching values up to 50 mmHg in the afternoon in spite of prescribed therapy so the patient was hospitalised again. IOP in *oculi sinstri* (S) was 13–16 mmHg without therapy. HRT II results during the second hospitalization showed progression and decompensation of glaucoma. Since IOP in *oculi dextri* (OD) was not normalized under further therapy, antiglaucomatous surgery (trabeculectomy – TTR), in general anesthesia, was performed during the third hospitalization (Figure 5). Mitomycin C was not used in the procedure in order to avoid postoperative hypotonia because IOP showed significant daily variations.

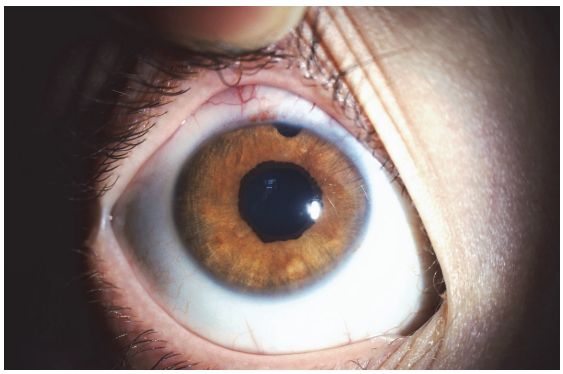
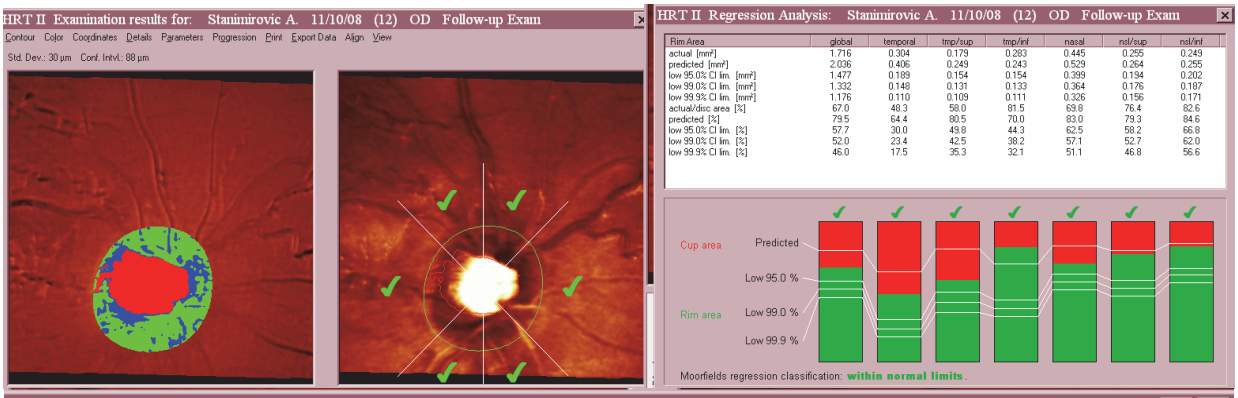


Fig. 5 – Anterior segment of the right eye after the performed antiglaucomatous surgery (trabeculectomy – TTR).

Upon discharge, IOP on the right eye was 8 mmHg with a somewhat enlarged filtration bleb, and 9 mmHg on the left eye.



Parameters	global	temporal	tmp/sup	tmp/inf	nasal	nsl/sup	nsl/inf
disc area [mm²]	2.560	0.630	0.309	0.348	0.638	0.334	0.302
cup area [mm²]	0.844	0.326	0.130	0.064	0.192	0.079	0.053
rim area [mm²]	1.716	0.304	0.179	0.283	0.445	0.255	0.249
cup/disc area ratio [ ]	0.330	0.517	0.420	0.185	0.302	0.236	0.174
rim/disc area ratio [ ]	0.670	0.483	0.580	0.815	0.698	0.764	0.826
cup volume [mm³]	0.436	0.149	0.067	0.031	0.116	0.048	0.026
rim volume [mm³]	0.666	0.062	0.046	0.143	0.189	0.096	0.130
mean cup depth [mm]	0.518	0.471	0.569	0.490	0.542	0.635	0.460
maximum cup depth [mm]	1.144	1.101	1.026	0.986	1.347	1.011	1.009
height variation contour [mm]	0.531	0.410	0.110	0.112	0.196	0.178	0.060
cup shape measure [ ]	-0.146	-0.157	0.023	-0.001	-0.259	0.162	-0.148
mean RNFL thickness [mm]	0.303	0.129	0.182	0.476	0.408	0.207	0.491
RNFL cross sectional area [mm²]	1.722	0.182	0.127	0.347	0.575	0.152	0.344
linear cup/disc ratio [ ]	0.574	-	-	-	-	-	-
maximum contour elevation [mm]	0.118	-	-	-	-	-	-
maximum contour depression [mm]	0.649	-	-	-	-	-	-
CLM temporal-superior [mm]	0.053	-	-	-	-	-	-
CLM temporal-inferior [mm]	0.347	-	-	-	-	-	-
average variability (SD) [mm]	0.031	-	-	-	-	-	-
reference height [mm]	0.638	-	-	-	-	-	-
FSM discriminant function value [ ]	1.636	-	-	-	-	-	-
RB discriminant function value [ ]	1.115	-	-	-	-	-	-

Fig. 6 – Hajdelberg Retina Tomograph II (HRT II) findings 18 months after the trabeculectomy. Values of stereometric parameters were: disc area: 2.560, rim area = 1.716 mm^2, cup volume = 0.436 mm^3, rim volume = 0.666 mm^3 mean cup depth = 0.518 mm, max. cup depth = 1.144 mm and cup/disc (C/D) ratio = 0.330 and linear C/D = 0.574. Neuroretinal rim (NR) was preserved in all its segments. Moorfields regression analysis were classified as "within normal limits".
RNFL – retinal nerve fiber layer; CLM – contour line modulation;
FSM – Mikelberg discrimination function; RB – right bone.

On the regular check-up 3 months later, IOP values were 10 mmHg on the right, and 11 mmHg on the left eye. Horizontal corneal diameter in OD was 10.5 mm and in OS 10 mm, with no Haab striae. Best corrected visual acuity (BCVA) was 20/20 on both eyes. Three months after the procedure, HRT II examination results were: cup area = 1.156 mm2, cup volume = 0.717 mm^3, rim area = 1.403 mm^2, rim volume = 0.495 mm^3 and C/D = 0.452, which is evidently lower C/D compared to the initial report.

Figure 6 shows the HRT II results 18 months after the trabeculectomy. The rim area increased to 1.716 mm^2 (0.958 mm^2 preoperatively) as well as rim volume which was 0.666 mm^3 (0.195 mm^3 preoperatively) while C/D decreased to 0.330 (0,626 preoperatively) as well as linear C/D =0.574 (0.791 preoperatively) (Figure 6). NR rim was found to be preserved in all its segments so the findings of Moorfields regression analysis were classified as "within normal limits". All this undoubtedly demonstrates the recovery of the NR rim on the optic disc.

Since the patient was without any therapy she had regular check-ups, first on 3, then on 6 months and then once a year. Last check-up was 6 months ago.

BCVA was 20/20 on both eyes, intraocular pressure was 14 mm Hg on the right, and 15 mm Hg on the left eye. Axial length of the right eye was 25 mm and of the left 23.5 mm.

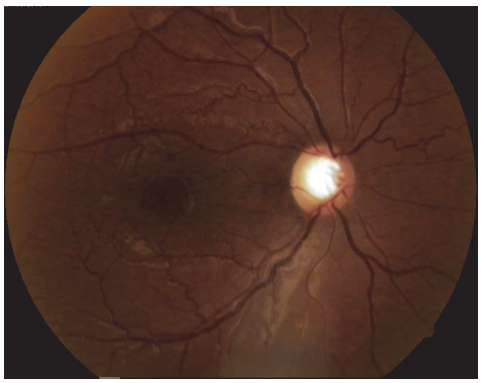


Fig. 7 – Foto fundus right eye – preserved neuroretinal rim (NR), cup/disc (C/D) ratio 0.4.

Foto fundus of the right eye showed preserved rim of the optic head with cup/disc ratio 0.4 and no pathology on the posterior segment of the eye (Figure 7).

Figure 8a presents HRT II of the right eye 9 years after the trabeculectomy. All the findings were within normal limits: HRT II left eye findings (6/1/2016) were C/D = 0.356, linear C/D = 0.597, rim area = 2.164, rim volume = 0.380 mm^3, disc area = 3.361 mm^2. Computerised visual field of the right eye showed generalised reduction of sensitivity MD-3.9 db; Bebie curve cumulative defect curve: diffuse loss of sensitivity under normal curve, but paralleled with displayed normal curve; left eye: normal sensitivity (Figure 8b).

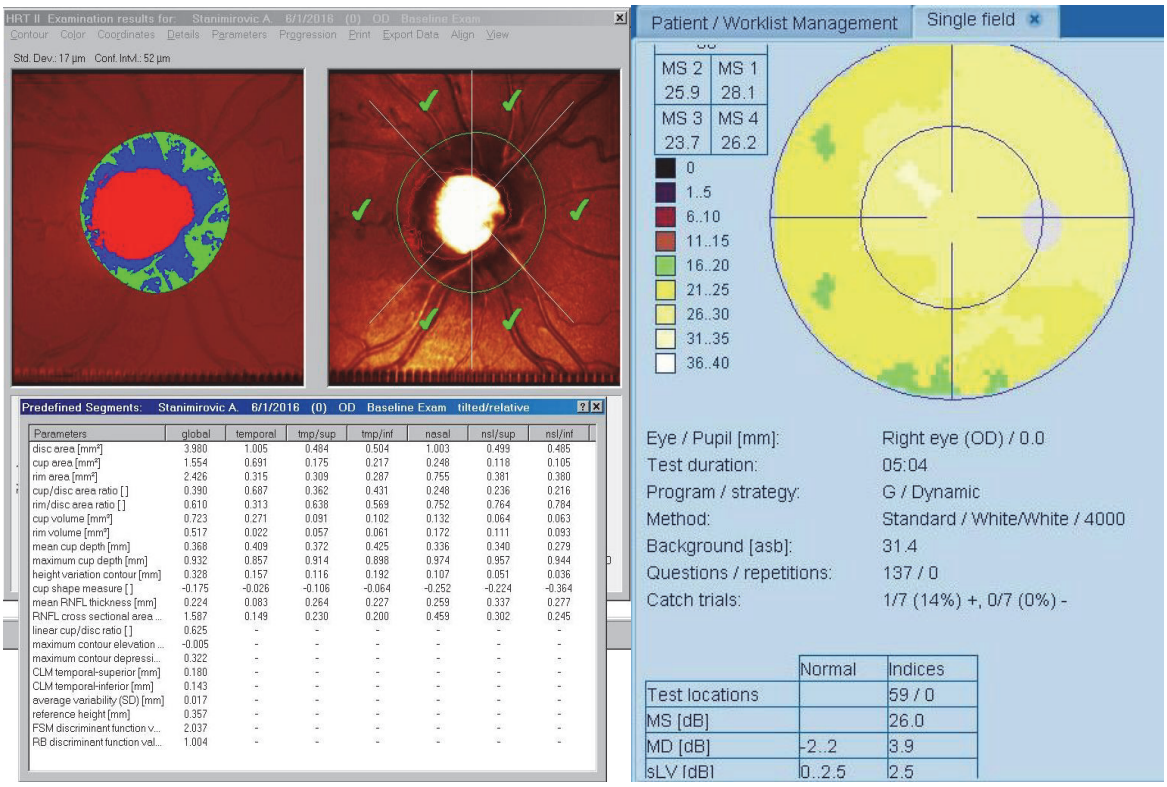


Fig. 8 – a) Haiedelberg Retina Tomograph II (HRTII) cup/disc (C/D) ratio: 0.390, linear C/D = 0.625, rim area = 2.426 mm^2, rim volume = 0.517 mm^3, disc area: 3.980 mm^2; b) Computerized visual field of the right eye showed generalised reduction of sensitivity MD-3.9 db, Bebie curve (cumulative defect curve): diffuse loss of sensitivity under normal curve, but paralleled with displayed normal curve.
RNFL – retinal nerve fiber layer; CLM – controur line modulation;
FSM – Mikelberg discrimination function; RB – right bank.

Discussion

In the majority of cases from the available literature CEU is associated with glaucoma. Typically, there is an initial drop of IOP following the induction of the topical therapy, like in the case presented, but eventually most cases require filtration surgery.

The origin of the CEU is not certain. Dowling et al. [1] suggested that abnormal migration of neural crest cells might be the reason for the development of the CEU, while Ritch et al. [2] suggested the possibility of a neural crest cell disorder. Some authors describe bilateral CEU with late onset primary glaucoma and bilateral prominent corneal nerves [3,4]. Wilson [5] concluded that, due to its embryological origin, this trinity supports the neural crest cell theory of the anterior segment dysgenesis.

Harasymowycz et al. [6] analysed a sample of an iris taken from the eye with CEU and glaucoma and found a fibro vascular membrane on the front surface of the iris. Its traction might be responsible for pulling the posterior pigmented epithelium and CEU formation. Embryonal heart studies [7] suggest the vascular factor being primary insult that leads to the secondary disorder of the neural crest cell migration.

The recovery of the optic nerve is widely discussed. Tavares et al. [8] found no significant change in anatomic and functional glaucoma evaluation 5–6 months after glaucoma surgery. Wright et al. [9] suggested that IOP lowering surgery enhances visual field (VF) sensitivity while Ventura et al. [10] found that the progressive loss of retinal ganglion cell function in early glaucoma may be alleviated after IOP lowering, as measured by pattern electroretinograms.

Lesk et al. [11] concluded that lowering of the IOP by 40% after glaucoma surgery had improved optic nerve morphology correlating highly with the percent reduction of IOP. Waisbourd et al. [12] also reported cupping reversal and visual field (VF) improvement, but results did not correlate to the amount of the IOP lowering.

Emery et al. [13] suggested that increased IOP causes bulging of the cribrous lamina posteriorly, rearrangement of the openings on it, strangulation of axons and stagnation of axoplasmatic transport. Quigley [14] demonstrated that decrease of the IOP induced cupping reversal and recovery of the optic nerve head. Mochizuki et al. [15] reported cupping reversal accompanied by the shrinkage of the scleral canal after successful IOP-lowering surgery. If the cupping reversal had not been observed, the scleral ring enlargement and ongoing stress on the optic nerve would have been continued. The cupping reversal is more frequent in children due to a larger number of elastic fibres in the fibrous layer of the globe.

Conclusion

In the presented case, normalisation of IOP induced a cupping reversal and normalized axoplasmatic transport resulting in the recovery of neuroretinal rim and the decrease of C/D ratio.

Even in the case of the secondary juvenile glaucoma due to congenital uveal ectropion, early detection and adequate treatment can prevent and reverse optic disc neuropathy.

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CASE REPORT

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Intraoperative diagnosis of an anterior sacral meningocele mimicking a giant ovarian cyst in an adult

Intraoperativna dijagnoza prednje sakralne meningokele koja imitira džinovsku cistu jajnika kod odrasle osobe

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Abstract

Introduction. Anterior sacral meningocele (ASM) is a very rare condition that is the herniation of the meningeal sac into the pelvic cavity through a developmental bone defect of the anterior wall of the sacrum. Most of the ASM are diagnosed in childhood but the reported cases that are diagnosed in adults exhibit a gamut of complications. **Case report.** We presented a case of ASM excision that was misdiagnosed as a giant ovarian cyst. A 28-year-old woman was admitted to the General Surgery Clinic and than to the Gynecology Department with suspect of ovarian cyst depending on ultrasonography (US) scans solely. Adnexial torsion was suspected and surgery to remove the cyst and adnexial exploration was planned. When the lesion was found out to be ASM, neurosurgery team tied the neck and excised the whole meningocele. Histopathologic evaluations confirmed dural sac. Neurological examinations right after the operation revealed 20% weakness in knee extension. It totally recovered in 6 months time. Her MR imaging studies and computed tomography (CT) scans revealed multiple ASM sacs and defects of the anterior wall of the sacrum. **Conclusion.** This case emphasizes the importance of utilizing available screening tools including CT andmagnetic resonance imaging (MRI) studies as the gold standard in addition to US scans in the preoperative period in order to accurately evaluate and characterize any pelvic lesion.

Key words:
ovarian cysts; diagnosis, differential; sacrococcygeal region; meningocele; diagnosis; neurosurgical procedures.

Apstrakt

Uvod. Prednja sakralna meningokela (PSA) je veoma retko stanje, koje podrazumeva herenijaciju meningealne vreće u šupljinu karlice kroz otvor kosti prednjeg zida sakruma. Najveći broj PSA dijagnostikuje se u detinjstvu, ali objavljeni slučajevi dijagnostikovani kod odraslih osoba prikazuju lepezu komplikacija. **Prikaz bolesnika.** Prikazali smo slučaj ekscizije PSA koja je bila pogrešno dijagnostikovana kao cista jajnika. Žena, životnog doba 28 godina, primljena je na Kliniku za opštu hirurgiju, a zatim na Odeljenje ginekologije, sa sumnjom na cistu jajnika samo na osnovu ultrasonografskog (US) nalaza. Postavljena je sumnja na torziju (uvrtanje) adneksa i planiran je hirurški zahvat radi odstranjivanja ciste i eksploracije andneksa. Kada je ustanovljeno da je u pitanju PSA, tim neurohirga podvezao je vrat meningokele i odstranio je u celosti. Patohistološkim ispitivanjem potvrđena je duralna vreća. Neurološkim ispitivanjem, neposredno posle operacije, otkrivena je slabost od 20% u ekstenziji kolena. Do oporavka je došlo tokom šest meseci. Ispitivanjem magnetnom rezonancom (MR) i kompjuterizovanom tomografijom (KT) otkrivene su vreće multiplih PSA i defekti prednjeg zida sakruma. **Zaključak.** Ovaj prikaz naglašava značaj korišćenja dostupnih metoda, uključujući CT i MR, kao zlatnog standarda u dodatku US nalaza u preoperativnom periodu, radi precizne procene i određivanje prirode bilo koje lezije karlice.

Ključne reči:
jajnik, cista; dijagnoza, diferencijalna; sakrokokcigealni predeo; meningokela; dijagnoza; neurohirurške procedure.

Introduction

Anterior sacral meningocele (ASM) is a very rare condition that may be present as a lower abdominal mass. It is the herniation of the meningeal sac into the pelvic cavity through a developmental bone defect of the anterior wall of the sacrum [1]. This anomaly might be either acquired or congenital. Acquired forms are extremely rare and are related

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with neurofibromatosis, Marfan's syndrome, Currarino syndrome and Ehler-Danlos syndrome, where, most of the AMS cases are congenital defects [1–7]. Most of the symptoms are due to the mass effect of the sac that sometimes can reach to enormous dimensions so as to interfere with the delivery in a pregnant woman [8–15]. As the mass grows into pelvis, genitourinary symptoms, radiculopathy, progressive weakness of the lower extremities and constipation may be other emerging symptoms. Most of the anterior sacral meningoceles are diagnosed in childhood but the reported cases diagnosed in adults exhibit a gamut of complications of the entity including meningitis and even deaths [16]. We presented a case of ASM excision that was misdiagnosed as a giant ovarian cyst.

Case report

A 28-year-old female patient was admitted to the General Surgery Clinic and than to the Gynecology Department with suspect of ovarian cyst depending on ultrasonography (US) scans. The patient with a history of a gestation otherwise healthy had a pelvic and urinary pain that started suddenly two days before and than spread to both inguinal quadrants of lower abdomen. She was evaluated by US and it was strongly suggestive of a single large ovarian cystic mass exceeding 12 cm that occupied the whole pelvis. As her complaints worsened shortly afterwards, adnexial torsion was suspected and surgery to remove the cyst and adnexial exploration was planned. The patient had no neurological complaints and deficits preoperatively. The US scan did not reveal any mural nodule, solid component, or septa (Figure 1).

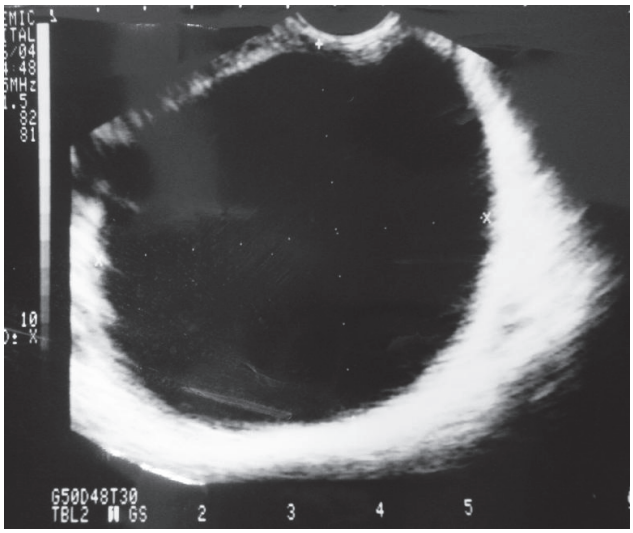

Fig. 1 – The gray scale ultrasonography (US) scan of the patient revealed a huge lesion (12 × 9 × 8 cm) in pelvis that was thought to be an ovarian cyst causing the abdominal pain probably because of the abdominal torsion. On the US scans, the cyst had no mural nodule, solid component or septa.

Cyst was a well-defined, non-echogenic giant simple cyst as identified on grayscale US by its unilocular appearance and lack of cyst wall papillae. Serum CA-125, CEA, CA 19-9, and CA 15-3 levels were within normal limits (18.6 U/mL, 7.9 ng/mL, 18.5 U/mL, and 8.8 U/mL, respectively). During the operation, uterus and both ovaries were observed anatomically normal. When the gynecologists gained access to the cystic lesion, they dissected it from the surrounding tissue and observed that ovaries did not have any connection with the cyst. It was attached to the posterior wall of the pelvic cavity. After opening the anterior wall of the cyst, the orifice that communicated the cyst with the spinal subarachnoid space was apparent. We could see the pulsation of the cerebrospinal fluid (CSF) through the orifice of the neck of the sac (Figure 2).

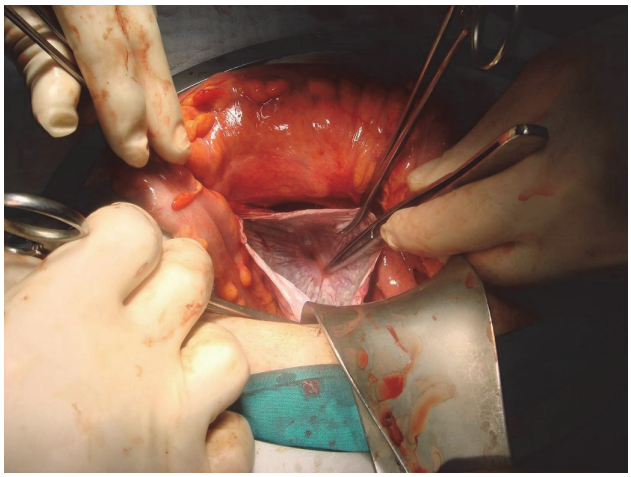

Fig. 2 – When the gynecologists gained access to the cystic lesion, they dissected it from the surrounding tissue and observed that ovaries did not have any connection with the cyst. It was attached to the posterior wall of the pelvic cavity. After opening the anterior wall of the cyst, the orifice that communicated the cyst with the spinal subarachnoid space was apparent.

After opening the sac and checking for any septa or neural tissue in the sac, we tied its neck and excised the whole meningocele (Figure 3).

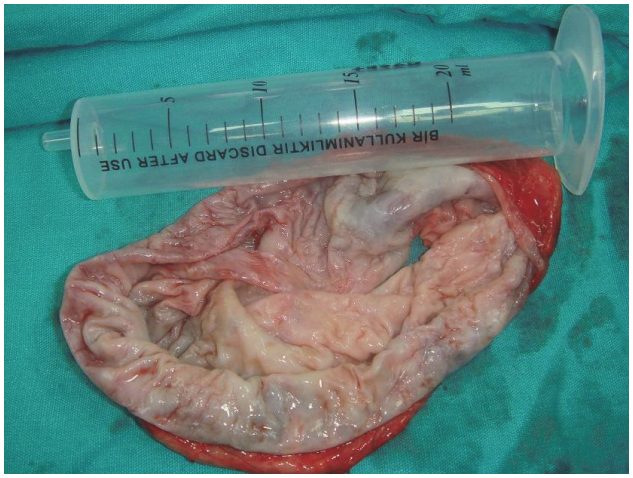

Fig. 3 – We tied the neck of the sac at the point closest to its entrance to the pelvic cavity and excised the cyst totally. The excised cyst material is seen and its dural structure was also histopathologically confirmed.

Histopathologic evaluations, as well, confirmed that it was dural sac. Neurological examinations of the patient after the operation revealed 20% weakness in knee extension, which totally recovered in 6 months time by means of intense physical treatment. Her magnetic resonance imaging (MRI) studies and computed tomography (CT) scans revealed multiple ASM sacs and defects of the anterior wall of the sacrum (Figure 4).

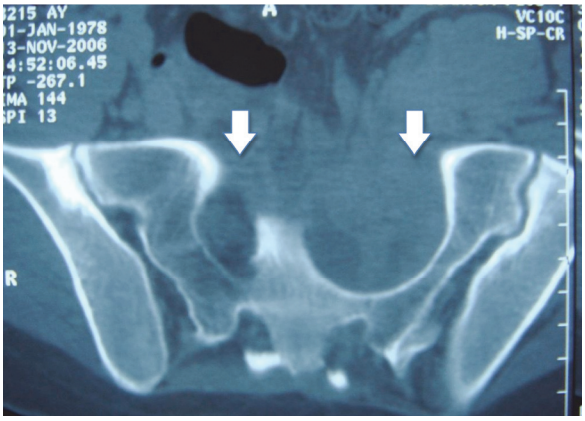

Fig. 4 – Postoperative sacral computed tomography (CT) scan of the patient discerns multiple bony defects in the anterior sacral wall (white arrows).

Her first-degree relatives (parents, sister, and two brothers and son) were also screened but none had any spinal structural deformity.

Discussion

ASM is a consequence of the sacral defect in the anterior wall through which the dural sac invaginates into the pelvic cavity presenting as a pelvic cystic mass that can attain remarkable dimensions is rarely accompanied by neural tissue [1, 15, 17]. The real incidence of the ASM is not clear because of the mostly asymptomatic characteristic of the anomaly. Most of them are diagnosed incidentally because of its insignificant clinical features. Even at that time, regular US features may lead to diagnostic errors unless investigated rigorously by using available tools to rule out mimicking other clinical entities [8, 10, 12–15, 18]. Therefore, history taking and detailed physical examination are initial steps of the utmost importance which will guide the physician through the accurate diagnosis so as to prompt one to use appropriate tools such as the MRI study as a gold standard. Since MRI study can discern detailed texture of the spinal and abdominal anatomy, it is the most frequently used diagnostic tool [10, 14, 18]. In the presented case, the patient had an inguinal pain that worsened in near past suggesting acute abdomen. It was accompanied by a chronic constipation that first began about three years before, and could not be solved permanently. She also had severe headache that emerged during the Valsalva maneuvers because of constipation. She was on analgesic medication for intractable headache recently. She had a delivery via physiologic route 3 years ago and has a healthy son.

Although ASM is usually asymptomatic and benign in nature, it should be investigated thoroughly and surgical treatment should be considered when diagnosed because it is liable to cause serious complications and can manifest abnormalities of the urinary and lower digestive systems. Anterior sacral defect may sometimes be accompanied by anorectal malformations and presacral space occupying mass lesion, composing the triad of the Currarino syndrome [2, 16, 17]. In some adult cases, obstetrical complications might be the situation that alerts the patient and the physician, however the presented patient had an uneventful pregnancy and delivery. Even during her control US scans in pregnancy did not reveal any remarkable finding of the sacral defect or a pelvic mass. Although she had been suffering from constipation for a quite long time, she did not undergo any imaging studies like CT or MRI else than the US scan that led to the false diagnose of ovarian cyst and adnexal torsion. Gynecologists operated on the patient without obtaining an MRI study which was the gold standard test for assessing most of the soft tissue and some of the bony lesions of the abdomen. This proves that assumption that an adnexal mass arises from the ovary may lead to diagnostic errors [19].

Conclusion

It is now well-known that success of any approach to correct the ASM and other concomitant malformations of the central nervous system or pelvic organs, strictly relies on the preoperative assessment of the MRI and/or CT scans. Therefore, one should make use of these tools initially not to further add new deficits to the patient. There is a female predominance of ASM and they are usually diagnosed at the routine gynecological screening procedures, unless their ASM was missed so far. The presented patient gave birth to a healthy baby, had an uneventful gravidity and still her ASM with several mild symptoms was overlooked. This case emphasizes the importance of utilizing available screening tools including CT and MRI studies as the gold standard in addition to US scans in order to accurately evaluate and characterize any pelvic lesion.

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CASE REPORT

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Primary malignant teratoma of the mediastinum with poor outcome: A case report

Primarni maligni teratom medijastinuma sa lošim ishodom

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Abstract

Introduction. About 5%–10% of mediastinal tumors in adults are teratomas and about 85% of them are benign. **Case report.** We report a case of extragonadal malignant teratoma in a 39-year old man. The computed tomography (CT) scan of the chest revealed the soft-tissue density mass in the middle lobe of the lung. The posterolateral thoracotomy was performed and a mediastinal tumor of 25 cm was completely resected. Histopathological findings confirmed malignant teratoma. One month after the resection the positron emission tomography-computed tomography (PET/CT) scan of the whole body was performed and showed progression of the disease. Patient developed signs of the superior vena cava obstruction and received radiotherapy of the mediastinum and the metastatic lesion of the lumbal vertebra. After radiotherapy, the patient developed paraplegia and urinary incontinence and received the best supportive treatment. **Conclusion.** Primary extragonadal germ cell tumors have poor prognosis due to their relative chemoresistance and frequent findings of advanced disease after establishing the diagnosis.

Key words:
teratoma; mediastinal neoplasms; diagnostic techniques and procedures; thoracic surgery; neoplasm metastasis; radiotherapy; palliative care.

Apstrakt

Uvod. Oko 5% –10% medijastinalnih tumora kod odraslih su teratomi, od kojih su prosečno 85% benigni. **Prikaz slučaja.** Prikazujemo slučaj ekstragonadalnog malignog teratoma kod bolesnika starog 39 godina. Kompjuterizovanom tomografijom (KT) grudnog koša uočena je mekotkivna promena u srednjem režnju desnog plućnog krila. Posterolateralnom torakotomijom viđen je medijastinalni tumor veličine 25 cm koji je u potpunosti odstranjen. Patohistološki nalaz resektata potvrdio je maligni teratom. Jedan mesec nakon resekcije pluća urađena je pozitron emisiona tomografija – kompjuterizovana tomografija (PET-CT) celog tela koja je ukazala na progresiju bolesti. Kod bolesnika je došlo do razvoja znakova kompresije na gornju šuplju venu, zbog čega je sprovedena radioterapija medijastinuma i palijativna radioterapija metastatske promene na lumbalnom pršljenu. Nakon radioterapije bolesnik postaje inkontinentan i paraplegičan, te je nadalje sprovedena najbolja potporna terapija. **Zaključak.** Primarni ekstragonadalni tumori imaju lošu prognozu usled njihove relativne hemorezistencije i čestog nalaza uznapredovale bolesti u momentu postavljanja dijagnoze.

Ključne reči:
teratom; medijastinum, neoplazme; dijagnostičke tehnike i procedure; hirurgija, grudna; neoplazme, metastaze; radioterapija; lečenje, palijativno.

Introduction

Teratoma is a special mixed tumor type that contains mature or immature cells or tissues derived from more than one germ cell layer, or sometimes of all three (ectoderm, endoderm, mesoderm) [1, 2]. Germ cells are those of the gonads and there are several theories explaining their presence in other locations. According to the theory that the origin of this tumor is anatomically related to the thymus, it is supposed that in an early embryo the primordial teratomatous focus is originally located in the thymus and may lie in such a position in the mediastinum that it is caught up and carried by the

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respiratory outgrowth from the foregut [3, 4]. Germ cell tumors are classified as extragonadal tumors when there is no presence of primary tumors of testes or ovary. The most common localization of extragonadal tumors in adults are pineal gland, mediastinum and retroperitoneum [5–7]. Teratoma is a common tumor of the anterior mediastinum and malignant teratoma seldom metastasizes to the lung. The most frequently involved extrathoracic metastatic site is the skeletal system [8, 9]. About 5%–10% of mediastinal tumors in adults are teratomas and about 85% of them are benign [1, 9–12]. The world wide annual incidence of teratomas is about 1 in 4000 live births [12, 13]. Risk factors for malignant extragonadal germ cell tumors include: a male sex, the age of 20 or older, Klinefelter syndrome [5, 14].

Case report

We report a case of a malignant teratoma in a 39-year-old man who presented with cough, high body temperature and shortness of breath. The chest radiography (X-ray) finding showed a massive, homogenous infiltration in the lower 2/3 of the left lung (Figure 1).

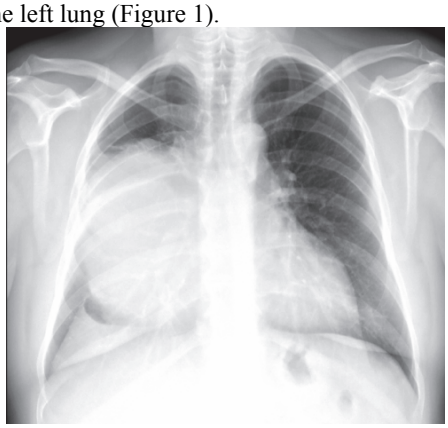

Fig. 1 – Chest X-ray finding at the admission: A massive, homogenous infiltration in the lower 2/3 of the right lung.

The computed tomography (CT) scan of the chest revealed a soft tissue density mass in the middle lung lobe sized 15×13×13 cm, with a compression of the right hilar region, left and right atria and superior vena (Figure 2).

Bronchoscopy with endoscopic biopsy and transthoracic needle biopsy were performed, but obtained samples were insufficient to diagnose radiologically found lesions. Posterorolateral thoracotomy was performed and a mediastinal tumor of 25 cm in size was seen on videoassisted thoracoscopy and it was completely resected. At the gross examination, the resected mass measured 17×14×13 cm and weighted 1,300 g, it was circumscribed, oval in shape, with a smooth surface. At cross section, it is mostly gray withish, rather firm and with some yellowish softend areas. Diffusely, small to medium cysts were present. On histology, the tumor consisted of various tissue types: fibrous, mature cartilage, smooth muscle bundles, nervous tissue with ganglionic cells, many cysts lined with columnar epithelial cells and malignant tumorous tissue. Some areas were necrotic and others with old and fresh haemorrhage (Figure 3).

The malignant tissue consisted of atypical, pleomorphic epithelial cells rather large with hyperchromatic nuclei and visible pathologic mitosis. The cells were arranged into diffuse sheets and some tubular or glandlike patterns. The zones of coagulative necrosis in the tumor nests, with some cellular debris were present. The immunohistochemical analysis showed the alpha-fetoprotein (AFP) and AE1/AE3 positivity in all tumor cells and CD30 focal positivity in about 10% of the tumor cells. The positive vimentin staining was in mesenchymal components and S100 in the nervous tissue. The negative staining was for placental-like alkaline phosphatase (PLAP) and CD117 (Figure 4). Based on the morphology and immunohistochemical characteristics of the tumor, the diagnosis of mediastinal teratoma with a component of a malignant mixed germ cell tumor has been established, with a predominating embryonal carcinoma as the main part.

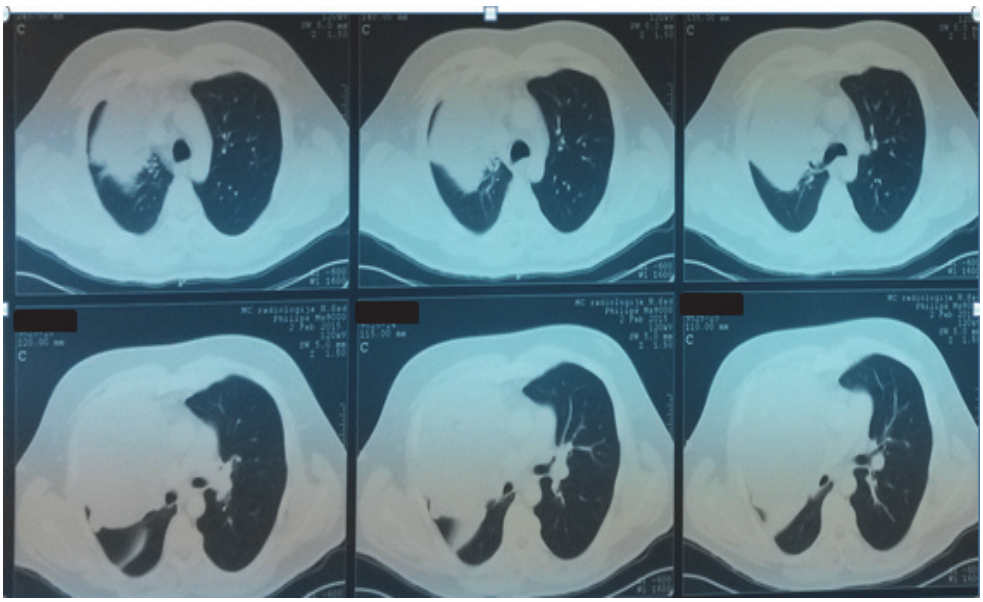

Fig. 2 – Chest computed tomography scan at the admission.

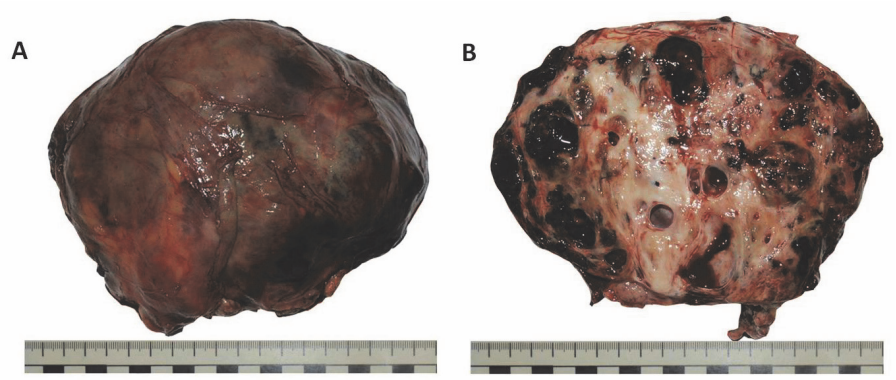

Fig. 3 – A) Gross appearance of the resected mass; B) Cross section of the tumor.

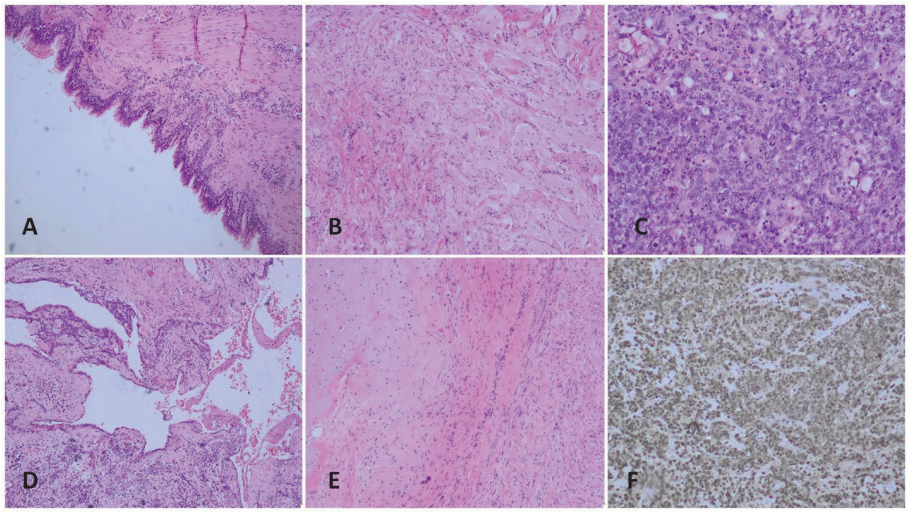

Fig. 4 – Respiratory epithelium [hematoxylin eosin (H&E), ×100]; B) Nervous tissue (H&E, ×40); C) Embryonal carcinoma (H&E, ×100); D) A cyst lined with cuboidal cells (H&E, ×40); E) Cartilaginous, fibrous changes in nervous tissue (H&E, ×40); F) Alpha-fetoprotein (AFP) positive carcinoma cells (AFP, ×100).

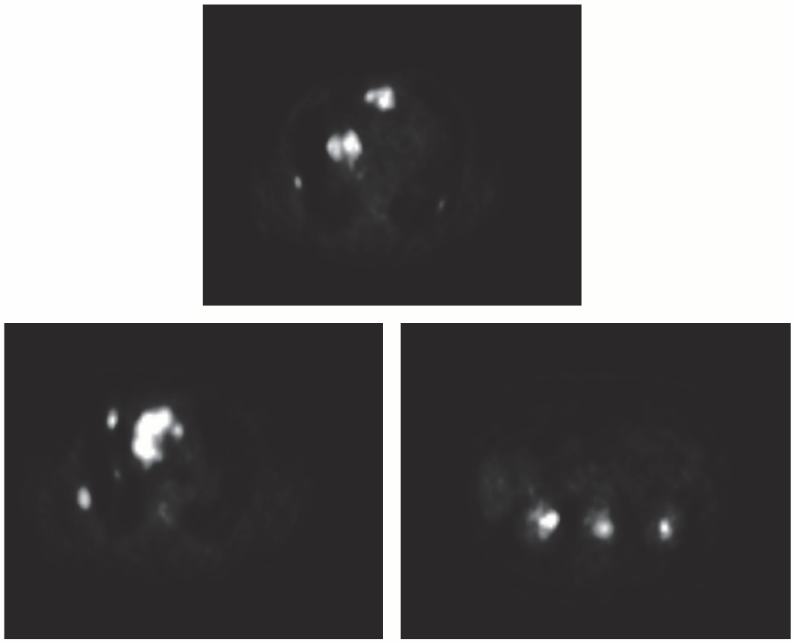

Fig. 5 – PET/CT scan of the whole body.
PET/CT – positron emission tomography/computed tomography.

One month after the resection, the patient underwent the positron emission tomography-computed tomography (PET/CT) scan of the whole body which showed multiple focuses of the fluorodeoxyglucosae (FDG) activity in the anterior mediastinum, multiple subpleural focuses in the right lung, micronodular lesions in both lungs and focuses of FDG activity in the 1st to 2nd lumbar vertebral body (Figure 5).

The serum AFP level was extremely high – 14,260 ng/mL (the normal value ranges from 0.6–6.6 ng/mL), and the serum beta human chorionic gonadotropin (β-HCG) value was within the normal range. Ultrasonography of the testis was normal. Having developed the signs of the superior vena cava obstruction, the patient received two series of radiotherapy of the mediastinum in the dose of 20 Gy/5 fractions (split-course) and palliative radiotherapy of the lumbar 1st–2nd vertebras in the dose of 20 Gy/10 fractions. In the course of radiotherapy, the patient developed paraplegia, urinary incontinence and his general condition worsened. After radiotherapy, the patient received the best supportive care.

Discussion

Extragonadal germ cell tumors can be benign (benign teratomas, teratodermoids) or malignant (seminoma or nonseminoma). Nonseminoma tumors are: teratocarcinoma, choriocarcinoma, embryonal carcinoma, endodermal sinus or yolk-sac tumors. Mixed nonseminoma tumors are the most common [4, 5, 11, 15]. Only 20% of extragonadal germ cell tumor are malignant. Malignant extragonadal teratomas are rare and are usually presented as a mediastinal mass. A rapid growth and invasive behavior are the rule [15]. The usual signs and symptoms of malignant extragonadal germ cell tumors due to a local extension of the tumor are: chest pain, breathing problems, cough, fever, headache, change in bowel habits, fatigue, walking problems, vision problems, symptoms due to elevated β-HCG levels (gynecomastia, hyperthyreoidism) [5, 8, 9]. Our patient developed the superior vena cava obstruction signs.

Diagnosis of extragonadal germ cell tumors is based on the: physical examination and history taking, chest X-ray, level of the serum tumor markers (AFP, β-HCG, lactate dehydrogenase – LDH), CT scan of the chest, abdomen or brain, magnetic resonance imaging, PET/CT scan of the whole body, ultrasonography of the testicles, tumor biopsy with patohistologycal and immunohistochemical analysis. Blood levels of the tumor markers signify if the tumor is seminoma or nonseminoma. Nonseminomatous germ cell tumors produce high levels of AFP, β-HCG or both, while in seminomatous tumors secretion of β-HCG is low [10]. The presence of tumor markers is of great value in the diagnosis and monitoring a patient's respond to treatment, as they tend to become negative if the treatment is appropriate [4]. CT scan of the chest is the imaging technique of choice in evaluating the mediastinum; teratomas are often presented as multi-locular cystic tumors, a heterogenous mass in the anterior mediastinum containing the soft-tissue, fluid, fat or calcium attenuation [13]. Magnetic resonance imaging (MRI) is used in adjunction to CT scanning in the evaluation of the nature, location and extent of the disease [11]. Varying proportion of mesodermal, ectodermal and endodermal elements are seen on the excised tumor microscopy [5, 13].

In our case, the diagnosis was confirmed by thoracotomy obtained biopsy sample. The serum levels of (AFP and LDH were elevated, while the serum β-HCG level was normal, as well as ultrasonography findings of the testicles.

Surgical excision is the treatment of choice in nonseminomatous tumors and a radical extirpation results in a long recurrence-free-survival [7, 16]. The postoperative irradiation therapy should be applied if a residual disease or nodal metastases are registered, whereas the preoperative irradiation therapy is indicated if there is evidence of the chest wall involvement [3]. Recommended chemotherapy protocols include PVB (cisplatin, vinbalstin, bleomycin), PEB (cisplatin, etoposide, bleomycin) and JEB (carboplatin, etoposide, bleomycin) [7].

Mediastinal mature teratomas have an excellent prognosis after complete resection in all age groups, while the prognosis of immature teratoma is still debated due to limited experience [1]. Primary extragonadal germ cell tumors have worse prognosis than that for testicular germ cell tumors due to their relative chemoresistance and frequent findings of the disseminated metastases at diagnosis [17].

Conclusion

Primary extragonadal germ cell tumors have poor prognosis due to their relative chemoresistance and frequent findings of advanced disease after establishing the diagnosis. We reported a case of a malignant teratoma of the mediastinum, characterized with a very rapid growth and poor prognoses.

Conflict of interest

We did not receive any financial support for this work and there is no conflict of interest.

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BOOK REVIEW/PRIKAZ KNJIGE

Odabrani metodi statističke analize za biomedicinska istraživanja

Autori: Jasmin Komić, Dubravko Bokonjić, Nemanja Rančić
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Štampa: Grafomark, Laktaši
Tiraž: 1 000
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Danas je, praktički, nemoguće zamisliti bavljenje naučnoistraživačkim radom bez dobrog poznavanja i razumevanja statistike. Ona, ne samo da pomaže u obradi i analizi rezultata sopstvenih istraživanja, već omogućava da se na pravi način razumeju i kritički analiziraju rezultati drugih autora objavljeni u naučnim i stručnim publikcijama.

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

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

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

Literatura

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.

Primeri referenci:

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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.

Detaljno uputstvo može se dobiti u redakciji ili na sajtu: www.vma.mod.gov.rs/vsp