Publishing Sector of the Society of Physicians of Vojvodina of the Medical Society of Serbia, Novi Sad, Vase Stajica 9

MEDICAL REVIEW

JOURNAL OF THE SOCIETY OF PHYSICIANS OF VOJVODINA OF THE MEDICAL SOCIETY OF SERBIA
THE FIRST ISSUE WAS PUBLISHED IN 1948

MEDICAL REVIEW is published bimonthly (six issues per year) with a circulation of 1.000 copies. The annual payment fee in 2018, for individuals from the territory of Serbia, is 3,000.00 dinars (the value-added tax included), 4,000.00 dinars for individuals from Serbia who are not members of the Society of Physicians of Vojvodina of the Medical Society of Serbia, 60 Euros for members outside the territory of Serbia, and 8,000.00 dinars (+ VAT) for institutions. The payment account is: 340-1861-70 or 115-13858-06, "Annual membership fee for Medical Review".

The manuscripts are submitted at: aseestant.ceon.rs/index.php/medpreg/. Editorial Office Address: Društvo lekara Vojvodine Srpskog lekarskog društva, 21000 Novi Sad, Vase Stajića 9, Tel. 021/521-096; 063/81 33 875, E-mail: dlv@sbb.rs; Website: www.dlv.org.rs

Printed by: Department for Joint Affairs of Provincial Authorities - Sector for Printing

Izdavačka delatnost Društva lekara Vojvodine Srpskog lekarskog društva, Novi Sad, Vase Stajića 9

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Štamparija: Uprava za zajedničke poslove pokrajinskih organa - Odsek za poslove štamparije

M E D I C A L R E V I E W
JOURNAL OF THE SOCIETY OF PHYSICIANS OF VOJVODINA OF THE MEDICAL SOCIETY OF SERBIA
Novi Sad Vase Stajića 9 Serbia

Med Pregl 2018; LXXI (9-10): 273-344. Novi Sad: September-October.

CONTENTS

M E D I C I N S K I P R E G L E D
ČASOPIS DRUŠTVA LEKARA VOJVODINE SRPSKOG LEKARSKOG DRUŠTVA
Novi Sad *Vase Stajića 9* *Srbija*

Med Pregl 2018; LXXI (9-10): 273-344. Novi Sad: septembar-oktobar.

SADRŽAJ

ORIGINAL STUDIES
ORIGINALNI NAUČNI RADOVI

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Original study
Originalni naučni rad
UDK 616.89-008.441.44-036.22(497.113)
https://doi.org/10.2298/MPNS1810277K

EPIDEMIOLOGICAL CHARACTERISTICS OF SUICIDE IN THE AUTONOMOUS PROVINCE OF VOJVODINA

EPIDEMIOLOŠKE KARAKTERISTIKE SAMOUBISTAVA U AUTONOMNOJ POKRAJINI VOJVODINI

Dragana KAČAVENDA BABOVIĆ[1], Predrag ĐURIĆ[2-4], Radomir BABOVIĆ[5], Tihomir DUGANDŽIJA[3,6], Jelena ĐEKIĆ MALBAŠA[3,4] and Smiljana RAJČEVIĆ[3,4]

Summary

Introduction. Suicide is defined as a conscious and deliberate taking of one's own life, or a self-destructive behavior with a fatal outcome. Every year, millions of people are affected by suicide or the feeling of grief. The aim of our research was to review the basic epidemiological characteristics of suicide in the Autonomous Province of Vojvodina, in order to assist in targeted prevention programs. **Material and Methods.** A retrospective, observational study was conducted. The data were analyzed in chronological order and in accordance with different demographic characteristics and topographic distribution. Basic statistical indicators were used as parameters: non-standardized, standardized and specific mortality rates. **Results.** During the observed period, from 1991 until the end of 2010, in the Autonomous Province of Vojvodina, the average annual non-standardized suicide rate was 27.9/100,000 inhabitants. The highest suicide rate was recorded in 1992 and 1993 (33.7/100,000 and 34.5/100,000, respectively) and in 1999 (31.5/100,000). The highest age-specific suicide rate was recorded in ≥ 80 year-old age group (120.5/100,000). The suicide rates were significantly higher among males, while the most common suicide method for both sexes was by hanging (69.9%). The highest average annual suicide rate was recorded among widowers (176.9/100,000) and widows (37.8/100,000). The lowest number of suicides was recorded in persons with higher level of education. **Conclusion**. Since in the Autonomous Province of Vojvodina persons at increased risk for suicide include males, the elderly population, persons with low education levels, and people who lost their partners, suicide prevention strategies should target these groups, including primary and secondary prevention measures.

Key words: Suicide; Epidemiology; Risk Factors; Demography; Social Class; Education; Age Factors; Cause of Death

Sažetak

Uvod. Samoubistvo se definiše kao svesno i namerno uništavanje sopstvenog života uz samodestruktivno ponašanje sa fatalnim ishodom. Svake godine milioni ljudi su pogođeni iskustvom samoubistva ili tugovanjem. Cilj ovog istraživanja je bio sagledavanje osnovnih epidemioloških karakteristika samoubistava u Autonomnoj Pokrajini Vojvodini, što bi doprinelo ciljanom usmerenju preventivnih programa. **Materijal i metode**. Sprovedena je deskriptivna epidemiološka studija. Podaci su analizirani hronološki, prema različitim demografskim karakteristikama i topografskoj distribuciji. Kao parametri korišćeni su osnovni statistički pokazatelji: nestandardizovane, standardizovane i specifične stope mortaliteta. **Rezultati**. U posmatranom periodu od 1991. do kraja 2010. godine u Autonomnoj Pokrajini Vojvodini prosečna godišnja nestandardizovana stopa samoubistava iznosila je 27,9/100.000 stanovnika. Najviše stope mortaliteta usled samoubistava zabeležene su 1992. i 1993. godine (33,7/100.000 i 34,5/100.000) i 1999. godine (31,5/100.000). Najviša uzrasno specifična stopa smrtnosti usled samoubistava zabeležena je u starosnoj grupi ≥ 80 godina (120,5/100,000). Stope smrtnosti usled samoubistva bile su značajno veće kod muškaraca, dok je najčešći metod samoubistva kod oba pola bio vešanjem (69,9%). Najveća prosečna godišnja stopa samoubistva zabeležena je kod udovaca (176,9/100.000) i udovica (37,8/100.000). Najmanji broj samoubistava registrovan je kod osoba sa višim stepenom obrazovanja. **Zaključak.** Kako su u Autonomnoj Pokrajini Vojvodini muškarci, starije osobe, osobe sa niskim nivoom obrazovanja i osobe koje su izgubile svog partnera imale povećan rizik od samoubistva, preventivni programi bi trebalo da budu usmereni na te grupe, uključujući mere primarne i sekundarne prevencije.

Ključne reči: samoubistvo; epidemiologija; faktori rizika; demografija; socijalni status; starost; uzrok smrti

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Acknowledgement
We would like to acknowledge and thank Josip Mihajlović, MBA for creating a topographic map for this paper.

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Abbreviations

APV – Autonomous Province of Vojvodina
WHO – World Health Organization
BiH – Bosnia and Herzegovina
PTSD – post-traumatic stress disorder

Introduction

The term suicide is used for a self-directed injurious behaviour with intent to die as a result of the behaviour [1]. Every year, more than 800,000 people die by suicide, while the number of attempted suicides is much greater. Globally, suicide is the second leading cause of death among people aged between 15–29 years, and in 2015 it accounted for 1.4% of all deaths in the world, making it the 17th leading cause of death [2].

The psychological and social impact of suicide on the family and society is immeasurable. On average, one suicide intimately affects at least six other persons. If it takes place at school or in the workplace, it affects hundreds of other people [3]. The American Centre for Disease Control and Prevention in Atlanta has assessed that annually the community costs related to suicide amount to about 56.9 billion dollars. On average, each suicide costs about 1.287,5 million dollars [4].

Europe has the highest suicide rates in the world. According to the latest World Health Organization (WHO) data, in 2015 the highest suicide rates (per 100,000 inhabitants) were recorded in Lithuania (32.7), Kazakhstan (27.5) and Belarus (22.8), while the lowest rates were recorded in Azerbaijan (3.3) and Albania (4.3). Serbia, with a suicide mortality rate of 17.0 per 100,000 inhabitants, was in the 12th place in Europe [5].

The study of Milicinski and Mrevlje from 1990, "Yugoslav suicide paradox" presented significant variations in suicide rates between different parts of former republics of Yugoslavia. Northern areas (Slovenia, Croatia, Vojvodina) showed a ten times higher suicide rates than the areas in the South (Kosovo, Macedonia) [6]. Serbia, one of the former republics of Yugoslavia, has different regional suicide rates; on average, the APV has 2 – 3 times higher suicide rate than Central Serbia, during the 20th century and at the beginning of the 21st century [7].

Suicide is a complex phenomenon affected by a large number of factors. Mental illnesses, especially affective disorders, are considered to be the most significant risk factors for serious suicide attempts and suicide [3, 8, 9]. It is estimated that their elimination would reduce the risk of serious attempts of suicide by up to 80% [9]. In almost all countries, with the exception of some countries in the East, committed suicides are more common among males. The risk factors that significantly increase the risk of suicide are more common in males: substance abuse, particularly of alcohol, as well as association of affective disorders with substance abuse. Men react more strongly to changes in socioeconomic conditions. They are more impulsive and more often choose more lethal methods when attempting suicide [10, 11]. Epidemiological data indicate that the risk of suicide attempt resulting in death is highest among the elderly almost everywhere in the world. Suicide mortality rates are highest among the elderly people, especially among men. Depression is a major risk factor for suicide among the elderly [12].

Suicide is a serious public health problem that may have lasting harmful consequences on individuals, their families and the entire community. Prevention programs should be developed at global, national and local levels, based on a multisectoral approach. The goal of suicide prevention is to reduce the factors that increase the risk and stimulate resistance factors [13].

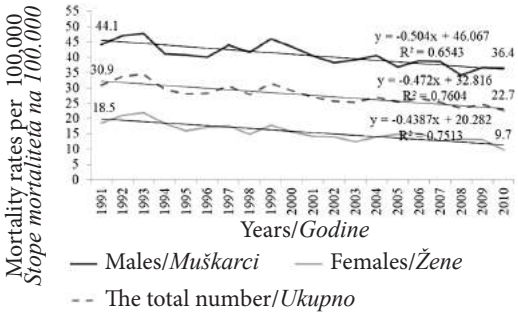
The objective of this paper is to review the basic epidemiological characteristics of suicide in the Autonomous Province of Vojvodina (APV), in order to assist in targeted prevention programs.

Material and Methods

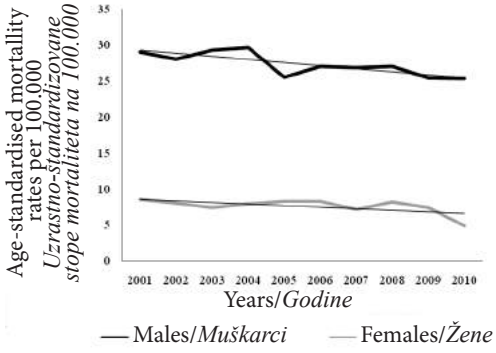
A descriptive epidemiological study was conducted with chronological and demographic analyses. Basic statistical indicators were used as parameters: non-standardized, standardized and specific mortality rates. The age standardized rates were calculated by using the direct standardization method based on the world standard population, and population of the APV (including age and sex) using the official data of the Statistical Office of the Republic of Serbia, based on census data and projections for a particular year. The number and rates of committed suicides were retrospectively reviewed by gender from 1991 to 2010, while data on age, methods of suicide, education level, and marital status were available for the period from 2001 to 2010. The overall non-standardized and gender-specific suicide rates were calculated based on the census data in the Republic of Serbia (1991, 2002) and projections for a particular year, while specific suicide rates in terms of age, marital status and municipalities were calculated based on the 2002 census in the Republic of Serbia. Chi-square test was used for comparison of different education levels and sex distribution of persons who committed suicides. We have used the data obtained from the Office for Vital Statistics of the Statistical Office of the Republic of Serbia on committed suicides during the period 1991–2010 in the APV.

Results

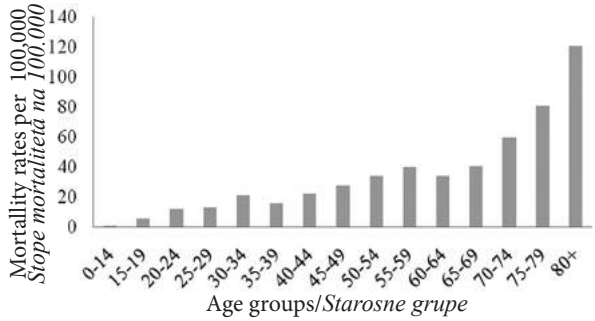
During the observed period, a total of 11,166 persons (7,940 males and 3,226 females) died of suicide. The average annual mortality rate for males (40.8/100,000) was by almost three times higher than in females (15.6/100,000). During the period from 1991 to the end of 2010, the annual suicide mortality rate in the APV ranged from 22.7 to 34.5 per 100,000 inhabitants, while the average annual non-standardized rate was 27.9/100,000. The high-



Graph 1. Overall and gender specific suicide rates in the APV, 1991–2010
Grafikon 1. *Opšte i rodno specifične stope samoubistava u Autonomnoj Pokrajini Vojvodini, 1991–2010*



Graph 2. Age- and sex-standardized suicide mortality rates in the APV, 2001–2010
Grafikon 2*. Uzrasno-standardizovane rodno specifične stope samoubistava u Autonomnoj Pokrajini Vojvodini, 2001–2010*



Graph 3. Age-specific suicide mortality rates in the APV, 2001–2010
Grafikon 3*. Uzrasno specifične stope mortaliteta samoubistava u Autonomnoj Pokrajini Vojvodini, 2001–2010*

est suicide rates were recorded in 1992 and 1993 (33.7/100,000 and 34.5/100,000, respectively) and in 1999 (31.5/100,000). The lowest suicide rates were recorded during the last three years of the study period (23.4/100,000, 24.6/100,000 and 22.7/100,000, respectively). There was a linear declining trend in the overall suicide rates and gender specific rates in the observed period **(Graph 1)**.

During the ten-year period (2001–2010), the highest standardized rate for males was recorded in 2004 (29.7/100,000), while the highest standardized rate for females was recorded in 2001 (8.6/100,000) **(Graph 2)**. The suicide rate increases with age. The highest age-specific suicide mortality rate was recorded in the ≥ 80 year olds (120.5/100,000) **(Graph 3)**.

In regard to the level of education and gender, there was a statistical difference between males and females for all levels of education. Except for those without any education ($p < 0.173$), much more males committed suicide ($p < 0.000$; $p < 0.010$), regardless of the level of education. After examining differences between individual education levels in relation to the total number of suicides for each level of education, the difference was statistically significant ($p < 0.001$). Most suicides were committed by persons with high education (1,770), followed by persons with elementary (1,522) and incomplete primary education (1,203), while the lowest number of suicides was recorded in persons with university education (155) and college (119) education **(Table 1)**.

The highest average annual suicide rate was recorded among widowers (176.9/100,000) and widows (37.8/100,000), while the lowest suicide rate was re-

Table 1. Number of deaths by suicide with gender and education level distribution in the APV, 2001–2010
Tabela 1. *Broj umrlih osoba usled samoubistva u odnosu na rodno specifičnu pripadnost i stepen školske spreme u Autonomnoj Pokrajini Vojvodini, 2001–2010*

Level of education/*Stepen školske spreme*	No education level *Bez škole*	Incomplete elementary school/*Nepotpuna osnovna škola*	Elementary School/*Osnovna škola*	High School *Srednja škola*	College *Viša škola*	University Degree *Fakultet*	Unknown *Nepoznato*
Total *Ukupno*	n = 238 4.7%	n = 1203 23.7%	n = 1522 30.0%	n = 1770 34.8%	n = 119 2.3%	n = 155 3.1%	n = 73 1.4%
Males *Muškarci*	108 2.9%	720 19.5%	1162 31.4%	1434 38.7%	98 2.7%	128 3.5%	48 1.3%
Females *Žene*	130 9.4%	483 34.9%	360 26.1%	336 24.3%	21 1.5%	27 2.0%	25 1.8%
χ^2 test	1.85	46.30	421.55	679.89	50.24	64.52	6.63
p	0.173	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.010

Legend**:** Chi-Square (DF) = 1, for all $\chi 2$ tests (Yates correction)
Legenda: Broj stepeni slobode (DF) = 1, za sve $\chi 2$ testove (Jejtsova korekcija)

Table 2. Suicide mortality rates per 100,000 inhabitants aged 15 years and over with gender and marital status distribution in the APV, 2001–2010
***Tabela 2**. Stope mortaliteta usled samoubistava na 100.000 stanovnika uzrasta 15 godina i starijih u odnosu na rodno specifičnu pripadnost i bračni status u Autonomnoj Pokrajini Vojvodini, 2001–2010*

	Unmarried *Neoženjen/Neudata*	Married *Oženjen/Udata*	Widower/Widow *Udovac/Udovica*	Divorced *Razveden/Razvedena*
Total/*Ukupno*	24.1	22.5	63.6	67.2
Males/*Muškarci*	34.8	35.9	176.9	118.6
Females/*Žene*	9.1	9.2	37.8	32.9

Table 3. Suicide methods and gender distribution in the APV, 2001–2010
***Tabela 3**. Distribucija načina izvršenja samoubistava po polu u Autonomnoj Pokrajini Vojvodini, 2001–2010*

Suicide methods *Način izvršenja suicida*	Males/*Muškarci* (n = 3 698) 100%	Females/*Žene* (n = 1382) 100%	Total/*Ukupno* (n = 5080) 100%
Hanging, strangulation, suffocation/*Vešanje, davljenje, gušenje*	2590 (70.1%)	963 (69.7%)	3553 (69.9%)
Drowning, submersion/*Utapanje, potapanje*	94 (2.5%)	94 (6.7%)	188 (3.7%)
Jumping from a high place/*Skok sa visine*	46 (1.2%)	25 (1.9%)	71 (1.4)
Jumping or lying before moving object *Skakanje ili leganje ispred predmeta u pokretu*	10 (0.3%)	3 (0.3%)	13 (0.3%)
Firearms, explosives/*Vatreno oružje, eksploziv*	581 (15.7%)	39 (2.8%)	620 (12.2%)
Sharp and blunt objects/*Oštri i tupi predmeti*	80 (2.2%)	24 (1.8%)	104 (2.0%)
Smoke, fire, flame and steam/*Dim, vatra, plamen i para*	7 (0.2%)	3 (0.2%)	10 (0.2%)
Motor vehicle crash/*Udar motornog vozila*	18 (0.5%)	6 (0.4%)	24 (0.5%)
Poisoning by solid or liquid substances *Trovanje čvrstim i tečnim supstancama*	157 (4.2%)	166 (12.0%)	323 (6.4%)
Poisoning by exposure to gases/*Trovanje gasovima*	11 (0.3%)	4 (0.3%)	15 (0.3%)
Other and unspecified means/*Druga i neoznačena sredstva*	105 (2.8%)	54 (3.9%)	159 (3.1)

corded among unmarried males (34.8/100,000) and females (9.1/100,000) **(Table 2)**.

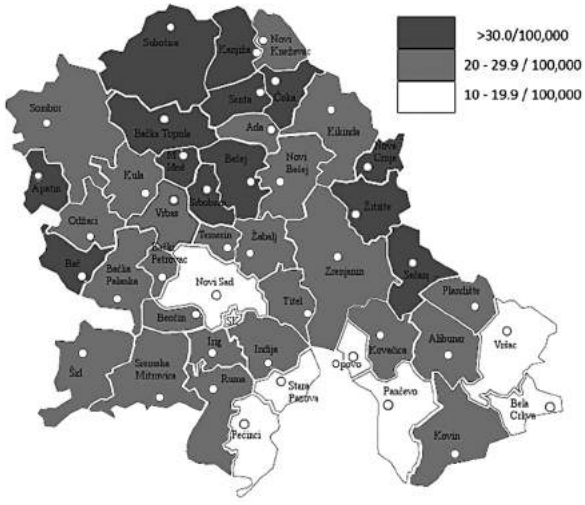


Figure 1. Non-standardized suicide rates per 100,000 inhabitants by municipalities of the APV, 2001–2010
***Slika 1.** Stope samoubistava na 100.000 stanovnika u opštinama, Autonomne Pokrajine Vojvodine, 2001-2010.*

The most frequent suicide methods among males were by hanging (70.1%) and firearms and explosives (15.7%), whereas among females the dominant suicide methods were by hanging (69.7%) and poisoning by solid or liquid substances (12%) **(Table 3)**.

In the APV municipalities, the average annual suicide rate per 100,000 inhabitants ranged from 14.4 (Novi Sad) to 41.8 (Kanjiža) in the ten-year period (2001–2010) **(Figure 1).**

Discussion

Our research shows that the suicide mortality rate in the APV was high during the last decade of the 20th century, while since 2000 it has shown a moderate decline. In the APV, during the observed period (from 1991 to the end of 2010), the average annual non-standardized suicide mortality rate was 27.9/100,000 inhabitants, while global average crude (non-standardized) suicide rates for both sexes ranged from 12.2/100,000 in 2002 to 11.2/100,000 in 2010. Non-standardized suicide rate for both sexes in Europe was 14.1/100,000 in 2015 [14].

According to the WHO data, in our neighboring countries in 2000, the crude suicide rates for both sexes ranged from 6.0/100,000 (Albania) and 9.8/100,000 (Bosnia and Herzegovina), to 21.1/100,000

(Croatia) and 32.4/100,000 (Hungary), and the same range but slightly lower rates in 2010 in those countries [5]. All of this indicates that the APV belongs to a group of countries with a high suicide rate.

The suicide mortality rate in low-income and middle-income countries is lower than in high-income countries (11.2 vs. 12.7 per 100,000 people) and 78% of deaths by suicide occur in low-income and middle-income countries [15].

The highest suicide mortality rates in the APV were recorded during three years (1992, 1993 and 1999). The mentioned period coincides with crisis, war and socio-economic disintegration in the former Yugoslav republics, as well as the NATO bombing of Serbia. This indicates a significant impact of the economic crisis, social instability, fear for one's own life and livelihood on the increase of suicides. These findings are in line with other studies that show rise in suicide rates during war and their decline after the war, linking this phenomenon with increased availability of firearms during the war and its reduction after the war. Also, increased alcohol consumption during the war was associated with higher suicide rates [16–18]. According to the data of Musić et al. [19], there were no differences in total suicide rates in BiH and Sarajevo in the pre-war and post-war period, but during the war data were unavailable, at the time when the highest suicide rates were recorded in the APV. This could be linked with a forced migration [20] of a large number of people from BiH to other countries during the mentioned period.

Several epidemiological studies are based on the analysis of correlation of the post-traumatic stress disorder (PTSD) and suicidal behavior, mainly on the sample of Vietnam veterans and displaced persons [21–23]. The PTSD is frequently comorbid with major depressive disorder, and in that case, the risk for suicidal behaviour is enhanced [22]. According to the Veterans Health Study, the prevalence of significant depressive symptoms among veterans was 31%, higher than among the general United States population [24]. The assessment and treatment of these comorbid conditions are likely to contribute to the reduction of suicide risk in this vulnerable population [22], but there is still an open question to what extent other factors may be associated with high suicide rates, such as divorce or separation, migration across state lines, cultural and economic factors or exposure to mass media [23].

A study conducted in 4 centres (Croatia, Serbia, Germany, and the United Kingdom) ten years after the war-related trauma in a sample from the former Yugoslavia, found that older age, more traumatic war-events, lower education, and living in post-conflict countries were associated with higher rates of current PTSD [25].

A decrease of suicide rates after the war may be linked to effective psychiatric and psychology therapy, and better socioeconomic conditions in our country. We can see that in 1999 there was an increase of suicide rates and the country was again facing a socio-economic crisis. On the other hand, causes may be in reduced access to firearms, and the displacement of the population from the territory of the former Yugoslavia.

Similar to the results of countries worldwide [5, 26] the suicide mortality rate in APV is significantly higher among males. The ratio of the mortality rate (from 1991 to 2010) between males and females was 3 : 1, and it was generally stable over the time. The difference in the male-to-female ratio shows that females are affected by different cultural and racial characteristics in regard to the individual populations [18, 26].

Suicide rates increase with age, which is in line with the global data of the WHO member countries [2]. We found that the highest specific suicide rates were recorded in the ≥ 80 year-old age group, and in the 75–79 year-old age group. According to the data from 2006, in the Republic of Serbia, almost every second deceased person who died by suicide was older than 60 (48.7%) and every third person was older than 70 years (33%) [27]. In addition to this trend, along with the increase in suicide rates from younger to older age, contrary to the results of our research, in some countries there is a higher incidence of suicide among young people in the 15–24 year age group [18]. Older people lose economic security, spouse, children leave the family, and physical and mental illness are more common at this age. Functional disability was shown to be associated with suicidal behaviour in older adults [28]. The low living standard of this group of inhabitants in our country with difficult access to healthcare, especially in rural areas where most of the elderly live, may be linked to high suicide rates.

Similar to the reports of many countries [29], the most common method of suicide among males in the APV was hanging (70.1%) and use of firearms (15.7%), and among females hanging (69.7%) and poisoning by solid and liquid substances (12.0%). Until twenty years ago, suicides by firearms were comparatively rare. However, after the start of the socio-economic crisis in Yugoslavia and the availability of large amounts of weapons among the population, this method of suicide has become more dominant. According to the official data, the number of suicides committed by use of firearms has increased by five times in mid-nineties and at the end of nineties compared to the period of the fifties of the last century [30], as described by other authors [16, 17, 19, 31]. During the war and post-war years, the number of suicides by firearms has significantly increased in neighboring Croatia, particularly among males. In 1985, the incidence of suicides committed by firearms in the total number of suicides was 7.2%, while in 1992 and 1995 firearms accounted for about 26%. This suicide method has declined in recent years [32]. The lowest number of suicides in the APV occurred among persons with college or university degrees, while the highest number of suicides occurred among persons with high school education, followed by persons with elementary and incomplete primary education. A lower level of education is associated with lower socioeconomic status, unemployment, alcohol abuse (especially in men), less availability and use of healthcare. The increase in unemployment is associated with poverty, higher inci-

dence of depression and it poses a higher risk of suicide. This is confirmed by the first European comparative study on socioeconomic inequalities in suicide, which includes the data from several European countries (Austria, Belgium, Denmark, Finland, Norway, Spain, Germany and Switzerland). A low level of education was a risk factor for both genders [33]. The highest rate of mortality due to suicide in the observed ten-year period was recorded among widowers and widows, and the lowest among unmarried males and females. It supports the fact that the loss of a spouse causes high psychological stress and consequently an increase in the suicide rates [10, 34].

In the municipalities of the APV, the average annual suicide rate per 100,000 inhabitants in the ten-year period (2001–2010) ranged from 14.4 (Novi Sad) to 41.8 (Kanjiža). There was no municipality in the APV with suicide mortality rate below 10 per 100,000 inhabitants, while 13 (app 30%) municipalities had suicide rates above 30 per 100,000 inhabitants. This distribution and the highest suicide rates in municipalities in the north part of the APV may be explained by the fact that in this territory of the Province a large part of the population consists of ethnic Hungarians, with the highest suicide rates in Serbia around the 2002 census [35].

The WHO highlighted suicide reduction as one of prime health policy goals back in 1984. Following this, and aiming to support suicide prevention, an initiative of the European Network for Suicide Prevention was established in 2000 [36]. After that, many countries have launched national action plans for suicide prevention, such as United Kingdom, Netherlands, Finland, Scotland, Northern Ireland, Austria, Switzerland, and they have achieved positive results [37]. However, a large number of countries have not entered the targeted suicide prevention process yet.

In 2013, the Mental Health Action Plan was adopted by the WHO, and one of its main objectives is to reduce suicide rate by 10% by 2020 (Preventing suicide: a global imperative). At the same time, the quality of statistical data related to suicide was also emphasized, because in the low and middle-income countries, there is still no good registration of vital statistics [15].

Despite high suicide rates, Serbia (and therefore Vojvodina), belongs to a group of countries that have not yet defined a national strategy for suicide prevention. Researches like this, examining epidemiological, demographic and socio-economic characteristics of suicide, should identify vulnerable groups that require special attention, as well as areas in which preventive actions need to be taken.

Conclusion

Since in the Autonomous Province of Vojvodina persons at increased risk for suicide include males, the elderly, persons with low level of education, and people who lost their partners, suicide prevention strategies should target these groups, including primary and secondary prevention measures.

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Rad je primljen 7. X 2017.
Recenziran 1. II 2018.
Prihvaćen za štampu 9. V 2018.
BIBLID.0025-8105:(2018):LXXI:9-10:277-283.

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Original study
Originalni naučni rad
UDK 616.97/.98-053.6:613.96
https://doi.org/10.2298/MPNS1810284R

AWARENESS, KNOWLEDGE AND BEHAVIOR OF HIGHSCHOOL STUDENTS CONCERNING SEXUALLY TRANSMITTED INFECTIONS

SVEST, ZNANJE I PONAŠANJE SREDNJOŠKOLACA U VEZI SA POLNO PRENOSIVIM INFEKCIJAMA

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Summary

Introduction. This paper presents the results of a research conducted among senior high school students in northern Kosovo and Metohija on their knowledge about sexually transmitted infections, emphasizing their awareness and sources of information, as well as their sexual behavior and use of contraceptives. **Material and Methods.** The survey of senior high school students was done using a previously prepared anonymous questionnaire which was followed by statistical processing of fully completed questionnaires. **Results.** The research included students aged 17 to 19; 63% were females and 37% males; 35.8% were sexually active. The correct definition of sexually transmitted infections was identified by 49%. School was the source of information on these infections for 45.6% of students and biology class for 45.7%. There were 40.6% sexually active male respondents and 33.0% of female (on average, 18 years old). Of the surveyed students of both sexes, 40.6% became sexually active at the age of 17. Condom use was reported by 49% of respondents of both sexes, whereas 50.8% of sexually active students always used condoms. There were 38.7% (38.9% girls, 38.4% boys) of students who used condoms for protection against sexually transmitted infections, and 58.1% of them personally decided whether to use them. **Conclusion.** The majority of our respondents were able to identify the correct definition of sexually transmitted infections, and they most often heard of the human immunodeficiency virus/acquired immune deficiency syndrome. School was their most common source of information, biology class, and a considerable number were informed about this issue on the Internet. The majority of sexually active girls did not use any contraceptives. School curricula and parent-child relationships should have a greater impact on the youth's awareness of reproductive health.

Key words: Sexually Transmitted Diseases; Adolescent; Health Knowledge, Attitudes, Practice; Schools; Contraception Behavior; Surveys and Questionnaires

Sažetak

Uvod. Rad prikazuje rezultate anketiranja učenika završnih razreda srednjih škola na severnom Kosovu i Metohiji o polno prenosivim infekcijama, njihovoj obaveštenosti i izvorima informisanja, njihovoj seksualnoj aktivnosti i korišćenju kontraceptivnih sredstava. **Materijal i metode.** Anketiranje učenika završnih razreda srednjih škola na severu Kosova i Metohije prethodno pripremljenim anonimnim upitnikom. Statistički su obrađeni kompletno popunjeni upitnici. **Rezultati.** Istraživanjem su obuhvaćeni učenici od 17 do 19 godina, 63% ženskog i 37% muškog pola; 35,8% ispitanika je seksualno aktivno. Tačnu definiciju polno prenosivih infekcija prepoznalo je 49% anketiranih učenika. Škola je izvor informisanosti o polno prenosivim infekcijama za 45,6% učenika, a za 45,7% anketiranih časovi biologije u školi. Seksualno je aktivno 40,6% učenika i 33% učenica; oni imaju prosečno 18 godina. Polno aktivno pre sedamnaeste godine postalo je 40,6% anketiranih učenika. Većina seksualno aktivnih ispitanika, 49%, oba pola koristi kondom kao kontraceptivno sredstvo. Uvek koristi kondom 50,8% polno aktivnih učenika. Seksualno aktivni ispitanici koriste kondom zbog zaštite od polno prenosivih infekcija − 38,7% (38,9% učenica i 38,4% učenika), a 58,1% lično odlučuje o njihovoj primeni. **Zaključak.** Većina naših ispitanika je prepoznala tačnu definiciju polno prenosivih infekcija, a najčešće su čuli za infekciju virusom humane imunodeficijencije/sindrom stečene imunodeficijencije. Ispitanici su najčešće bili obavešteni u školi, na časovima biologije, a znatan broj je informisan o ovom problemu putem interneta. Školski sadržaji i razgovor sa roditeljima bi trebalo značajnije da utiču na svest mladih o reproduktivnom zdravlju.

Ključne reči: polno prenosive infekcije; adolescent; znanje o zdravlju, stavovi, praksa; škole; kontraceptivno ponašanje; istraživanja i upitnici

Introduction

Adolescence is the period of transition from childhood to adulthood and it is a very important part of life of every individual. The World Health Organization defined adolescence as the period between 10 and 19 years of age [1, 2]. Recently, adolescent sexuality has been considered a risky behavior with the following consequences: reproductive health disorders, unwanted pregnancies, and sexually transmitted infections (STIs) that may lead to epidemics [3]. STIs are becoming a major public health issue. The incidence of STIs is on the rise in the whole world, especially among adolescents whose

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Abbreviations

WHO – World Health Organization
STIs – sexually transmitted infections
HIV – human immunodeficiency virus
AIDS – acquired immunodeficiency syndrome
IUD – intrauterine device

sexual behavior is changing [1, 3]. The increase of STIs is followed by an increase of complications. The most dangerous complications for women are sterility, ectopic pregnancies, development of neoplasms, and for fetuses - intrauterine and perinatal mortality, infections and malformations [4]. A significant factor in the prevention of STIs and their complications is a higher level of knowledge about them, their characteristics and precautions.

The aims of the study were to analyze the knowledge of senior high school students from North Kosovo about STIs, sources of their information, measures of preventions, methods of contraception, as well as their sexual activity and their first sexual experience.

Material and Methods

During a four-month period, October 2014 - February 2015, a survey was performed among high school students aged 17 to 19 years. The survey was conducted in the cities of North Kosovo: North Mitrovica, Zvečan and Leposavić. The following high schools were involved in the study: Medical High School, High School of Economics and Trade, High School of Mechanical Engineering, Gymnasium, High School of Vučitrn (temporarily relocated to Kosovska Mitrovica), Zvečan High School Center (laboratory technicians and sanitary-ecological technicians) and Leposavić High School Center (economics and law and Gymnasium).

Our survey was approved by our institutions (project: "Sexually transmitted infections – knowledge of secondary school students in northern Kosovo and Metohija") and by the Ministry of Education, Science and Technological Development of the Republic of Serbia. We also provided a consent from the head of school administration in northern Kosovo and Metohija as well as a consent from all principals of all schools and parent school councils. All students that were included in the survey had to sign an informed consent for an anonymous questionnaire. In agreement with all the institutions, the confidentiality of research results was guaranteed and the results can be used for study purposes only. The survey was performed during a regular school day.

The anonymous questionnaire was previously prepared. At the beginning of the survey, the examinees were informed that the questionnaire was anonymous, how to fill it in and what the goals of the research were. The first part of questionnaire referred to the basic information (place of residence, age, gender, high school, place of education, grades in high school, level of parents' education, parental marital status). The second part of questionnaire was intended to determine the actual knowledge of teenagers about STIs. It contained questions about STIs, their definitions and measures of prevention. In this part the examinees were supposed to mention STIs that they have heard of. There were questions about sources of information about STIs, their sexual activity (if they were sexually active), first sexual experience, knowledge about contraceptives and their usage, as well as awareness of risky sexual behavior. Most of the questions were multiple choice questions and examinees were supposed to circle only one answer per question. At the end of the questionnaire, students had the opportunity to ask questions and give suggestions regarding this issue. After collecting completed questionnaires, the survey examiners responded to the examinees' questions.

Statistical analysis was performed using the Statistical package for social sciences (SPSS software package, version 18.0; SPSS Inc., Chicago, IL, USA). Descriptive data were expressed as mean values ± standard deviation (SD) or percentage for coefficient of variation. Nonparametric data were tested using Mann-Whitney test. Categorical variables were compared using Chi-square test (2 x 2). P value less than 0.05 was considered statistically significant.

The univariate logistic regression was done using 12 independent variables (gender, age, maternal education, paternal education, marital status of parents, grades, answers to questions: have you ever heard of STIs at school, have you ever read about STIs on the Internet, have you ever heard of STIs on television, have you ever heard of STIs from your parents, did you have sexual intercourse, and so on).

Results

The study included 433 students from 17 to 19 years of age. The average age was 17.8 ± 0.5 years. On average, the respondents of both sexes were 17.8 years old. Two thirds of examinees were females (63%) and 37% were males. The majority of examinees were 18 years old (68.1%), every fourth (25.4%) was 17 years old, and about 6.5% of examinees were 19 years old. Three quarters of female (75.1%) and more than half of male students (56.2%) were 18 years old. The majority of female students attended the Medical High School in Kosovska Mitrovica (79.6%). There was a statistically significant difference ($p < 0,001$) between the number of female and male students.

Most respondents' mothers had a secondary level of education (70.4%). The education of mothers and gender of examinees showed a statistically significant difference ($p < 0.05$). The highest percentage of examinees' fathers also had secondary education (78.3%). The parents' level of education and gender of examinees showed a statistically significant difference ($p < 0.01$) as did the level of paternal ($p < 0.01$) and maternal knowledge about STIs ($p = 0.001$). Most parents were married (84.5%), while parents of every sixth female student were divorced – 15.5%. There was a statistically significant difference between gender of examinees and the number of divorced parents ($p < 0.01$).

Most students of both genders have heard about STIs (94.9%), 96.3% of female and 92.5% of male examinees. Every fifth student (20.3%) thought that

Anonymous questionnaire – SEXUALLY TRANSMITTED INFECTIONS

Short introductory notice: Please read all the questions carefully and answer them sincerely and to the best of your knowledge, since the survey is anonymous and the data concern rather serious sexually transmitted infections (STIs). World literature data are warning about the increase of these infections among youth worldwide, so we would hereby like to examine the following - your understanding of the issue, your awareness**,** having had and/or been treated for these infections, as well as about taking measures for preventing STIs.

Note before filling out the questionnaire: students who are not sexually active do not respond to questions in section 5 to 18**!**

I GENERAL INFORMATION:

Initials (anonymous poll):
Gender: M F
Age: Place of residence:
School you attend:
Education level of your mother: Basic; Secondary; College; Bachelor Degree or higher
Education level of your father: Basic; Secondary; College; Bachelor Degree or higher
Parental marital status: married; divorced; single parents; you have only a mother; you have only a father; your parents are deceased

II SEXUALLY TRANSMITTED INFECTIONS (STIs)

1. Do you know what sexually transmitted infections are:
a. Yes
b. No

2. If your answer to the previous question is affirmative, please circle the answer you think defines STIs:
a) Infections which are transmitted only through sexual contact
b) Infections which are transmitted by any kind of physical contact between two people
c) Infections most commonly transmitted through sexual contact, but it is not the only route of transmission
d) Infections exclusively transmitted by sexual intercourse without any protection

3. Specify the STIs you have heard about: _____

4. Have you heard of any of the listed STIs?

a. Human immunodeficiency virus (HIV) infection:	Yes	No
b. Acquired Immune Deficiency Syndrome (AIDS):	Yes	No
c. Syphilis:	Yes	No
d. Gonorrhea:	Yes	No
e. Condyloma (genital warts):	Yes	No
f. Hepatitis B, C:	Yes	No
g. Chlamydia infection:	Yes	No
h. Genital herpes:	Yes	No
i. Trichomonas vaginalis:	Yes	No

5. Are the following diseases also STIs?

a. HIV infection:	Yes	No
b. AIDS:	Yes	No
c. Brucellosis:	Yes	No
d. Syphilis:	Yes	No
e. Scabies:	Yes	No
f. Pediculosis pubis:	Yes	No
g. Eczema:	Yes	No
h. Gonorrhea:	Yes	No
i. Condyloma (genital warts):	Yes	No
j. Influenza:	Yes	No
k. Chickenpox:	Yes	No
l. Lyme disease:	Yes	No
m. Hepatitis B, C:	Yes	No
n. Genital herpes:	Yes	No
o. Trichomonas vaginalis:	Yes	No

6. Where have you first heard about STIs?
a. At school
b. From a friend
c. From a youth counselor
d. From books
e. On television
f. From newspapers
g. From my parents

7. At school you first heard about STIs:
a. At biology class
b. Talking to my class teacher
c. Events and lectures on this topic

8. You got information on STDs:
a. On the Internet
b. On TV
c. From newspapers

9. Have you heard about events related to this medical problem? a. Yes b. No

10. Do you know the International Day Against some of the STIs? a. Yes b. No

11. Do you know any preventive measures for these infections? a. Yes b. No

12. Have you heard or do you know something about contraception? a. Yes b. No

13. Do contraceptive measures prevent pregnancy? a. Yes b. No

14. Do contraceptives prevent transmission of STIs? a. Yes b. No

15. Indicate some contraceptives/methods: _____

16. Do contraceptives/methods include?
a. Condoms/Preservatives: Yes No
b. Contraceptive pills: Yes No
c. Coils: Yes No
d. Spermicides: Yes No
e. Coitus interruptus: Yes No
f. Others (specify)_____

III SEXUALLY TRANSMITTED INFECTIONS AND YOUR SEXUAL ACTIVITY

1. Have you ever been examined for some STIs? a. Yes b. No

2. Have you been examined before for the following reasons?
a. Syphilis: Yes No
b. Gonorrhea: Yes No
c. HIV: Yes No
d. Genital herpes: Yes No
e. Condyloma (genital warts): Yes No
f. Trichomonas vaginalis: Yes No

3. Have you ever had?
a. Genital redness: a. Yes b. No
b. Pain, burning sensation: a. Yes b. No
c. Changes of the urinary color due to genital inflammation: Yes No
d. Enlarged lymph nodes in the groove: Yes No

4. Are you sexually active? Yes No

5. How old were you when you had your first sexual intercourse?
a. > 12
b. 13
c. 14
d. 15
e. 16
f. 17
g. 18
h. 19

6. Your first sexual intercourse was:
a. Voluntary: Yes No
b. Due to the insistence of my current boyfriend/girlfriend: Yes No
c. Under the influence of alcohol: Yes No
d. Under the influence of drugs: Yes No
e. At a party: Yes No

7. What do you use when having sex?
a. A condom/preservative: Yes No
b. Contraceptive pills: Yes No

c. Coitus interruptus: Yes No
d. A coil: Yes No
e. Spermicides: Yes No
f. Morning after pill: Yes No
g. Other (specify) _____

8. How often do you use the aforementioned methods?
a. Regularly: Yes No
b. Irregularly: Yes No
c. Never: Yes No
d. Never, because you have a regular partner (trust): Yes No

9. What are your reasons for using aforementioned contraceptive methods?
a. Protection from STIs: Yes No
b. Pregnancy protection: Yes No

10. Who makes the decision whether to use contraceptives?
a. You personally make the decision: Yes No
b. You let your partner to make the decision: Yes No
c. You have a steady partner who you trust: Yes No

11. Do you have a steady sexual partner?
a. Yes
b. No

12. You have no steady sexual partner and you change partners:
a. Occasionally: Yes No
b. Frequently: Yes No

13. Do you use contraception when engaging in sexual intercourse with a non-steady partner?
a. Yes No
b. Yes No

14. Do you engage in sexual intercourse with a stranger or a casual acquaintance?
a. Yes No
b. Yes No

15. If your answer to the previous question is affirmative, do you use contraceptives when engaging in sexual intercourse with a stranger or an acquaintance?
a. Yes No
b. Yes No

16. Have you engaged in sexual intercourse in short-term relationships (shorter than 7 days)?
a. Yes No
b. Yes No

17. Have you had a sexual intercourse with a person you have only met (a one-night stand)?
a. Yes No
b. Yes No

18. Do you think there is a risky sexual behavior?
a. Yes No
b. Yes No

19. If your answer to the previous question is affirmative, can you define such a behavior?
a. Yes No
b. Yes No

20. If your answer to the previous question is affirmative, is risky sexual behavior related to the following?
a. Changing sexual partners: Yes No
b. Sexually transmitted infections: Yes No
c. Sexual intercourse without any protection: Yes No
d. Sexual intercourse under the influence of alcohol: Yes No
e. Sexual intercourse under the influence of drugs: Yes No
f. Sexual relationship outside marriage: Yes No
g. Kissing: Yes No
h. Insufficient knowledge about STIs: Yes No
i. Other (please specify)_____

21. Do you have any questions about the above issues/questions?
If you have a question, please feel free to ask!_____

22. Your suggestions on this issue_____

Table 1. Students' distribution regarding the definition of STIs and gender
Tabela 1. *Distribucija učenika prema definiciji polno prenosivih infekcija (PPI) i polu*

STIs definition *Definicija PPI*	Total *Ukupno*		Schoolgirls *Učenice*		Boys *Učenici*	
	No/*Br.*	%	No/*Br.*	%	No/*Br.*	%
Infections transmitted only through sexual contact *Infekcije koje se prenose samo polnim kontaktom*	88	20,3	55	*%*	33	7,6
Infections which are transmitted by any kind of physical contact between two people/*Infekcije koje se prenose bilo kojim fizičkim kontaktom između dve osobe*	66	15,2	41	9,5	25	5,8
Infections most commonly transmitted through sexual contact, but it is not the only route of transmission/*Infekcije koje se najčešće prenose polnim kontaktom, ali to nije jedini način prenošenja infekcije*	212	49	141	32,6	71	16,4
Infections exclusively transmitted by sexual intercourse without any protection/*Infekcije koje se prenose samo polnim odnosom bez zaštite*	67	15,5	36	8,3	31	7,2
Total/*Ukupno*	433	100,0	273	63,0	160	37,0

STIs are infections that can only be transmitted during sexual contact (12.7% of girls and 7.6% of boys) **(Table 1)**. The exact definition of STIs was recognized by two thirds of students that lived in Kosovska Mitrovica (49%). There was a statistically significant difference between respondents who recognized the exact definition of STIs and their place of living ($p < 0.01$).

Students had to fill in blank questions and list STIs they knew: 42% of examinees knew only one STI (human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) and syphilis; 25.6% of students listed two STIs (AIDS and syphilis or syphilis and gonorrhea), whereas 11.32% of respondents have not heard of any STIs. Of all the students, 82.4% have heard of HIV/AIDS **(Table 2)**. The awareness of students of HIV/AIDS by the place of education has shown a statistically significant difference ($p = 0,001$). Of all the students, 83.9% of female and 80% of male students were aware of HIV/AIDS. There were 80.6% sexually active examinees: 84.4% of girls and 75.4% of boys. Only 40.9% of examinees have heard of syphilis. The analysis of students who have and who have not heard of syphilis showed a statistically significant difference ($p = 0.004$). The awareness of respondents of gonorrhea was very low. Only 15.9% of examinees have heard about this STI, 13.5% of them were sexually active, 17.8% of girls and 7.7% of boys. Just a few students have heard about genital warts (6.5%), 8.4% of sexually active examinees (10% of girls and 6.2% of boys). The awareness of genital warts according to sexual activity has not showed a statistically significant difference ($p = 0.225$). A few students mentioned chlamydia, herpes virus infection, pubic lice, hepatitis B and C (6.7%). None of the examinees have heard

Table 2. Students' distribution regarding their knowledge of STIs and gender
Tabela 2. *Distribucija učenika prema znanju o PPI i polu*

	Total/*Ukupno*	Schoolgirls/*Učenice*	Boys/*Učenici*
Have you heard of HIV/AIDS?/*Da li ste čuli za HIV/AIDS?*	No %/*Br. %*	No %/*Br. %*	No %/*Br. %*
Yes/*Da*	357 (82,4)	229 (83,9)	128 (80,0)
No/*Ne*	76 (17,6)	44 (16,1)	32 (20,0)
Have you heard of syphilis?/*Da li ste čuli za sifilis?*			
Yes/*Da*	177 (40,9)	126 (46,2)	51 (31,9)
No/*Ne*	256 (59,1)	147 (53,8)	109 (68,1)
Have you heard of gonorrhea?/*Da li ste čuli za gonoreju?*			
Yes/*Da*	69 (15,9)	42 (15,4)	27 (16,9)
No/*Ne*	364 (84,1)	231 (84,6)	133(83,1)
Have you heard of HPV infection? *Da li ste čuli za HPV infekciju?*			
Yes/*Da*	28 (6,5)	21 (7,7)	7 (4,4)
No/*Ne*	405 (93,5)	252 (92,3)	153 (95,6)
Have you heard of chlamydia infection? *Da li ste čuli za infekciju hlamidijom?*			
Yes/*Da*	29 (6,7)	19 (7,0)	10 (6,3)
No/*Ne*	404 (93,3)	254 (93,0)	150 (93,7)

Table 3. Sources of information about STIs and gender distribution
Tabela 3. *Izvori informisanosti o PPI i pol ispitanika*

Gender/*Pol*	School/*Škola*		Internet/*Internet*		TV/*TV*		Parents/*Roditelji*	
	No/*Br.*	%	No/*Br.*	%	No/*Br.*	%	No/*Br.*	%
Female/*Učenice*	124	45,4	80	29,3	23	8,4	23	9,2
Male/*Učenici*	74	46,3	32	20,0	15	4,7	8	4,7
Total/*Ukupno*	198	45,7	112	25,8	38	8,7	31	7,1

Table 4. Students' distribution regarding sexual activity, age at first sexual intercourse and gender
Tabela 4. *Distribucija učenika prema seksualnoj aktivnosti, godinama prvog seksualnog odnosa i polu*

Sexual activity (age at first sexual intercourse) *Seksualna aktivnost (starost prilikom prvog seksualnog odnosa)*	Total/*Ukupno*		Schoolgirls/*Učenice*		Schoolboys/*Učenici*	
	No/*Br.*	%	No/*Br.*	%	No/*Br.*	%
17	42	27,1	19	21,1	23	35,4
18	103	66,5	68	75,6	35	53,8
19	10	6,4	3	3,3	7	10,8
Total number of sexually active/*Ukupna seksualna aktivnost*	155	35,8	90	33,0	65	40,6

about Trichomonas vaginalis infection. A small number of respondents have listed infections that are not sexually transmitted (measles, meningitis, some allergies, Candida infection).

The univariate logistic regression analysis has been done to evaluate the impact of different factors on the probability to define STIs correctly. This analysis showed that three factors impacted the probability: age of respondents (correlation 3.79, 95% CI [1:59 9.07] p = 0.003), level of mothers' knowledge (correlation 2.47, 95% CI [1.07, 5.72], p = 0,035) and answers of respondents to the question "Have you ever heard about STIs at school?" (correlation 3,54, 95% CI [1,17,10,78], p = 0,026). These three factors were examined using multivariate logistic regression analysis. The results showed that the age of respondents had the strongest impact on defining STIs correctly (correlation 2,89, 95% CI [1,20, 6,94], p = 0,018), showing that the knowledge about STIs increased 2,8 times each year.

There was a statistically significant difference between sexually active students who learned about STIs at school (36.8%) and those who were not sexually active and also heard about STIs at school (63.2%) (p = 0.005). A quarter of our examinees have found data about STIs on the Internet (25.8%), every third girl (29.3%) and every fifth boy (20%) **(Table 3)**. There was a statistically significant difference between the gender and Internet as a source of information (p = 0.033). TV was the source of information for a small part of our examinees (8.7%), 4.7% of male and 8.4% of female students. The lowest percentage of our examinees has been informed about STIs by talking to their parents (7.1%), 9.2% of girls and 4.7% of boys.

Every third respondent was sexually active (35.8%), 40.6% of boys and 33.0% of girls **(Table 4)**. There was a statistically significant difference between sexual activity of our respondents and place of living (p = 0.034). The highest percentage of sexually active examinees was attending a high school in Kosovska Mitrovica (78.1%). The frequency of sexual activity was variable from school to school and it showed a statistically significant difference (p < 0.000). The average age of sexually active respondents was 18 years. The majority of examinees became sexually active at the age of 17 years (40.6%), every third examinee at the age of 16 years (35.5%) and 12.3 % around the age of 15. The sexual activity started around the age of 13.5 and 18 years in both genders, more frequently in males. There was a statistically significant difference between the respondents that had their first sexual intercourse (sexarche) at the age of 17 years and those who changed sex partners (p = 0.031). The same was established between students with sexarche at 16 years of age and changed their sex partners (p = 0,000) and students who changed their sex partners and had sexarche when they were younger than 15 years of age (p = 0.000). Most students had their first sexual experience under the influence of alcohol (82.5%), psychoactive substances at the party (14.8%) or because of the pressure from their girlfriend/boyfriend (2.6%).

Table 5. Measures for preventing STIs
Tabela 5. *Mere prevencije PPI*

Measures for preventing STIs/*Mere prevencije PPI*	%
Informed/*Informisani*	31,9
Not informed/*Neinformisani*	68,1
Total/*Ukupno*	100,0

Of all the examinees, 31.9% were informed about measures for preventing STIs, and 68.1% were not **(Table 5)**. A statistically significant difference (p = 0.001) was established between the place of education and knowledge about measures for preventing STIs. Condoms were used by 51% of sexually active boys and 49% of girls. More than a half of sexually active examinees (51%) used condoms regularly (44.5% of females and 21.3% of males) and 49% used them irregularly. Contraceptive pills were used by 24.5% of sexually active respondents, 20.6% of females and 3.2% of males said that their partners were using them. Of all the examinees, 5.9% of female examinees have heard about contraceptive intrauterine device (IUD) or coil, equally by sexually active and inactive students. They were students of a secondary medical school. None of the examinees named a spermicide or a morning-after pill as a contraceptive method. Our respondents were asked about the reason for using contraceptive devices/methods: 14.8% said they were for preventing STIs/pregnancy and 13.9% said they were for STIs prevention. Just a few respondents (1.2%) used contraceptive methods for avoiding unwanted pregnancy.

About 58.1% of respondents have decided to use contraception on their own (61.1% of girls and 53.8% of boys). Since 30% of respondents, more boys (40%) than girls (35.6%), have a regular partner, it did not matter who made the decision to use contraception. Every other examinee had a regular sex partner (46.5%), 47% of boys and 45.6% of girls. Every fifth respondent (21.3%) changed sex partners and those were more often boys (23.1%) than girls (20%). Most sexually active respondents, equal percentage of both genders (60%) used protection with an irregular partner, but 40% did not. Over 20% of sexually active students (23.2%) visited a doctor due to some of these symptoms: genital redness - 14.2%, urinary color changes - 8.4% or genital secretions - 10.3%. There was a statistically significant difference for symptoms like genital redness (p = 0.000) and genital secretions (p = 0.000), as well as urinary color changes (p = 0.000). Our results indicated that there was a statistically significant difference among students that became sexually active at 18 years of age and had urinary color changes (p = 0,039). Genital warts had been earlier reported by 12.3% of sexually active respondents, with a statistically significant difference (p = 0.000). A statistically significant difference was also present in sexually active students with genital redness who changed their sex partners (p = 0.009), as well as in students with genital warts (p = 0.000). The results showed that there was a statistically significant difference in respondents that became sexually active at the age of 17 and had genital redness (p = 0.001) and genital warts earlier (p = 0.031). The same goes for the examinees that had first sex when they were 16 years old and had genital redness (p = 0.000), genital secretions (p = 0.007) and urinary color changes (p = 0.000). A statistically significant difference was found in students who had sexarche when they were younger than 15 years and had genital warts earlier (p = 0.000).

About 35.6% of boys and 30% of girls considered that risky behavior was related to STIs. More than a half of the examinees (67.9%) did not agree about it, 70% of boys and 64.4% of girls. Similar results were obtained from sexually active respondents. Almost every third sexually active examinee (27.7%) thought that there was risky sexual behavior, but most of them (72.3%) denied it. We asked those who thought that there was risky sexual behavior to define it, and every third examinee did it successfully (29.7%), with a statistically significant difference between genders - 36.7% of females and 20% of males. They thought that this kind of behavior was connected to change of sexual partners, unprotected sex or having sex with strangers and without protection.

Discussion

The average age of students of both sexes in this study was 17.8 years. More than two thirds of examinees (63%) were females and 37% were males. In a similar survey, Kisić-Tepavčević D. et al. [5] there were 56.2% of female and 43.8% of male students. Oni et al. conducted a survey of students; the average age of male examinees was 18.1 years and 16.1 years of female students [6]. In another research, the examinees were 15 years of age on average, 55% of females and 45% of males [7]. Bergamini et al. surveyed students from 14 to 19 years of age [8]. In a research of students from 15 to 19 years, 19% were sexually active [9]. The examinees of Manaf et al. were 18 years old (97%) on average, 56% of them were females and 4.5% of them were sexually active [10]. Kaptanoglu et al. studied 49% of males and 50% of girls, aged 15.6 years on average [11]. The respondents of Oliveira-Campos et al. were a little bit younger than ours - 79.8% of them were younger than 14 years, 52.5% of girls and 47.5% of boys. Every third examinee was sexually active, most of them before 13 years of age [12]. The study of Devine S. et al. included 60.4% of girls and 39.6% of boys, from 14 to 18 years of age, and a lot of them had already had more than one risky sexual intercourse [13].

Most students have heard about STIs, with a small difference between genders. The exact definition of STIs was recognized by every other student, more often by female students. Every other respondent recognized the exact offered definition of STIs, without gender difference [8]. The highest percentage of examinees was familiar with STIs (91%) while 8.75% did not know about them [11]. A research on the awareness of STIs of high school students in Germany, the authors also included the mothers' level of education in demographic variables, apart from students' age and gender. In their earlier researches, these factors were connected with the awareness and knowledge of adolescents about STIs. A significant association was established only between high and basic level of education [7]. Successful development and improvement of sex education in schools depends on parents' attitudes as well. The parents of

students had limited knowledge about STIs and they very rarely talked about this issue with their daughters [14]. We found a statistically significant difference between the level of education of our examinees parents based on gender and also their knowledge on the exact definition of STIs.

A statistically significant difference was found on the awareness of students about HIV/AIDS in regard to the place of school. A high percentage of examinees have heard about HIV/AIDS and most of them attended a school in Zvečan (92.6%). The girls were a little bit better informed about HIV/AIDS than boys were. Our examinees showed less knowledge about other SITs, like syphilis, gonorrhea and genital warts. A research in Germany showed that 99% of examinees have heard about HIV/AIDS, 51% about syphilis, 23% for chlamydia and 17% for gonorrhea [7]. About 91% of students from Cyprus have heard about AIDS and 48% about gonorrhea. Molluscum contagiosum was mentioned by 52% and bacterial vaginosis by 44% of examinees [11]. Because of insufficient knowledge about STIs and contraception, the incidence of STIs is still growing among young people [15].

Our examinees were asked to list all STIs that they have heard about: 42% have heard only about one STI, 25% about two STIs and 11% listed none. A little bit less than a half of our examinees have heard about STIs in Biology class at school. The majority of examinees that were informed about STIs at school were still sexually inactive (36.8%). We hope that the information have affected them to delay the start of sexual activity. Adolescents in Ethiopia have sexarche at younger age than in the past. They most often have unprotected sexual intercourse, with an increased risk of developing or transmitting STIs (HIV infection) and unwanted pregnancy [9]. The teacher's role is very important to increase the level of knowledge about sexual and reproductive health [16]. Only 7.6% of examinees talked about STIs with their parents in study of Lindberg et al. [17]. Over 95% of respondents knew about AIDS. The main sources of information were television and school (21% each) [8]. Every other female examinee was informed about reproductive health talking to her mother and every fourth male examinee was informed by his father. About 67% of examinees have not heard about contraception. For 30% of boys the source of information about STIs were parents and for 23% of them school. For 70% of girls the source of information were parents and for 60% of them the Internet. The majority of respondents (80%) answered the questions about STIs and 79% of them knew about AIDS [6]. Most of the students were well informed about STIs because they learned about it at schools and they considered that sex education at schools should be better [18]. About 87% of students talk openly about sex with other students and friends, and just a few of them talk about it with brothers and sisters, parents and teachers [19]. About 42% of students listed school as source of information about STIs [9] and for 78% of them the main source was biology class. Sexual education should include other STIs besides HIV/AIDS [7]. The respondents of Oni et al. have been informed about contraception via media, TV/radio/magazines - 54.5% of boys and 21.5% of girls [6]. Every third male examinee was informed by a teacher/professor (34%) and 19% of female students were informed talking to their parents. The main sources of information about contraception were mass media [8].

The school sex education programs have not reduced the number of teenage pregnancies and their consequences [20]. The awareness and knowledge about reproductive health and STDs should be improved, especially in the younger population (10 – 14 years old) [21]. Poorly-informed teenagers are at higher risk of unwanted pregnancy and STIs [5]). The respondents from Brazil become sexually active at very young age and they very rarely use condoms during sexual intercourse [12]. Over 31% of surveyed students were informed about measures/methods of STI prevention. Practice is not always followed by adequate knowledge and behaviors related to STIs and contraceptive pills [22].

Every third respondent of both genders is sexually active. More than a half of sexually active students of both genders live in Kosovska Mitrovica. Sexually active students are 18 years old, on average. Our results are mostly in agreement with literature data. The research of Salih et al. showed that 29% of girls at the age between 14 and 20 years were sexually active, with sexarche at 16.6 years [2]. Of all the examined students, 5% had a STI, and one girl (0.44%) had an unwanted pregnancy [5]. The high school students included in the study had first sexual experience at 15 years of age and 2 sexual partners on average [7]. The sexual activity began between 15 and 18 years, at 16 in girls and at 17 in boys [23]. The sexarche in 42% of male and 9% of female students was before 14 years of age [24]. In order to improve the sexual health of young people it is important to delay the first sexual intercourse and to increase the parents' level of knowledge [20]. About 42% of girls and 44% of boys between 15 and 19 years of age had a sexual intercourse at least once [25]. About 54% of pregnant women were between 15 and 17 years old and 54% of them had sexarche before the age of 14 years [26]. Increased and earlier sexual activity followed by rare use of contraceptive methods lead to more unwanted pregnancies and STIs [19]. In our research, 20% of sexually active persons visited a doctor because of genital symptoms (genital redness, urinary color changes and secretion from urethra). Of course, these symptoms may be caused by other causes, and our respondents do not need to know or recall the diagnosis that was then set. A statistically significant difference was obtained for sexually active examinees, for those who changed their sex partners, and examinees that became sexually active before the age of 18 years. They usually used no contraceptives. The boys had multiple sex partners (4.2%) compared to girls (2.4%). Chlamydia was diagnosed in 8% of boys and 15% of girls and gonorrhea in 2% of boys and 4% of girls [24].

Although the majority of sexually active students did not agree with it, 27.7% of them thought that there was risky sexual behavior. Risky sexual behavior was precisely defined by 36.7% of sexually active girls and 20% of sexually active boys. They associated this behavior with multiple sex partners, sexual relations without contraceptive means, having sex with strangers and using no protection.

Most students of both genders had voluntary first sex experience. A small percentage of respondents said that their first sex experience was at a party under the influence of drugs or alcohol. The smallest percentage of respondents had their first sexual intercourse at their partner's insistence.

Every third examinee has heard about measures of STIs prevention. Two thirds of examinees knew about contraceptive methods. High school students claimed that condoms were the most frequently used contraceptive method (89.8%) [19]. The students of both sexes used condoms more frequently than contraceptive pills. A certain number of them frequently changed sex partners without using contraceptives [2]. More than 90% of sexually active examinees used contraception during the first sexual intercourse, 37% of girls and 51% of boys used only condoms [7]. About 22% of students had only one sex partner, and 21% of examinees had five partners. Condom use as a contraceptive method was reported by 74% of examinees and 14.9% of examinees used oral hormonal contraception [27]. About 50% of sexually active students did not use any kind of contraception during their first sexual intercourse. About 23% of studied girls had an unwanted pregnancy and 91% had an abortion [25]. Sexarche was often the result of pressure from peers or partners [28].

Conclusion

Our examinees showed poor knowledge about sexually transmitted infections and measures of their prevention, which can be explained by insufficient and improper education about reproductive health. This can be changed by educating parents, health professionals and teachers, as well as by organizing more informative events.

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Rad je primljen 18. II 2018.
Recenziran 15. VI 2018.
Prihvaćen za štampu 18. VI 2018.
BIBLID.0025-8105:(2018):LXXI:9-10:284-294.

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Original study
Originalni naučni rad
UDK 616.379-008.64-06:617.7
UDK 616.379-008.64-06:616.61
https://doi.org/10.2298/MPNS1810296A

CORRELATION BETWEEN EYE AND RENAL COMPLICATIONS OF DIABETES

KORELACIJA OČNIH I BUBREŽNIH KOMPLIKACIJA ŠEĆERNE BOLESTI

Katarina ANIŠIĆ[1, 2] and Sofija DAVIDOVIĆ[2, 3]

Summary

Introduction. Complications of diabetes can affect almost all tissues and organs, causing high morbidity, disability and mortality. The aim of this study was to examine eye and kidney disorders of patients with patients with diabetes, and assess the correlation between them. **Material and Methods.** This retrospective study included 45 patients suffering from type 2 diabetes for more than ten years. The patients were divided into three groups of 15 subjects each: patients without diabetic retinopathy, patients with non-proliferative retinopathy, and patients with proliferative diabetic retinopathy. **Results.** The average levels of fasting blood glucose and glycosylated hemoglobin were highest in patients with proliferative diabetic retinopathy (11.27 mmol/l and 8.48%, respectively). Of 30 patients with diabetic eye diseases, diabetic maculopathy was found in 60% of cases; of those, 20% had non-proliferative retinopathy and 40% had proliferative retinopathy. The mean values of best corrected visual acuity, in both eyes, were 0.45 in patients with proliferative diabetic retinopathy, while mean values of serum urea and creatinine, creatinine clearance, and 24-h albuminuria in this group were 7.37 mmol/l, 106.13 μmol/l, 72.80 ml/min, and 346.31 mg/24h, respectively. **Conclusion.** Severe forms of diabetic retinopathy and nephropathy were found in patients with poor metabolic regulation. A correlation between diabetic eye and kidney diseases was established, and the level of visual damage correlated with the degree of renal function impairment.

Key words: Diabetes Mellitus, Type 2; Diabetes Complications; Diabetic Retinopathy; Diabetic Nephropathies; Blood Glucose; Urine; Visual Acuity

Sažetak

Uvod. Komplikacije šećerne bolesti mogu zahvatiti praktično sva tkiva i organe, uzrokujući veliki morbiditet, invalidnost i mortalitet. Cilj rada je bio da se ispitaju promene na očima i bubrezima kod pacijenata obolelih od šećerne bolesti, kao i da li postoji međusobna povezanost. **Materijal i metode**. Retrospektivna analiza podataka 45 pacijenata koji su imali dijabetes melitus tip 2, duže od deset godina. Svi pacijenti su podeljeni u tri grupe od po 15 ispitanika: pacijenti bez dijabetesne retinopatije, sa neproliferativnom i oni sa proliferativnom dijabetesnom retinopatijom. **Rezultati.** Prosečne vrednosti jutarnjeg („našte“) šećera u krvi kao i glikoziliranog hemoglobina bile su najviše kod pacijenata sa proliferativnom dijabetesnom retinopatijom (11,27 mmol/l i 8,48%). Od 30 pacijenata koji su imali promene na očima uzrokovane šećernom bolešću, dijabetesna makulopatija nađena je kod 60% pacijenata. Od toga, 20% u grupi sa neproliferativnom, a 40% u grupi pacijenata sa proliferativnom dijabetesnom retinopatijom. Srednje vrednosti najbolje korigovane vidne oštrine, prosek za oba oka, iznosile su 0,45 kod pacijenata sa proliferativnom dijabetesnom retinopatijom, dok su srednje vrednosti uree i kreatinina u serumu, klirensa kreatinina i 24-časovne albuminurije u ovoj grupi iznosile 7,37 mmol/l, 106,13 μmol/l, 72,80 ml/min. i 346,31 mg/24 h respektivno. **Zaključak.** Teži oblici dijabetesne retinopatije i nefropatije nalazili su se kod metabolički najlošije regulisanih bolesnika. Utvrđeno je da postoji međusobna povezanost promena od šećerne bolesti na očima i bubrezima, kao i da je stepen oštećenja na oku srazmeran stepenu oštećenja bubrežne funkcije.

Ključne reči: dijabetes melitus, tip 2; komplikacije dijabetesa; dijabetička retinopatija; dijabetička nefropatija; šećer u krvi; urin; vidna oštrina

Introduction

Diabetes mellitus is one of the most common metabolic diseases with an increasing incidence, and its complications can affect almost all tissues and organs, causing high morbidity, disability and mortality [1]. Diabetic retinopathy is an eye complication of diabetes and it is the main cause of visual function loss in the working-age population [2]. Blindness is 25 times more common in diabetics than in the general population [3]. The consistent risk factors for diabetic retinopathy include duration of diabetes, age of the patient, nephropathy/albuminuria, genetic factors, pregnancy and degree of retinopathy, and variable risk factors including hyperglycemia/glycosylated hemoglobin (HbA1c), hypertension, dyslipidemia, physical inactivity and obesity [4]. Two major processes are involved in the development of diabetic retinopathy and clinically significant macular edema: occlusion of the retinal capillaries and other blood vessels, resulting in ischemia, and abnormal vascular permeability [5]. Another significant chronic complication of diabetes

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Abbreviations

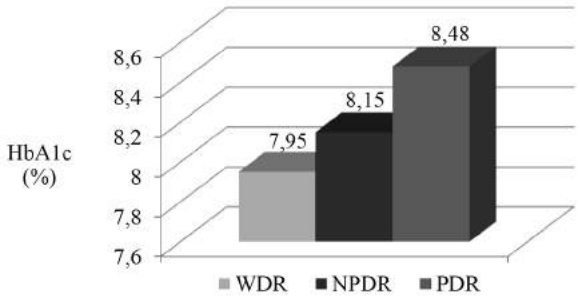
WDR	– without diabetic retinopathy
NPDR	– non-proliferative diabetic retinopathy
PDR	– proliferative diabetic retinopathy
FBG	– fasting blood glucose
HbA1c	– glycosylated hemoglobin
BCVA	– best corrected visual acuity
IOP	– intraocular pressure
VA	– visual acuity

is diabetic nephropathy and it is one of the leading causes of terminal renal failure, as well as mortality at this stage of the disease [3]. Kidney damage rarely occurs in the first 10 years of diabetes, usually taking 15 – 25 years to develop [6]. It is important to inform and educate people with diabetes about the illness, risk factors and complications that accompany it, in order to actively participate in the screening process, to regularly monitor their vision and renal function, and to get treated in a timely manner.

The aim of this study was to examine eye and kidney disorders of patients with diabetes, and assess the correlation between them.

Material and Methods

A retrospective study included 45 patients with diabetes mellitus who underwent a routine ophthalmological examination at the Eye Clinic of the Clinical Center of Vojvodina in Novi Sad. The study was approved by the Ethics Committee of the Clinical Center of Vojvodina, Novi Sad, Serbia. Patients were divided into three groups (according to the International Clinical Diabetic Retinopathy Disease Severity Scale) [7]: patients without diabetic retinopathy (WDR), patients with non-proliferative diabetic retinopathy (NPDR), and patients with proliferative diabetic retinopathy (PDR). Each group included 15 patients who suffered from type 2 diabetes mellitus for more than ten years.

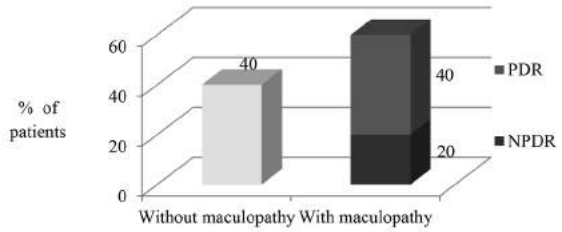


Graph 1. Mean HbA1c levels (%) in blood in groups: WDR, NPDR, PDR

Grafikon 1. *Prosečna koncentracija HbA1c (%) u krvi u odnosu na grupe:* WDR*, NPDR, PDR*

Legend: WDR - without diabetic retinopathy; NPDR - non-proliferative diabetic retinopathy; PDR - proliferative diabetic retinopathy; HbA1c - glycosylated hemoglobin

Legenda: *WDR – bez dijabetesne retinopatije; NPDR – neproliferativna dijabetesna retinopatija; PDR - proliferativna dijabetesna retinopatija; HbA1c - glikozilirani hemoglobin*

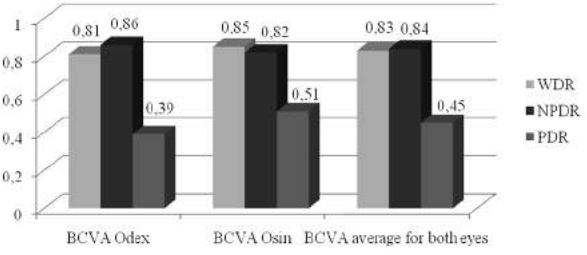


Graph 2. Percentage (%) of patients without and with maculopathy

Grafikon 2. *Procenat (%) pacijenata bez i sa makulopatijom*

The following data were recorded: patient's name and surname; gender; fasting blood glucose (FBG) and HbA1c from venous blood (according to Guidelines for Diabetes Mellitus [3] it should not exceed 7 mmol/l for FBG and 6.5% for HbA1c); level of diabetic retinopathy and presence or absence of maculopathy (based on fundus biomicroscopy with the slit lamp in artificial mydriasis, fluorescein angiography and optical coherence tomography); best corrected visual acuity (BCVA) using the Snellen optotype (in decimal values); intraocular pressure (IOP) measured by applanation tonometry; urea and creatinine in the serum (reference values for serum urea 2 – 7 mmol/l, and serum creatinine 45 – 90 μmol/l in women, and 60 – 110 μmol/l in men); creatinine clearance (reference values 97 – 137 ml/min in men, and 88 – 128 ml/min in women) and screening of 24-hour albuminuria after 24-hour urine collection.

Statistica 12.0 software was used for statistical data analysis. Statistical significance of the difference between the arithmetic means of three samples, with one variable of variance, was calculated using the analysis of variance (ANOVA). The F0

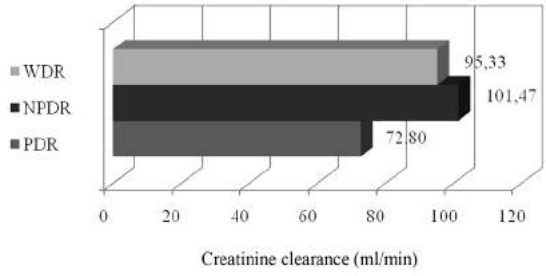


Graph 3. Mean values of the best corrected visual acuity (BCVA) for the right eye, left eye and average for both eyes in groups: WDR, NPDR, PDR

Grafikon 3. *Srednje vrednosti najbolje korigovane vidne oštrine (BCVA) za desno, levo i prosek za oba oka u grupama: WDR, NPDR, PDR*

Legend: WDR - without diabetic retinopathy; NPDR - non-proliferative diabetic retinopathy; PDR - proliferative diabetic retinopathy; HbA1c - glycosylated hemoglobin

Legenda: *WDR – bez dijabetesne retinopatije; NPDR – neproliferativna dijabetesna retinopatija; PDR - proliferativna dijabetesna retinopatija; HbA1c - glikozilirani hemoglobin*



Graph 4. Mean creatinine clearance in groups: WDR, NPDR, PDR

Grafikon 4. Srednje vrednosti klirensa kreatinina u odnosu na grupe: Bez DR, NPDR, PDR

Legend: WDR - without diabetic retinopathy; NPDR - non-proliferative diabetic retinopathy; PDR - proliferative diabetic retinopathy; HbA1c - glycosylated hemoglobin

Legenda: *WDR – bez dijabetesne retinopatije; NPDR – neproliferativna dijabetesna retinopatija; PDR - proliferativna dijabetesna retinopatija; HbA1c - glikozilirani hemoglobin*

test of freedom, variance between groups divided by variance within groups, was compared with Snedecor's F-distribution, which is the probability test. If F0 < F, there was no statistically significant difference ($p > 0.05$), whereas if F0 > F, there was a statistically significant difference ($p < 0.05$) with a risk error of 5%.

Results

This study included a total of 45 patients, of which 17 (37.78%) were female and 28 (62.22%) male.

The average FBG levels in all three groups of patients exceeded the reference ranges. The highest levels were recorded in patients with PDR (11.27 mmol/l), followed by patients with NPDR (10.63 mmol/l) and finally in patients with WDR (9.37 mmol/l). There was no statistically significant difference between the groups in regard to FBG (F0 = 1.02, F = 3.22, F0 < F, $p > 0.05$).

The highest mean HbA1c level was observed in patients with PDR – 8.48%, while it was 8.15% in the group of patients with NPDR, and 7.95% in patients with WDR **(Graph 1)**. There was no statistically significant difference between the observed groups (F0 = 1.69, F = 19.5, F0 < F, $p > 0.05$).

Of the 30 patients with diabetes-induced eye diseases, diabetic maculopathy was found in 18 patients (60%). Of those, 6 patients (20%) were in the NPDR group and 12 of them (40%) in the PDR group **(Graph 2)**.

Visual acuity (VA) was measured by Snellen optotype, and the results were expressed in decimals. The mean values of BCVA in both eyes were significantly lower in the PDR group (mean value of 0.45) in comparison with the values in patients with NPDR (mean value of 0.84) and WDR group (mean value of 0.83) **(Graph 3)**. There was a statistically significant difference in mean BCVA values in both eyes (F0 = 9.99, F = 3.19, F0 > F, $p < 0.05$) in the observed groups.

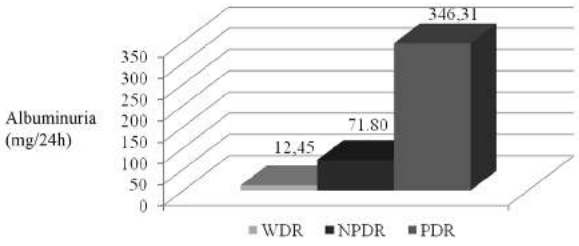
Mean values of IOP, measured by applanation tonometer, were not elevated in any patient, i. e. in all patients values ranged from 10 to 21 mmHg.

Mean serum urea was 6.61 mmol/l in the WDR group, in the NPDR group it was 6.16 mmol/l, and the highest value was 7.37 mmol/l, in the PDR group. There was no statistically significant difference in mean values of urea between these three groups (F0 = 1.41, F = 19.5, F0 < F, $p > 0.05$).

Mean serum creatinine levels were highest in the PDR group (106.13 μmol/l), followed by the NPDR group (94 μmol/l) and lowest among the patients in the WDR group (90.73 μmol/l). There was no statistically significant difference in creatinine levels between these groups (F0 = 1.05, F = 19.5, F0 < F, $p > 0.05$).

The lowest mean creatinine clearance was found in the PDR group – 72.80 ml/min. In the NPDR group, it was 101.47 ml/min, and in the WDR group it was 95.33 ml/min **(Graph 4)**. There was a statistically significant difference in mean levels of creatinine clearance in the studied groups (F0 = 3.36, F = 3.22, F0 > F, $p < 0.05$).

The mean albuminuria level was highest in patients with PDR – 346.31 mg/24h, followed by NPDR patients – 71.80 mg/24h, while the lowest level was observed in patients in the WDR group – 12.45 mg/24h **(Graph 5)**. There was a statistically significant difference in mean albuminuria level in the studied groups (F0 = 43.67, F = 3.22, F0 > F, $p < 0.05$).



Graph 5. Mean values of 24-h albuminuria in groups: WDR, NPDR, PDR

Grafikon 5. *Srednje vrednosti 24h albuminurije u odnosu na grupe: Bez DR, NPDR, PDR*

Legend: WDR - without diabetic retinopathy; NPDR - non-proliferative diabetic retinopathy; PDR - proliferative diabetic retinopathy; HbA1c - glycosylated hemoglobin

Legenda: *WDR – bez dijabetesne retinopatije; NPDR – neproliferativna dijabetesna retinopatija; PDR - proliferativna dijabetesna retinopatija; HbA1c - glikozilirani hemoglobin*

Discussion

Complications of diabetes are directly related to disease control [8]. Mean values of FBG and HbA1c were highest in the PDR group (11.27 mmol/l and 8.48%, respectively), which corresponds to the fact that severe forms of diabetic retinopathy are found in patients with poor metabolic regulation [9, 10]. The mean values of HbA1c in the FinnDiane study

were similar to values found in our patients and amounted to 8.6% in patients with PDR [11]. It has been proven that glycemic control, especially when initiated in the early stage of the disease, can prevent or delay development of diabetic retinopathy [9]. It has been established that 1% reduction of HbA1c reduces the risk of retinopathy by 40% and mortality due to diabetic complications by 25% [8, 12].

Macular edema that occurs in both forms of diabetic retinopathy is the most common cause of visual impairment. The Wisconsin Epidemiological Study of Diabetic Retinopathy found that in patients with moderately severe and severe NPDR, macular edema occurred in 38% of cases, while in patients with proliferative form it occurred in 71% of cases [13]. In our study, of 30 patients with diabetes-induced diseases, diabetic maculopathy was found in 60% of patients. Of these, 20% of patients were in the NPDR group and 40% in the PDR group. A statistically significant difference in the mean values of the BCVA in both eyes was established between the groups; values were significantly lower in the PDR group, which is in accordance with the study from India, where the BCVA was proportionally reduced with the severity of diabetic retinopathy [14].

Diabetic nephropathy is a very significant chronic complication of diabetes and is one of the leading causes of terminal renal failure and mortality in this stage of the disease [3]. In our research, PDR patients had a mean serum urea levels above reference ranges, amounting to 7,37 mmol/l. These levels were significantly higher in the study carried out in India – 16,17 mmol/l [14]. In our study, the mean serum creatinine levels were 94 μmol/l in the NPDR group and 106.13 μmol/l in the PDR group. These results are very similar to the Indian study which reported the mean value of 95,92 μmol/l in the NPDR group and 112,64 μmol/l in the PDR group [14]. In this study there was a statistically significant difference in mean creatinine clearance level in the observed groups (WDR, NPDR and PDR). The lowest mean creatinine clearance was observed in the PDR group – 72,80 ml/min. In the NPDR group, it amounted to 101,47 ml/min, and in the WDR group it was 95,33 ml/min. In the FinnDiane study, mean creatinine clearance in WDR, NPDR, and PDR groups was 93 ml/min, 79 ml/min and 59 ml/min, respectively; in the Chronic Renal Insufficiency Cohort study, values for the same groups were 42.8 ml/min, 36.6 ml/min and 32.2 ml/min, respectively, while the Indian study reported a mean creatinine clearance of 95.2 ml/min in patients with NPDR and 80.2 ml/min in patients with PDR [11, 14, 15]. Although the values are quite different among the studies, it can be seen that creatinine clearance was significantly lower in severe forms of retinopathy. Mean albuminuria levels in the WDR, NPDR and PDR groups were 12.45 mg/24h, 71.80 mg/24h and 346.31 mg/24h, respectively. There was a statistically significant difference among the groups, which points to the fact that in patients with advanced diabetic proliferative eye diseases there was proportional renal function impairment, manifesting in 24-h albuminuria. In FinnDiane study, these values were significantly lower (8 mg/24h, 31 mg/24h and 135 mg/24h) [11].

Our findings support the hypothesis that common mechanisms may cause both retinal and renal vascular changes and that there is a correlation between the degree of diabetic retinopathy, 24-h albuminuria and kidney function [11, 15–17].

Conclusions

1. Severe forms of diabetic retinopathy were found in patients with poor metabolic regulation, i. e. in patients with higher blood sugar levels and glycosylated hemoglobin.
2. Patients with proliferative diabetic retinopathy had a higher incidence of macular edema as well as significantly reduced best-corrected visual acuity in both eyes, while the values of intraocular pressure were within normal limits.
3. Serum urea and creatinine, as well as 24-h albuminuria, showed the highest levels in patients with proliferative diabetic retinopathy, whereas creatinine clearance was the lowest in this group.
4. It has been established that there is a correlation between diabetic eye and kidney diseases, and that the degree of eye damage was proportional to the degree of renal function damage; this opens up a possibility that there may be a link between the pathogenetic mechanisms causing diabetic retinopathy and nephropathy.

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Rad je primljen 21. VI 2018.
Recenziran 17. VII 2018.
Prihvaćen za štampu 20. VIII 2018.
BIBLID.0025-8105:(2018):LXXI:9-10:295-299.

PROFESSIONAL ARTICLES
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Professional article
Stručni članak
UDK 618.146:[616.988:578.82/.83
https://doi.org/10.2298/MPNS1810301D

ASSOCIATION BETWEEN HUMAN PAPILLOMAVIRUS INFECTION AND ATYPICAL CERVICAL SQUAMOUS CELLS

UDRUŽENOST INFEKCIJE HUMANIM PAPILOMA VIRUSOM I ABNORMALNOSTI SKVAMOZNIH ĆELIJA GRLIĆA MATERICE

Drage DABESKI

Summary

Introduction. The aim of the study was to confirm the association between human papillomavirus infection and atypical cervical squamous cells. **Material and Methods.** This cross-sectional study, conducted in the period from January 2016 to June 2017, included 128 sexually active women, aged 20 to 59 years with squamous cell abnormalities of the cervical cytology, who came to their annual gynecological exam at the University Clinic of Gynecology and Obstetrics in Skopje. All patients underwent human papillomavirus testing and colposcopic cervical biopsy with endocervical curettage for histopathological analysis. **Results.** Data analysis showed an increase in the human papillomavirus infection alongside with cytological ($p = 0.029296$) and histopathological ($p = 0.029443$) increasing grades of cervical lesions. It showed an association between the oncogenic potential of the virus and the cytological ($p = 0.000086$) and histopathological ($p = 0.00001$) grades of cervical lesions. A human papillomavirus infection was detected in 75.00% of the examined women. The relationship between the prevalence of high-risk and low-risk human papillomavirus genotypes was 56.25%: 10.94%. Mixed human papillomavirus infection was detected in 32.03% of all patients, in 42.71% of human papillomavirus positive patients. The most common human papillomavirus genotypes, in descending order, were human papillomavirus-16 (43.75%), human papillomavirus-31 (15.62%), human papillomavirus-18 10.4%), human papillomavirus-45 (9.37%), human papillomavirus-33 (7.29%), etc. **Conclusion.** This study has confirmed an association between human papillomavirus infection and squamous cell abnormalities of the uterine cervix. Young women under 30 years of age were the most affected group.

Key words: Papillomavirus Infections; Uterine Cervical Dysplasia; Squamous Intraepithelial Lesions of the Cervix; Polymerase Chain Reaction; Human Papillomavirus DNA Tests; Colposcopy

Sažetak

Uvod. Cilj studije bio je da se potvrdi postojanje udruženosti između infekcije humanim papiloma virusom i abnormalnosti skvamoznih ćelija grlića materice. **Materijal i metode.** Studija preseka, sprovedena u periodu od januara 2016. do juna 2017. godine na 128 seksualno aktivnih žena, starosti od 20 do 59 godina, sa abnormalnostima skvamoznih ćelija na cervikalnoj citologiji, koje su došle na godišnji ginekološki pregled na Univerzitetsku kliniku za ginekologiju i akušerstvo u Skoplju. Kod svih žena je urađeno testiranje infekcije humanim papiloma virusom i kolposkopska biopsija grlića materice sa endocervikalnom kiretažom za histopatološku analizu. **Rezultati.** Analiza podataka pokazala je povećanje prisustva infekcije humanim papiloma virusom paralelno sa povećanjem citopatološkog ($p = 0.029296$) i histopatološkog ($p = 0.029443$) stepena lezije grlića materice. Analiza podataka pokazala je udruženost između onkogenog potencijala virusa i citopatološkog ($p = 0.000086$) i histopatološkog ($p = 0.0001$) stepena lezije grlića materice. Infekcija humanim papiloma virusom otkrivena je kod 75% ispitanih žena. Odnos između prevalencije visokorizičnih i niskorizičnih genotipova humanih papiloma virusa iznosio je 56,25 : 10,94%. Mešovita infekcija humanim papiloma virusom otkrivena je kod 32,03% od svih žena, odnosno 42,71% kod žena pozitivnih na humani papiloma virus. Najčešći genotipovi infekcije humanim papiloma virusom u opadajućem redosledu bili su: humani papiloma virus-16 (43,75%), humani papiloma virus-31 (15,62%), humani papiloma virus-18 (10,4%), humani papiloma virus-45 (9,37%), humani papiloma virus-33 (7,29%) itd. **Zaključak.** Postoji udruženost između infekcije humanim papiloma virusom i abnormalnosti skvamoznih ćelija grlića materice. Žene mlađe od 30 godina bile su najugroženija starosna grupa.

Ključne reči: papiloma virusne infekcije; displazija grlića materice; skvamozne intraepitelijalne lezije grlića materice; PCR; HPV-DNK testiranje; kolposkopija

Introduction

Invasive cervical cancer is the fourth most common cancer in women and seventh most common cancer in general, with 527.624 new cases and 265.672 deaths in 2012, accounting for 7.5% of all cancer deaths in women [1]. According to the latest data from Global Cancer Observatory, Macedonia has an estimated incidence of

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Abbreviations

HPV	– human papillomavirus
DNA	– deoxyribonucleic acid
PCR	– polymerase chain reaction
ASC-US	– atypical squamous cells of undetermined significance
ASC-H	– atypical squamous cells, cannot exclude a high-grade squamous intraepithelial lesion
LSIL	– low grade squamous intraepithelial lesion
CIN1	– cervical intraepithelial neoplasia grade 1
HSIL	– high grade squamous intraepithelial lesion
CIN2	– cervical intraepithelial neoplasia grade 2
CIN3	– cervical intraepithelial neoplasia grade 3
CIS	– carcinoma *in situ*

cervical cancer of 12.4 per 100,000 inhabitants and it ranks 17th in Europe, which is close to the European average of 11.4 per 100,000 [2]. Squamous cell carcinoma of the cervix is the most common histological subtype of cervical cancer. About 90% of cervical cancer cases are squamous carcinomas, 10% are adenocarcinomas, and a small number are other types [3]. The occurrence of cervical cancer is preceded by various types of intraepithelial lesions including a series of progressive morphological changes, from productive human papillomavirus (HPV) infection/mild dysplasia to in situ carcinoma [4]. The most common risk factor for squamous cells abnormalities of the uterine cervix is HPV infection, especially with high-risk HPV genotypes. Only persistent, high-risk HPV infection represents a major risk factor for squamous cell abnormalities of the uterine cervix [5]. Deoxyribonucleic acid (DNA) from HPV has been found in 99.7% of cases of cervical carcinoma [6]. There are different classifications of HPV: by genetic similarity, by oncogenic potential and by affinity for certain tissues. According to their oncogenic potential, they are divided into high-risk and low-risk [7]. The prevalence of HPV genotypes varies by geographical regions. In Europe and North America, HPV-16 is still the most common high-risk genotype [8]. The population of young women, from 18 to 25, has the highest rates of HPV infection. After the age of 25 years, the incidence of HPV infection is reduced to reach its second highest level after the age of 45 years [9]. Detection of HPV can be done using two methods; the first one is direct hybridization or in situ hybridization, and the other is amplification or polymerase chain reaction (PCR) [10]. The aims of the study were to confirm the association between HPV infection and squamous cells abnormalities of the uterine cervix, detection and typing HPV genotypes, which are the most common causes of intraepithelial lesions and cervical cancer, to determine the prevalence of HPV infections and the most affected age groups.

Material and Methods

This cross-sectional study included 128 sexually active women aged 20 to 59 years, with abnormal cervical cytological findings, i.e. a finding of Papanicolaou (PAP) test showing a squamous intraepithelial lesion or invasive squamous cervical cancer.

The study did not include pregnant women, women with previous surgery of the uterine cervix (cervical conization, carbon dioxide laser vaporization and total abdominal hysterectomy) as well as previous abnormal cytological and histopathological findings of the uterine cervix.

The study was conducted in the period from January 2016 to June 2017.

The study was conducted at the University Clinics of Gynecology and Obstetrics and Radiotherapy and Oncology in Skopje, Republic of Macedonia.

All women underwent HPV testing and colposcopic cervical biopsy with endocervical curettage for histopathological analysis.

All samples for cytological analysis were taken using the ThinPrep PAP test and were analyzed in the cytological laboratory at the University Clinic of Gynecology and Obstetrics in Skopje by a cytopathologist. Cytological results were classified according to the revised Bethesda classification [11, 12] including atypical squamous cells of undetermined significance (ASC-US); atypical squamous cells, cannot exclude a high-grade squamous intraepithelial lesion (ASC-H); low grade squamous intraepithelial lesion (LSIL), cervical intraepithelial neoplasia grade 1 (CIN1); high grade squamous intraepithelial lesion (HSIL), cervical intraepithelial neoplasia grade 2 (CIN2), cervical intraepithelial neoplasia grade 3 (CIN3), carcinoma *in situ* (CIS) and invasive squamous cell carcinoma.

Samples for histopathological analysis were taken at the University Clinic of Gynecology and Obstetrics in Skopje and were analyzed at the University Clinic of Radiotherapy and Oncology in Skopje, at the Department of Histopathology and Clinical Cytology by an experienced histopathologist. According to the morphology determined in bioptic samples, cervical findings were characterized as normal findings (non-specific cervicitis); LSIL (cervicitis chronica virosa, flat condyloma, mild dysplasia); HSIL (moderate dysplasia, severe dysplasia, *in situ* squamous cell carcinoma) and invasive squamous cell carcinoma [13].

Cervical biopsy samples were taken for HPV testing and analyzed at the University Clinic of Gynecology and Obstetrics in Skopje, at the Laboratory for HPV testing. HPV detection and typing were done using multiple polymerase chain reaction (Multiplex PCR) and reverse hybridization. The results of the HPV test were analyzed and demonstrated based on the presence or absence of DNA from HPV and the specified genotype [14]. The first step in HPV testing was the isolation of DNA from the collected cells from cervical biopsies. For isolation of DNA series, three paraffin cuts were prepared. The cuts were incubated in 1 ml xylene for 5 minutes at 55°C and centrifuged at 10.000 G for 5 minutes at room temperature. The same procedure was repeated two more times. After careful removal of the remains of xylene, the samples were briefly incubated twice in 1 ml of 100% ethanol, and centrifuged for 5 minutes at room temperature. After removal of ethanol and a short air dry, the cuts were incubated overnight in a buffer with freshly added proteinase K at 55°C. The second step was the detection of DNA in HPV by using

PCR. To verify the quality and integrity of the isolated DNA, actually of a present inhibitor, a reaction of multiplication of primers for beta globin PC04 and GH20 was first made for each sample. Three pairs of primers were used, common to a larger number of HPV types: degenerate beginners My09/My11 and CPI/CPII G and Gp5/6+. The samples were carried through all reactions with primers specific to high-risk and low-risk HPV genotypes. The third step was genotyping by using reverse hybridization. It is a method that is based on the hybridization of specific DNA probes that are immobilized on nitrocellulose or nylon tapes. It is a set of beginners (SPF 10) with aim-propagation of the L1 gene on the viral DNA. The product of amplification with SPF beginners of 65 bp allows detection of 25 new genotypes. Denatured biotinylated PCR products are hybridized with specific oligonucleotide probes that are immobilized as parallel lines on membrane strips. After hybridization and washing with streptavidin, alkaline phosphatase is added, which binds to the biotinylated hybrids formed previously. Incubation with BCIP (5-bromo-4-chloro-3-indolyl-phosphate)/NBT (nitro blue tetrazolium) chromogens give purple precipitate and the results are interpreted visually.

Data analysis was done using the Excel database. Statistical analysis of the established statistical series was done by the statistical package for the social sciences, version 23.0. The structure of numerical signs was analyzed by determining the measures of central tendency (arithmetic mean) and measures of dispersion (standard deviation).

The analysis of the relationship (existence of association) between two sets of attribute variables was performed using the Chi-square test. Statistical significance was defined as p value <0.05.

Results

Of the 128 examined patients, aged 20 to 59 years (40.50 ± 10.85), 28 (21.87%) were aged 20 – 29; 38 (29.69%) 30 – 39 years; 30 (23.44%) 40 – 49 years, and 32 (25.00%) were aged 50 – 59 years.

The distribution of HPV infection in 128 patients, correlated with cytopathological diagnosis, is shown in **Table 1**.

Data analysis showed an increase of HPV infection with an increase in the cytopathological grade of the cervical lesion. There were 46.15% (6/13) of samples with ASC-US, 57.14% (4/7) with ASC-H, 70.97% (22/31) with LSIL, 80.36% (45/56) with HSIL, and 90.48% (19/21) with invasive squamous cell carcinoma (chi-square test = 10.7682, p = 0.029296, p < 0.05).

Data analysis showed an association between the oncogenic potential of the virus and the cytopathological grade of cervical lesions (chi-square test = 23.8298, p = 0.000086, p < 0.05).

Distribution of HPV infection in 128 patients in correlation with histopathological diagnosis is shown in **Table 2**.

HPV infection was detected in 75.00% (96/128) of studied patients. The lowest percentage was observed in LSIL – 63.41% (26/41), with an increase to 83.33% (45/54) in HSIL and 87.50% (21/24) in invasive squa-

Table 1. Correlation between the HPV infection and cytopathologic diagnosis
Tabela 1. *Korelacija između HPV infekcije i citopatološke dijagnoze*

HPV infection *HPV infekcija*	Cytopathologic diagnosis/*Citopatološka dijagnoza*							
			LSIL (n = 31)		HSIL (n = 56)		Invasive squamous cell carcinoma *Invazivni skvamozni karcinom* (n = 21)	Total *Ukupno* (n = 128)
	ASC-US (n = 13)	ASC-H (n = 7)	CIN1 (n = 31)	CIN2 (n = 20)	CIN3 (n = 21)	*In situ* squamous cell carcinoma/*In situ skvamozni karcinom* (n = 15)		
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
HPV negative *HPV negativne*	7 (53.85)	3 (42.86)	9 (29.03)	4 (20.00)	4 (19.05)	3 (20.00)	2 (9.52)	32 (25.00)
HPV positive *HPV pozitivne*	6 (46.15)	4 (57.14)	22 (70.97)	16 (80.00)	17 (80.95)	12 (80.00)	19 (90.48)	96 (75.00)
H-R HPV positive/*Visoko rizične HPV pozitivne*	0 (0)	3 (42.86)	12 (38.71)	11 (55.00)	17 (80.95)	12 (80.00)	18 (85.71)	73 (57.03)
L-R HPV positive/*Niskorizične HPV pozitivne*	4 (30.77)	1 (14.28)	4 (12.90)	2 (10.00)	0 (0)	0 (0)	0 (0)	11 (8.59)
H-R and L-R HPV positive/*Visokorizične i niskorizične HPV pozitivne*	2 (23.08)	0 (0)	6 (19.35)	3 (15.00)	0 (0)	0 (0)	1 (4.76)	12 (9.37)

Legend: n - number; ASC-US - atypical squamous cells of undetermined significance; ASC-H - atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; LSIL - low-grade squamous intraepithelial lesion; HSIL - high-grade squamous intraepithelial lesion; CIN - cervical intraepithelial neoplasia; HPV - human papillomavirus; H-R - high-risk; L-R - low-risk
Legenda: n - broj; ASC-US - atipične skvamozne ćelije neodređenog značaja; ASC-H - atipične skvamozne ćelije, ne isključuju skvamoznu intraepitelijalnu leziju visokog stepena; LSIL - skvamozna intraepitelijalna lezija niskog stepena; HSIL - skvamozna intraepitelijalna lezija visokog stepena; CIN - cervikalna intraepitelna neoplazija; HPV - humani papiloma virus; H-R - visokorizičan; L-R - niskorizičan

Table 2. Correlation between the HPV infections and histopathological diagnosis
Tabela 2. *Korelacija između HPV infekcija i histopatološke dijagnoze*

HPV infection *HPV infekcija*	Histopathological diagnosis/*Histopatološka dijagnoza*								
	Normal finding *Normalni nalaz* (n = 9)	LSIL (n = 41)			HSIL (n = 54)			Invasive squamous cell carcinoma *Invazivni skvamozni karcinom* (n = 24)	Total *Ukupno* (n = 128)
		Cervicitis chronicavirosa *Hronični virusni cervicitis* (n = 20)	Flat condyloma *Pljosnata bradavica* (n = 2)	Mild dysplasia *Blaga displazija* (n = 19)	Moderate dysplasia *Umerena displazija* (n = 15)	Severe dysplasia *Teška displazija* (n = 23)	*In situ* squamous cell/carcinoma/*In situ skvamozni karcinom* (n = 16)		
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
HPV negative *HPV negativne*	5 (55.56)	7 (35.00)	0 (0)	8 (42.10)	3 (20.00)	4 (17.39)	2 (12.50)	3 (12.50)	32 (25.00)
HPV positive *HPV pozitivne*	4 (44.44)	13 (65.00)	2 (100)	11 (57.89)	12 (20.00)	19 (82.61)	14 (87.50)	21 (87.50)	96 (75.00)
H-R HPV positive *Visokorizične HPV pozitivne*	1 (11.11)	3 (15.00)	0 (0)	6 (31.58)	11 (73.33)	17 (73.91)	14 (87.50)	20 (83.33)	72 (56.25)
L-R HPV positive *Niskorizične HPV pozitivne*	2 (22.22)	8 (40.00)	1 (50.00)	3 (15.79)	0 (0)	0 (0)	0 (0)	0 (0)	14 (10.94)
H-R and L-R HPV positive/ *Visokorizične i niskorizične HPV pozitivne*	1 (11.11)	2 (10.00)	1 (50.00)	2 (10.53)	1 (6.67)	2 (8.70)	0 (0)	1 (4.17)	10 (7.81)

Legend: n - number; LSIL - low-grade squamous intraepithelial lesion; HSIL - high-grade squamous intraepithelial lesion; HPV - human papillomavirus; H-R - high-risk; L-R - low-risk
Legenda: n - broj; LSIL - skvamozna intraepitelijalna lezija niskog stepena; HSIL - skvamozna intraepitelijalna lezija visokog stepena; HPV - humani papiloma virus; H-R - visokorizičan; L-R - niskorizičan

mous cell carcinoma (chi-square test = 7.0506, p = 0.029443, p < 0.05).

Data analysis showed an association between the HPV infection and the appearance of squamous cell abnormalities of the uterine cervix (chi-square test = 4.8204, p = 0.028125, p < 0.05).

The prevalence of high-risk and low-risk HPV positive tests was 56.25%: 10.94% (i.e. 75.00%: 14.58% among HPV positive patients).

Data analysis showed an increase of high-risk HPV associated with increased histopathological lesions, from 21.95% (9/41) in LSIL, over 77.78% (42/54) in HSIL and up to 83.33% (20/24) in invasive squamous cell carcinoma.

The analysis also showed an association between the oncogenic potential of the virus and the histopathological grade of cervical lesion (chi-square test = 31.5089, p = 0.00001, p <0.05). The incidence of HPV infection was 85.71% (24/28) in patients aged 20 – 29 years; 65.79% (25/38) in 30 – 39 years; 73.33% (22/30) in 40 – 49 years, and 78.12% (25/32) in patients aged 50 – 59 years **(Table 3)**.

A single HPV infection was detected in 42.97% (55/128) of all patients (i.e. in 57.29% of HPV positive patients). The most common single HPV infection was high-risk HPV in 53.12% (51/96) **(Table 4)**.

Mixed HPV infection was detected in 32.03% (41/128) of all patients (i.e. 42.71% of HPV positive patients). The most common co-infection was high risk with high-risk HPV: 22.45% (22/96). Co-infection with high-risk and low-risk HPV was found in 11.46% (11/96), and co-infection with low-risk and low-risk

Table 3. Age group distribution of HPV infection in 128 patients
Tabela 3. *Distribucija HPV infekcije po starosnim grupama kod 128 pacijenata*

HPV infection/*HPV infekcija*	Age groups/*Starosne grupe*							
	20–29		30–39		40–49		50–59	
	n	(%)	n	(%)	n	(%)	n	(%)
HPV positive/*HPV pozitivne*	24	(85.71)	25	(65.79)	22	(73.33)	25	(78.12)
HPV negative/*HPV negativne*	4	(14.29)	13	(34.21)	8	(26.67)	7	(21.88)
Total/*Ukupno*	28	(21.87)	38	(29.69)	30	(23.44)	32	(25.00)

Legend: n - number; HPV - human papillomavirus/*Legenda: n - broj; HPV - humani papiloma virus*

Table 4. Correlation between single and mixed HPV infections and histopathological diagnosis in 96 HPV positive patients
Tabela 4. *Korelacija između pojedinačnih i mešanih HPV infekcija i histopatološke dijagnoze kod 96 HPV pozitivnih pacijentkinja*

Type of HPV infection *Tip HPV infekcije*	Histopathological diagnosis/*Histopatološka dijagnoza*								
	Normal finding *Normalni nalaz* (n = 4)	LSIL (n = 26)			HSIL (n = 45)			Invasive squamous cell carcinoma *Invazivni skvamozni karcinom* (n = 21)	Total *Ukupno* (n = 96)
		Cervicitis chronica virosa *Hronični virusni cervicitis* (n = 13)	Flat condyloma *Pljosnata bradavica* (n = 2)	Mild dysplasia *Blaga displazija* (n = 11)	Moderate dysplasia *Umerena displazija* (n = 12)	Severe dysplasia *Teška displazija* (n = 19)	*In situ* squamous cell carcinoma *In situ skvamozni karcinom* (n = 14)		
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Single infection *Pojedinačna infekcija*	1 (25.00)	7 (53.85)	0 (0)	3 (27.27)	6 (50.00)	13 (68.42)	11 (78.57)	14 (66.67)	55 (57.29)
Single H-R/*Pojedinačna visokorizična*	1 (25.00)	4 (30.77)	0 (0)	2 (18.18)	6 (50.00)	13 (68.42)	11 (78.57)	14 (66.67)	51 (53.12)
Single L-R/*Pojedinačna niskorizična*	0 (0)	3 (23.08)	0 (0)	1 (9.09)	0 (0)	0(0)	0 (0)	0 (0)	4 (4.17)
Mixed infection *Mešovita infekcija*	3 (7.00)	6 (46.15)	2 (100)	8 (72.73)	6 (50.00)	6 (31.58)	3 (21.43)	7 (33.33)	41 (42.71)
Mixed H-R – H-R *Mešovita visokorizična-visokorizična*	0 (0)	0 (0)	0 (0)	4 (36.36)	5 (41.67)	4 (21.05)	3 (21.43)	6 (28.57)	22 (22.92)
Mixed H-R – L-R *Mešovita visokorizična-niskorizična*	1 (25.00)	2 (15.38)	1 (50.00)	3 (27.27)	1 (8.33)	2 (10.53)	0 (0)	1 (4.76)	11 (11.46)
Mixed L-R – L-R *Mešovita niskorizična-niskorizična*	2 (50.00)	4 (30.77)	1 (50.00)	1 (9.09)	0 (0)	0 (0)	0 (0)	0 (0)	8 (8.33)

Legend: n - number; LSIL - low-grade squamous intraepithelial lesion; HSIL - high-grade squamous intraepithelial lesion; HPV - human papillomavirus; H-R - high-risk; L-R - low-risk
Legenda: n - broj; LSIL - skvamozna intraepitelijalna lezija niskog stepena; HSIL - skvamozna intraepitelijalna lezija visokog stepena; HPV - humani papiloma virus; H-R - visokorizičan; L-R - niskorizičan

HPV was detected in 8.16% (8/96). In correlation with histopathological diagnosis, the prevalence of mixed HPV infections was 75.00% (3/4) in normal findings, 61.54% (16/26) in LSIL, 33.33% (15/45) in HSIL and 33.33% (7/21) in invasive squamous cell carcinoma **(Table 4)**.

Data analysis showed that mixed HPV infections are the most frequent in patients under 30 years of age (58.33%; 14/24) **(Table 5)**.

HPV typing identified a total of 20 HPV genotypes, of which 15 were high-risk (HPV -16, -18, -31, -33, -35, -39, -45, -52, -53, -56 , -58 -59, -66, -68, and -73) and 5 low-risk (-6, -11, -40, -42 and -61). The prevalence of 20 HPV genotypes in single and mixed HPV infections in correlation with histopathological diagnosis is shown in **Table 6**.

Among high-risk HPV genotypes, HPV-16 was the most common (43.75%; 42/96), followed by (in descending order) HPV-31 (15.62%; 15/96), HPV-18 10.4%, 10/96), HPV-45 (9.37%, 9/96), HPV-33 (7.29%, 7/96), HPV-35, -52 and -56 (5.21%; 5/96), etc. Among the low-risk HPV genotypes, the most common was

Table 5. Age group distribution of single and mixed HPV infections in 96 HPV positive patients
Tabela 5. *Distribucija pojedinačnih i mešovitih HPV infekcija po starosnim grupama kod 96 HPV pozitivnih pacijenata*

HPV infection *HPV infekcija*	Age groups/*Starosne grupe*							
	20–29		30–39		40–49		50–59	
	n	(%)	n	(%)	n	(%)	n	(%)
Single/*Pojedinačna*	10	(41.67)	13	(52.00)	14	(63.64)	18	(72.00)
Mixed/*Mešana*	14	(58.33)	12	(48.00)	8	(36.36)	7	(28.00)
Total/*Ukupno*	24	(25.00)	25	(26.04)	22	(22.92)	25	(25.00)

Legend: n - number; HPV - human papillomavirus/*Legenda: n - broj; HPV - humani papiloma virus*

HPV-6 (14.58%; 14/96), followed by HPV-11 (9.37%; 9/96). HPV-16 was most common in patients with HSIL and invasive squamous cell carcinoma, while HPV-6 in patients with LSIL **(Table 6)**.

Discussion

In 1976, Harald zur Hausen published his hypothesis about the probable association of cervical cancer

Table 6. Prevalence of HPV genotypes in regard to histopathological diagnosis
Tabela 6. *Prevalencija HPV genotipova u korelaciji sa histopatološkom dijagnozom*

HPV genotype *HPV genotip* Type of HPV infection *Tip HPV infekcije*	Histopathological diagnosis/*Histopatološka dijagnoza*								
	Normal finding *Normalni nalaz* (n = 4)	LSIL (n = 26)			HSIL (n = 45)			Invasive squamous cell carcinoma *Invazivni skvamozni karcinom* (n = 21)	Total *Ukupno* (n = 96)
		Cervicitis chronica virosa *Hronični virusni cervicitis* (n = 13)	Flat condyloma *Pljosnata bradavica* (n = 2)	Mild dysplasia *Blaga displazija* (n = 11)	Moderate dysplasia *Umerena displazija* (n = 12)	Severe dysplasia *Teška displazija* (n = 19)	*In situ* squamous cell carcinoma/*In situ skvamozni karcinom* (n = 14)		
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
16 Single/*Pojedinačna*	1 (25.0)	0 (0)	0 (0)	0 (0)	4 (33.33)	9 (47.37)	5 (35.71)	6 (28.57)	25 (26.04)
Mixed/*Mešoviti*	0 (0)	1 (7.69)	0 (0)	3 (27.27)	3 (25.00)	3 (15.79)	3 (21.43)	4 (19.05	17 (17.71)
18 Single/*Pojedinačna*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (14.28)	3 (3.12))
Mixed/*Mešoviti*	0 (0)	0 (0)	0 (0)	1 (9.09)	1 (8.33)	2 (10.53)	0 (0)	3 (14.28)	7 (7.29)
31 Single/*Pojedinačna*	0 (0)	0 (0)	0 (0)	0 (0)	1 (8.33)	3 (15.79)	3 (21.43)	1 (4.76)	8 (8.33)
Mixed/*Mešoviti*	0 (0)	0 (0)	0 (0)	1 (9.09)	1 (8.33)	1 (5.26)	2 (14.29)	2 (9.52)	7 (7.29)
33 Single/*Pojedinačna*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5.26)	1 (7.14)	0 (0)	2 (2.08)
Mixed/*Mešoviti*	0 (0)	1 (7.69)	0 (0)	0 (0)	2 (16.77)	0 (0)	0 (0)	2 (9.52)	5 (5.21)
35 Single/*Pojedinačna*	0 (0)	1 (7.69)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.04)
Mixed/*Mešoviti*	0 (0)	0 (0)	0 (0)	2 (18.18)	1 (8.33)	1 (5.26)	0 (0)	0 (0)	4 (4.17)
39 Single/*Pojedinačna*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Mixed/*Mešoviti*	0 (0)	0 (0)	0 (0)	1 (9.09)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.04)
45 Single/*Pojedinačna*	0 (0)	0 (0)	0 (0)	0 (0)	1 (8.33)	0 (0)	2 (14.29)	4 (19.05)	7 (7.29)
Mixed/*Mešoviti*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (9.52)	2 (2.08)
52 Single/*Pojedinačna*	0 (0)	1 (7.69)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.04)
Mixed/*Mešoviti*	0 (0)	0 (0)	0 (0)	1 (9.09)	1 (8.33)	2 (10.53)	0 (0)	0 (0)	4 (4.17)
53 Single/*Pojedinačna*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Mixed/*Mešoviti*	0 (0)	0 (0)	1 (50.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.04)
56 Single/*Pojedinačna*	0 (0)	1 (7.69)	0 (0)	1 (9.09)	0 (0)	0 (0)	0 (0)	0 (0)	2 (2.08)
Mixed/*Mešoviti*	1 (25.0)	0 (0)	0 (0)	1 (9.09)	0 (0)	1 (5.26)	0 (0)	0 (0)	3 (3.12)
58 Single/*Pojedinačna*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Mixed/*Mešoviti*	0 (0)	0 (0)	0 (0)	0 (0)	1 (8.33)	0 (0)	0 (0)	0 (00)	1 (1.04)
59 Single/*Pojedinačna*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Mixed/*Mešoviti*	0 (0)	0 (0)	0 (0)	2 (18.18)	0 (0)	0 (0)	0 (0)	0 (0)	2 (2.08)
66 Single/*Pojedinačna*	0 (0)	1 (7.69)	0(0)	1 (9.09)	0 (0)	0 (0)	0 (0)	0 (0)	2 (2.08)
Mixed/*Mešoviti*	0 (0)	0 (0)	0(0)	1 (9.09)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.04)
68 Single/*Pojedinačna*	0 (0)	0 (0)	0(0)	0(0)	0(0)	0 (0)	0 (0)	0 (0)	0 (0)
Mixed/*Mešoviti*	0 (0)	0 (0)	0(0)	1(9.09)	1(8.33)	1 (5.26)	0 (0)	0 (0)	3 (3.12)
73 Single/*Pojedinačna*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Mixed/*Mešoviti*	0 (0)	0 (0)	0 (0)	1 (9.09)	0 (0)	0 (0)	1 (7.14)	0 (0)	2 (2.08)
6 Single/*Pojedinačna*	0 (0)	1 (7.69)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.04)
Mixed/*Mešoviti*	2 (50.0)	4 (30.77)	2 (100)	4 (36.36)	0 (0)	1 (5.26)	0 (0)	0 (0)	13 (13.54)
11 Single/*Pojedinačna*	0 (0)	1 (7.69)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.04)
Mixed/*Mešoviti*	2 (50.0)	4 (30.77)	1 (50.0)	1 (9.09)	0 (0)	0 (0)	0 (0)	0 (0)	8 (8.33)
40 Single/*Pojedinačna*	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	0 (0)	0 (0)	0 (0)	0 (0)
Mixed/*Mešoviti*	0 (0)	1 (7.69)	0 (0)	0 (0)	1 (8.33)	0 (0)	0 (0)	0 (0)	2 (2.08)
42 Single/*Pojedinačna*	0 (0)	1 (7.69)	0 (0)	1 (9.09)	0 (0)	0 (0)	0 (0)	0 (0)	2 (2.08)
Mixed/*Mešoviti*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.76)	1 (4.76)
61 Single/*Pojedinačna*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Mixed/*Mešoviti*	1 (25.0)	1 (7.69)	1 (50.0)	0 (0)	0 (0)	1 (5.26)	0 (0)	0 (0)	4 (4.17)

Legend: n - number; LSIL - low-grade squamous intraepithelial lesion; HSIL - high-grade squamous intraepithelial lesion; HPV - human papillomavirus
Legenda: n - broj; LSIL - skvamozna intraepitelijalna lezija niskog stepena; HSIL - skvamozna intraepitelijalna lezija visokog stepena; HPV - humani papiloma virus

and intraepithelial lesions with identical cause (HPV), which also causes hyperproliferative changes in the genital tract [15]. In 1996, the World Health Association recognized the importance of HPV for cervical cancer [6]. Early detection and treatment of squamous cell abnormalities of the uterine cervix can be crucial in the prevention of cervical cancer [16]. About 75% of the sexually active population has been in contact with one or more HPV genotypes in the course of their lives [17]. Depending on the geographical region, the study population and the method used, the frequency of HPV genotypes varies considerably in various cervical lesions. In this study, HPV infection was detected in 75% of the examined women. This relatively high percentage of HPV infection in women with squamous cell abnormalities of the uterine cervix corresponds with some previously published studies; in the study of Mazarico et al. (2012), HPV infection was detected in 73.20% of women with squamous cell abnormalities of the uterine cervix [18], while in the study of Pista et al. (2013), HPV infection was detected in 77.4% of studied women [19]. HPV-16 was the most common genotype accounting for 43.75%. In addition to HPV-16, the most common genotypes were HPV-31 (15.62%), HPV-6 (14.58%), HPV-18 (10.41%), HPV-45 and HPV-11 (9.37%), HPV-33 (7.29%) and HPV-35, -52, -56 (5.21%). The retrospective study of Andonovska J. (2014) that included 7.411 women, detected the following distribution of the most common genotypes: HPV-16 (23.39%), HPV-31 (10.68%), HPV-53 (10.60%) and HPV-18 (6.19%) [20]. The study of Stojanovska V. et al. (2009) included 6.988 patients and established the following distribution of the most common genotypes: HPV-16 (32.1%), HPV-31 (14%), HPV-53 (12.6%), HPV-18 9.9%), HPV-58 (5%), etc. [21], whereas the study of Duvlis S. (2000) included patients from Macedonia and detected the following distribution of the most common genotypes: HPV-16 (27.5%), HPV-31 (13.1%), HPV-66 (10.3%), HPV-6 (9.4%), HPV-18 (8.4%), etc. [22]. In these four studies, the most common HPV genotypes were HPV-16 and HPV-31, but there were discrepancies in the distribution of other most common HPV genotypes. In this study, a significant association was found between the HPV infection and the incidence of squamous intraepithelial lesions and squamous invasive cervical cancer ($p = 0.028125$). High percentage of high-risk HPV genotypes found in severe dysplasia (73.91%), in *in situ* squamous cell carcinoma (87.50%) and invasive squamous cell carcinoma (83.33%) once again confirmed a strong relationship between the oncogenic potential of the virus, the development of squamous intraepithelial lesions, and squamous invasive carcinoma of the cervix ($p < 0.00001$). The relationship between high-risk and low-risk HPV-genotypes in HPV positive patients was 75.00%:14.58%. In the Spanish study conducted by Garcia-Garcia in 2010, the ratio was 79.80%:19.70% [23]. The high percentage of HPV-16 in severe dysplasia (63.16%), in *in situ* squamous cell carcinoma (57.14%) and invasive squamous carcinoma (47.62%), distinguishes HPV-16 as a high risk genotype with the highest oncogenic potential. In this study, mixed HPV infection was found in 32.03% of patients, i.e. in 42.71% of HPV-positive DNA. This prevalence of mixed HPV infections corresponds with previously published studies of Sandri et al., 2009 (43.00%) [24], Cuschieri et al., 2001 (43.30%) [25], and Vujošević et al., 2012 (42.00%) [26]. The highest prevalence of HPV infection was found in patients under 30 years of age (85.71%), and the lowest in patients aged 30 to 39 (65.79%). Our results are in agreement with the results of some previously published studies: Italian study of Agodi et al., 2009, where the highest prevalence of HPV infection was detected in patients under the age of 25 (73.2%) [27]; American study of Evans, 2006, showed the highest prevalence of HPV infection in patients under the age of 30 (96.00%; 458/475) [28]; the same percentage of HPV infection (96.00%) was also found by Hariri et al., 2012, in a study that included 3.058 American women aged 18 to 39 [29]. The highest prevalence of mixed HPV infection was detected (58.33%) in the group of patients under 30 years of age. A high prevalence in the young population can be explained by their sexual behavior, that is, promiscuity. A Serbian study by Kovačević G. et al., 2016, including students from the University of Novi Sad found mixed HPV infection in 49.4% [30]. Relatively high rates of mixed HPV infection among the young population were also detected in the Bulgarian studies of Grozdanov P., 2014 (63.00%) [31] and Kovachev S., 2013 (53.00%) [32], as well as in the Romanian study of Moga M., 2014 (54.93%) [33].

Conclusion

This study has confirmed that there is an association between human papillomavirus deoxyribonucleic acid infection and atypical cervical squamous cells; the young population under 30 years of age is the most affected, and human papillomavirus-16 is the most common genotype in our environment.

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Rad je primljen 10. VII 2018.
Recenziran 5. IX 2018.
Prihvaćen za štampu 7. IX 2018.
BIBLID.0025-8105:(2018):LXXI:9-10:301-308.

CASE REPORTS
PRIKAZI SLUČAJEVA

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Case report
Prikaz slučaja
UDK 616.89-008.434:615.8
UDK 81'23
https://doi.org/10.2298/MPNS1810311T

NEUROREHABILITATION OF ALEXIA WITHOUT AGRAPHIA – A CASE REPORT

NEUROREHABILITACIJA ALEKSIJE BEZ AGRAFIJE – PRIKAZ SLUČAJA

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Summary

Introduction. Alexia without agraphia is an impairment of reading ability. Speech, auditory comprehension, repetition and writing are relatively intact. Due to a damage of the splenium of corpus callosum, alexia without agraphia is considered to be an interhemispheric disconnection syndrome. **Case Report.** We presented a 71-year-old male, with chronic hypertension, diabetes mellitus and dyslipidemia. The magnetic resonance imaging showed a lesion in the left medial temporal region, including the equilateral thalamus, posterior cingulate gyrus, splenium of corpus callosum, lingual occipital gyrus, and the tail of the hippocampus. Lacunar ischemia was found on the right side of cerebellum. The neuro-linguistic diagnostic protocol included the Mini Mental State Examination, Boston Diagnostic Aphasia Examination, Boston Naming Test and phonemic and category fluency tests. We have also designed a clinical protocol for color recognition assessment. The results showed a mild cognitive impairment related to the time and space orientation, delayed memory and reading. On the speech and language levels, a severe acquired alexia without agraphia was registered which was not associated with other language modalities. **Conclusion.** The neuro-linguistic tests and clinical techniques provide a rather reliable diagnostic criteria, which is the basis for neuro-rehabilitation. The rehabilitation protocol refers to training techniques: tactile-kinesthetic recognition of graphemes and application of various reading techniques, such as letter-by-letter reading, Multiple Oral Re-reading, melodic intonation therapy and oral reading technique in order to facilitate rehabilitation of reading.

Key words: Neurological Rehabilitation; Alexia, Pure; Corpus Callosum; Cognition Disorders; Stroke; Hemianopsia; Mental Status and Dementia Tests

Sažetak

Uvod. Aleksija bez agrafije se manifestuje oštećenjem sposobnosti čitanja uz relativnu očuvanost ostalih modaliteta jezičkih funkcija: spontanog govora, auditivnog razumevanja, ponavljanja i pisanja. Zbog oštećenog splenijuma korpusa kalosum aleksija bez agrafije se smatra primerom interhemisfernog diskonekcionog sindroma. **Prikaz slučaja.** Prikazujemo 71-godišnjeg muškarca, sa višegodišnjom hipertenzijom, dijabetesom melitus i dislipidemijom. Magnetna rezonancija pokazuje leziju mediotemporalno levo sa zahvatanjem istostranog talamusa, posteriornog cingularnog girusa, splenijuma korpusa kalosuma, lingvalnog okcipitalnog girusa i repa hipokampa ishemijske etiologije; lakunarna ishemija cerebelarno desno. Neurolingvistički dijagnostički protokol uključuje *Mini mental test*, *Bostonski test* za dijagnozu afazije, *Bostonski test imenovanja* i testove fonemske i kategorijalne fluentnosti. Posebno je strukturisan klinički protokol za procenu sposobnosti prepoznavanja i imenovanja boja. Analiza rezultata pokazuje blagi kognitivni pad koji se odnosi na poremećaj vremenske i prostorne orijentacije, odloženog pamćenja i čitanja. Na govorno-jezičkom planu se registruje stečena aleksija teškog stepena bez agrafije koja, klinički značajno, ne remeti ostale modalitete jezika. **Zaključak.** Primena neurolingvističkih testova i kliničkih tehnika omogućava pouzdanije dijagnostičke kriterijume na osnovu kojih je strukturisana neurorehabilitacija. Rehabilitacioni protokol se odnosi na trening tehnika taktilno-kinestetskog prepoznavanja grafema i primenu različitih tehnika čitanja, kao što su tehnika „slovo po slovo", tehnika višestrukog glasnog ponovljenog čitanja i melodijski intonirana tehnika glasnog čitanja sa ciljem restitucije stečenog poremećaja čitanja.

Ključne reči: neurološka rehabilitacija; aleksija bez agrafije; korpus kalosum; poremećaji kognicije; moždani udar; hemianopsija; testovi mentalnog statusa i demencije

Introduction

Alexia without agraphia syndrome was first described by Dejerine in a patient who had a stroke in

Acknowledgement
The research was financed in part by the Ministry of Education, Science and Technological Development of the Republic of Serbia, within the projects No. 178027.

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Abbreviations
MMSE – mini mental state examination
BDAE – Boston diagnostic aphasia examination
VWFA – visual word form area
LBL – letter by letter
BNT – Boston naming test
ARNC – ability for recognition and naming of colors
MRI – magnetic resonance imaging
MCI – mild cognitive impairment

the medial part of the occipital lobe and the splenium of corpus callosum, impairing activation of orthographic word representations [1–3]. Alexia without agraphia is an impairment of reading ability with relatively preserved other linguistic functions: spontaneous speech, auditory comprehension, repetition and writing. Although other linguistic functions are mainly intact, these patients have a severe form of alexia, with acquired reading deficit, impaired comprehension of written text and no ability to directly read what they have written [2, 4].

Alexia without agraphia is a clinical syndrome that most often manifests as verbal alexia (inability to read words), and in most severe cases as a literal alexia (inability to read individual graphemes) [5]. A recent research on the neurorehabilitation of alexia without agraphia has emphasized the existence of neuro-anatomical correlates, defined as the Visual Word Form

Table 1. Neurolinguistic testing
Tabela 1. *Neurolingvističko testiranje*

Mini Mental State Examination *Mini mentalni test*	23/30 Loss of points: time orientation (3) space orientation (2) memory (1) reading (1) *Gubitak poena: vremenska orijentacija (3) prostorna orijentacija (2) pamćenje (1) čitanje (1)*
Boston Diagnostic Aphasia Examination *Bostonski test za dijagnozu afazija*	Achievement: *Postignuće:*
Phonemic fluency (S, K, L)/*Fonemska fluentnost (S, K, L)*	11, 15, 14
Category fluency/*Kategorijalna fluentnost*	17
Words differentiation/*Prepoznavanje reči*	62/72 >15 SD
Body parts repetition/*Delovi tela*	20/20
Commands/*Nalozi*	15/15
Complex ideational material/*Kompleksni ideacioni material*	8/12 > 2 SD
Oral nonverbal skill/*Oralna neverbalna spretnost*	9/12
Oral verbal skill/*Oralna verbalna spretnost*	14/14
Automatized sequences/*Automatizovani nizovi*	8/8
Word repetition/*Ponavljanje reči*	10/10
Great probability sentences repetition *Ponavljanje rečenica velike verovatnoće*	8/8
Small probability sentences repetition *Ponavljanje rečenica male verovatnoće*	8/8
Words reading/*Čitanje reči*	11/30
Sentences reading/*Čitanje rečenica*	0/10 > 20 SD
Naming induced by questions/*Imenovanje izazvano pitanjima*	30/30
Naming induced by images/*Imenovanje izazvano slikama*	89/114
Sentences and paragraph reading/*Čitanje rečenica i pasusa*	0/10 >10 SD
Boston Naming Test: correct answers *Bostonski test imenovanja: tačni odgovori*	47/60
Semantic support/*Semantička podrška*	28
Correct answers after semantic support *Tačni odgovori posle semantičke podrške*	13
Phonemic support/*Fonemska podrška*	17
Correct answers after phonemic support *Tačni odgovori posle fonemske podrške*	8
Mistakes of educational nature/*Greške edukativne prirode*	6
Visual perceptive mistakes/*Vizuo-perceptivne greške*	17
Verbal paraphasia/*Verbalna parafazija*	/
Phonological paraphasia/*Fonološka parafazija*	/

Table 2. Color recognition and color naming results
Tabela 2. *Rezultati na zadacima prepoznavanja i imenovanja boja*

Verbal-verbal tasks/*Verbalno-verbalni zadaci*		
Color fluency *Fluentnost boja*	10/1 min.	Successful *Uspešno*
Naming familiar colors after stimuli/*Imenovanje poznatih boja nakon stimulusa*	9/10	90%
Given color units naming/*Imenovanje jedinica zadate boje*	3,3,2,3	Difficult *Otežano*
Visual-visual tasks/*Vizuelno-vizuelni zadaci*		
Color adjustment - same quality stimulus/*Usklađivanje boja – stimulusi istog kvaliteta*	3/6	50%
Color adjustment - different quality stimulus/*Usklađivanje boja – stimulusi različitog kvaliteta*	6/10	60%
Linking images and matching colors/*Povezivanje slika i odgovarajućih boja*	3/5	60%
Verbal-visual tasks/*Verbalno-vizuelni zadaci*		
Color naming/*Imenovanje boja*	3/7	43%
Color names comprehension/*Razumevanje naziva boja*	4/9	44%
Pointing colors of a familiar stimulus/*Pokazivanje boja poznatih stimulusa*	3/8	37.5%

Area (VWFA), which participates in the restitution of of letter-by-letter (LBL) reading technique [6]. Alexia without agraphia often includes abnormalities in visual field (hemianopia), color anomia [2, 7], number alexia, and diverse degrees of verbal memory impairments. However, patients with number alexia can recognize numbers in a tactile way [5].

The aim of this paper is to show the original diagnostic protocol and the neuro-rehabilitation program for the clinical syndrome of alexia without agraphia of vascular etiology.

Case Report

We are presenting a 71-year-old male, a retired high-school teacher of Serbian language, an ambidexter. The first symptoms the patient described included inability to recognize numbers, read newspapers and use the phone. The personal history data showed a long-standing hypertension, diabetes mellitus and dyslipidemia, which were pharmaceutically controlled. He was a former smoker. The family history was burdened by cardiovascular diseases. Neurological findings revealed homonymous hemianopsia on the right side and positive pronation and toning of the right limbs, without asymmetry of reflexes.

Magnetic resonance imaging indicated a lesion in the left medial temporal region, including the equilateral thalamus, posterior cingulate gyrus, splenium of corpus callosum, lingual occipital gyrus, and the tail of the hippocampus. The lesion had an ischemic etiol-

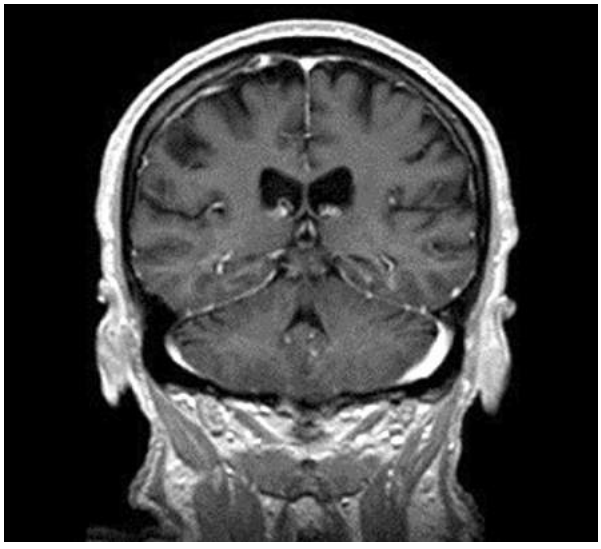

Figure 1. Magnetic resonance tomogram in the coronal plane in the FLAIR sequence
Slika 1. *Magnetno-rezonantni tomogram u koronarnoj ravni u FLAIR sekvenciji*

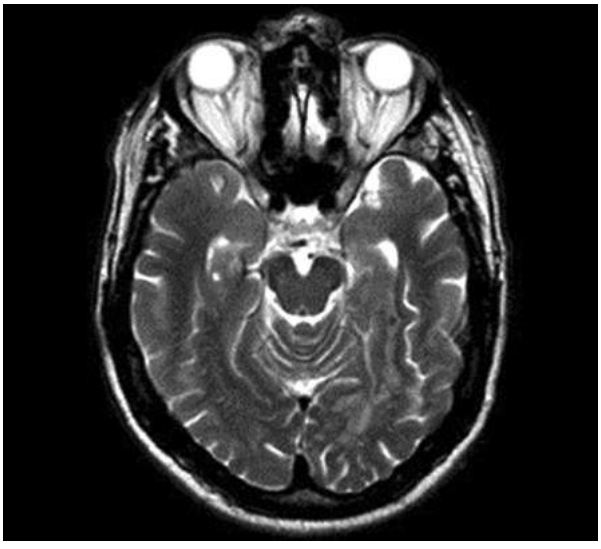

Figure 2. Magnetic resonance tomogram in the axial plane in the T2W TSE sequence
Slika 2. *Magnetno-rezonantni tomogram u aksijalnoj ravni u T2W TSE sekvenciji*

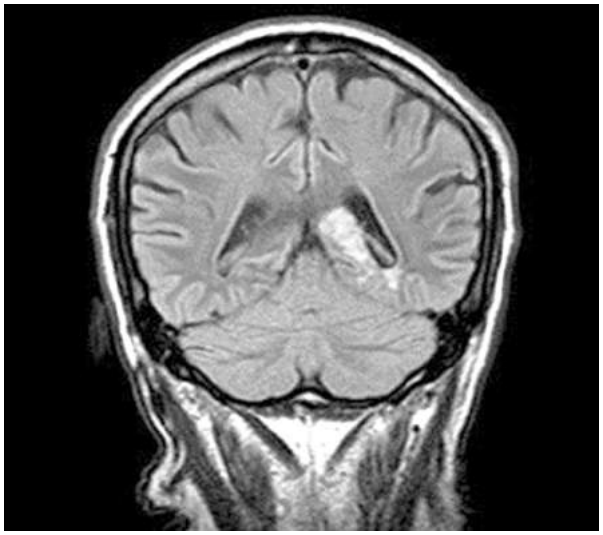

Figure 3. Postcontrast image in the coronal plane
Slika 3. *Postkontrastna slika u koronarnoj ravni*

ogy. Lacunar ischemia was found on the right side of the cerebellum **(Figures 1, 2 and 3)**.

Neuropsychological findings revealed a mild cognitive impairment (MCI) (Mini Mental State Examination - MMSE 23/30) manifesting with incomplete orientation in space and time, delayed verbal memory and a reading disorder. A severe alexia without agraphia and abnormalities in visual field significantly compromised the assessment of complex attention modalities, all reading modalities and comprehension of written text. A significant reduction in visual-perceptual and visual-constructive abilities and different modalities of visual gnosis (visual agnosia for colors, objects and numbers) were recorded. These findings were in line with a MCI and clinical picture of a severe alexia without agraphia, of a vascular etiology.

The neuro-linguistic diagnostic protocol used for the assessment of speech and language status [8] included MMSE [9], Boston Diagnostic Aphasia Examination (BDAE) [10], Boston Naming Test (BNT) [11] and phonemic and category fluency tests [12]. For the purposes of this research, we have designed a specific protocol for the assessment of the ability for recognition and naming of colors (ARNC) (verbal-verbal, visual-visual and visual-verbal tasks).

The analysis of gathered results showed a MCI (MMSE 23/30) related to the orientation in time and space, delayed memory and reading disorder **(Table 1)**. Neuro-linguistic evaluation showed mildly reduced fluency of spontaneous speech, difficulties in spontaneous naming, delayed initiation and tempo, normal articulation and preserved grammar. The voice was slightly rough and hypophonic. Evaluation of different modalities of listening comprehension was done by BDAE subtests showing slow and difficult auditory object recognition, while motor performance of complex verbal commands and comprehension of complex ideational material (CIM) remained preserved. All modalities of repetitive speech were intact. Verbal divergent thinking was preserved for phonemic and category fluency tasks **(Table 1)**. The analysis of BDAE subtests, which examined different modalities of reading showed a severe alexia without agraphia. Partially preserved analytic–synthetic ability enabled the patient to use the LBL reading technique relatively well so he successfully read five out of ten given words. Reading of longer words and sentences was completely unsuccessful **(Table 1)**. Also, the patient was not able to read his own handwriting, although just before that, he was writing correctly by dictation. The writing mechanism was not impaired. Transcription was significantly impaired with the tendency for omission, substitution and transposition of graphemes.

Based on the clinical examinations, it was confirmed that the patient had a visual agnosia for numbers and objects. The assessment of tactile number recognition (graphesthesia) was completely preserved (10/10), and mildly reduced for graphemes (8/10). Confrontational naming (BNT) was preserved, even though a large number of visual-perceptive errors were observed (for example: for a pretzel, the patient said "the thing you put your keys on"), which confirmed the clinical picture of visual agnosia for objects.

For clinical examination purposes, we have designed special tasks for the assessment of ARNC **(Table 2)**. The difficulty in solving these tasks confirmed the assumption that, in our patient, the clinical picture of alexia without agraphia was accompanied by color agnosia. By assessing this disorder in visual perception, discrimination, and color naming, we noted difficulties in verbal-visual tasks when the patient was asked to name the visually presented colors. He also had difficulties understanding the color names when he was asked to find the named color on the offered color palette, and failed to show the color of audibly presented familiar stimuli (for example: a pallet of ten colors was placed before the patient, and he was asked to show the color of the sun, tomato, sky...) **(Table 2)**.

Discussion

We have presented a patient with a relatively rare syndrome of alexia without agraphia, which occurs as a consequence of vascular lesion in the left medial occipitotemporal gyrus and splenium of corpus callosum **(Figures 1, 2 and 3)** with abnormalities in the visual field (right sided homonymous hemianopsia), visual agnosia and anomia for colors, objects and numbers [2, 7], with all modalities of writing preserved. Right after the patient wrote by dictation, he was not able to read his own handwriting [2, 4]. The writing automatisms were completely preserved while the transcription was significantly impaired due to the impaired capacity for graphic-phonetic conversion and the comprehension of the read text.

Our results confirmed the clinical picture of alexia without agraphia which arises as a consequence of a lesion in medial occipitotemporal gyrus in dominant hemisphere [3], which is also known as an anatomic correlate for Visual Word Form Area (VWFA) [13].

This VWFA is included in the simultaneous recognition of complete words, and therefore also into the fluent, conventional reading.

The reading disorder in our patient manifested as a severe degree of verbal alexia, inability of reading words, and as literal alexia, an inability of reading individual graphemes [5]. Detailed quantitative and qualitative analysis of achievements on the applied neurolinguistic tests (BDAE, BNT) and clinical examination (ARNC) determined techniques for neurorehabilitation of the reading disorder.

In the initial phase of rehabilitation, our patient was not able to recognize most of the presented individual graphemes. Based on our findings, the starting point of reading rehabilitation included two types of treatment, tactile and kinesthetic. Tactile treatment uses the sense of touch, by imprinting graphemes on the patient's skin, with the request to name the imprinted grapheme. Kinesthetic treatment for graphemes identification uses muscle movements, and is applied by imprinting or scanning a grapheme by patient's finger [5]. The daily practice of recognizing graphemes presented in visual, audible and tactile ways resulted in the restoration of the ability to name all the graphemes of the mother tongue. We have especially structured exercises of pairing the same graphemes written differently and orthographically similar graphemes. A significant part of the neurorehabilitation program was related to graphic-phonemic conversion exercises and analytical-synthetic skills. Further, the tasks were more and more complicated, so the patient was asked to read the syllables, then monosyllabic and afterwards polysyllabic words. In the initial phase of rehabilitation, the patient was using the LBL technique. The next phase was "syllabic reading". On higher levels of rehabilitation, the multiple oral re-reading technique was used. After mastering reading polysyllabic words, the patient was given a task to read simple, and then complex sentences. After that, he was requested to read short stories, using melodically intonated oral reading technique, with a task of immediate and delayed recall. Afterwards, the following exercises were introduced to the patient: lexical decision, filling blanks (typing missing letters/words), execution of written commands, and making decisions whether the written words or sentences were written correctly.

Conclusion

Alexia without agraphia of vascular etiology with visual field abnormalities (hemianopsia), visual agnosia and anomia for colors, objects and numbers with preserved writing is most commonly a consequence of a stroke in the area of internal and lower part of occipital lobe. Ischemic lesion of the splenium of corpus callosum is always present, and it leads to the disruption of information flow from occipital lobe to corresponding speech areas.

In conclusion, we believe that the application of presented neurolinguistic tests (Boston diagnostic aphasia examination, Boston naming test) and the ability for recognition and naming of colors technique provided reliable diagnostic criteria, as well as developing individual neurorehabilitation. The basic therapy protocol included a cognitive training of tactile-kinesthetic techniques for graphemes recognition and application of various reading techniques, such as letter-by-letter technique, multiple oral re-reading technique, and melodically intonated oral reading technique in order to facilitate rehabilitation of an acquired reading disorder.

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Rad je primljen 10. VIII 2018.
Recenziran 20. VIII 2018.
Prihvaćen za štampu 21. IX 2018.
BIBLID.0025-8105:(2018):LXXI:9-10:309-313.

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Case report
Prikaz slučaja
UDK 616.155.392:616.992-08]-053.2
https://doi.org/10.2298/MPNS1810316K

DISSEMINATED FUSARIOSIS IN A PEDIATRIC PATIENT WITH ACUTE LYMPHOBLASTIC LEUKEMIA AND PROLONGED FEVER – A CASE REPORT

DISEMINOVANA FUZARIOZA KOD PEDIJATRIJSKOG BOLESNIKA SA AKUTNOM LIMFOBLASTNOM LEUKEMIJOM I PRODUŽENOM FEBRILNOŠĆU – PRIKAZ SLUČAJA

Nataša KAĆANSKI[1], Branislava RADIŠIĆ[1] and Jovanka KOLAROVIĆ[1, 2]

Summary

Introduction. Infections caused by fungi of Fusarium species occur in immunocompromised individuals as disseminated diseases. **Case Report.** This case report presents a 5-year-old boy with acute lymphoblastic leukemia who developed a disseminated fusarium infection during reinduction chemotherapy. Fever was the main symptom and it lasted for 15 weeks. Refractory fever despite broad-spectrum antibiotics, as well as nausea, myalgia, pulmonary symptoms with detection of pulmonary infiltrates, liver and spleen involvement indicated an invasive fungal infection. The patient received fluconazole, voriconazole, liposomal amphotericin B and caspofungin. Since high temperature was persistent, diagnostic laparoscopy of the abdomen was done. Scattered lesions, up to 2 mm in diameter, were observed macroscopically on the surface of the liver and spleen. The liver culture was positive for Acinetobacter and Fusarium species. After 38 days of therapy with liposomal amphotericin B and 3 days of ciprofloxacin, the patient became afebrile. Itraconazole (according to the antimycogram) was continued during maintenance therapy. Abdominal ultrasound was completely normal after 5 months of treatment with itraconazole. This boy was our first patient with a disseminated fusarium infection. At that time, Fusarium was detected in the hospital water system and in hospital air samples. **Conclusion.** A timely diagnosis of invasive fungal diseases in children is a big challenge. Over the past decade, there has been an increase in survival rate of patients with invasive fusariosis due to much more common use of voriconazole or combined antifungal therapy.

Key words: Fusariosis; Immunocompromised Host; Fever; Invasive Fungal Infections; Signs and Symptoms; Laparoscopy; Antifungal Agents

Sažetak

Uvod. Infekcije prouzrokovane plesnima iz roda *Fusarium* javljaju se kod imunokompromitovanih bolesnika kao diseminovana bolest. **Prikaz slučaja.** U ovom prikazu slučaja je predstavljen petogodišnji dečak sa akutnom limfoblastnom leukemijom kod koga se razvila diseminovana fuzarijum infekcija tokom reindukcije. Glavni simptom je bila povišena temperatura koja je trajala 15 nedelja. Temperatura koja nije reagovala na antibiotike širokog spektra, mučnina, bolovi u mišićima, plućni simptomi sa prisutnim plućnim infiltratima, zahvatanje jetre i slezine upućivali su na gljivičnu infekciju kod našeg bolesnika. On je dobijao flukonazol, vorikonazol, lipozomalni amfotericin B i kaspofungin. Pošto je visoka temperatura i dalje bila prisutna, urađena je dijagnostička abdominalna laparoskopija. Makroskopski su uočene rasute, tačkaste promene promera do 2 mm na površini jetre i slezine. Iz uzorka jetre našeg bolesnika iskultivisani su *Acinetobakter* i *Fuzarijum*. Nakon 38 dana primene lipozomalnog amfotericina B i tri dana terapije ciprofloksacinom, bolesnik je postao afebrilan. Itrakonazol (prema antimikogramu) primenjivan je tokom terapije održavanja. Ultrazvuk abdomena je bio uredan nakon pet meseci primene itrakonazola. Ovaj dečak je bio naš prvi bolesnik sa diseminovanom fuzarijum infekcijom. U to vreme, fuzarijum je nađen u bolničkom vodovodu i vazduhu. **Zaključak.** Pravovremena dijagnoza invazivne gljivične infekcije kod dece je veliki izazov. U protekloj deceniji, preživljavanje bolesnika sa invazivnom fuzariozom je poraslo zahvaljujući mnogo češćoj primeni vorikonazola ili kombinovane antigljivične terapije.

Ključne reči: fusarioza; imunokompromitovani bolesnik; febrilnost; invazivne fungalne infekcije; znaci i simptomi; laparoskopija; antimikotici

Introduction

Fusarium species (spp.) are environmental fungi widely distributed in the soil, organic substrates and water. They also cause a broad spectrum of human infections [1].

Infections caused by fungi of Fusarium spp. occur in immunocompromised individuals as a disseminated disease. A high resistance of Fusarium spp. to most antifungal agents leads to mortality rate over 50% among immunocompromised patients [1, 2].

We report a case of a pediatric patient with acute lymphoblastic leukemia (ALL) and prolonged fever due to a disseminated infection with fusarium with unusual properties.

Case Report

A 5-year-old boy was diagnosed with ALL. The previous treatment (induction, consolidation and most of the reinduction chemotherapy) lasted about 9 months and there were no complications or delays.

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Abbreviations

ALL – acute lymphoblastic leukemia
CRP – C-reactive protein
FLU – fluconazole
US – ultrasonography
VRC – voriconazole
CPFG – caspofungin
L-AMB – liposomal amphotericin B
CT – computed tomography
GMI – galactomannan index
WBC – white blood cells

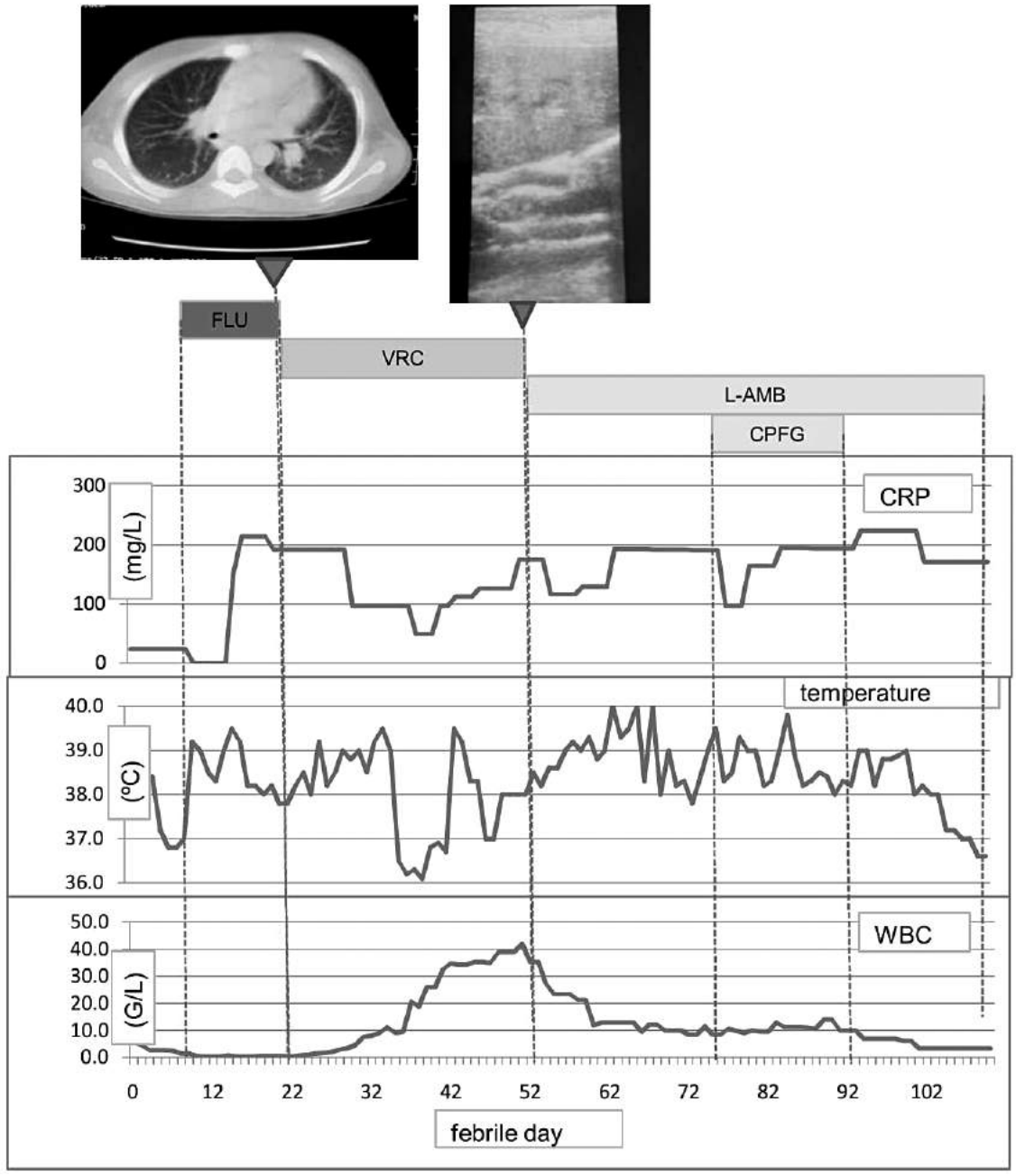
At the end of the reinduction phase, the boy developed fever and it lasted for the next 15 weeks.

In the beginning, the boy had a fever, but he was in a good condition with normal physical findings. The white blood cells (WBC) count was 2.4 G/l and C-reactive protein (CRP) level was 24 mg/l. Blood, urine and stool cultures were negative. He was initially treated with ceftriaxone and at the same time the reinduction phase was finished.

After that, in the next 4 weeks, the patient presented with high temperature every day **(Graph 1)**, (taken 2 to 3 times per day) associated with malaise, loss of appetite, and occasional vomiting without diarrhea. During the first two weeks he had a non-productive cough. At the end of the fourth week, decreased breath sounds were noted in the right lung. In this period, the boy had severe granulocytopenia for 3 weeks. The CRP ranged from 96 – 192 mg/l. Urine analysis, chest X-ray, X-ray of the paranasal sinuses, echocardiography and abdominal ultrasonography (US) revealed no abnormal findings. Repeated blood, urine, and stool cultures were also negative. After 28 days of treatment with broad-spectrum antibiotics and a 10-day course of intravenous fluconazole (FLU), the patient still had daily fevers. Extensive infectious and autoimmune workup was negative and the source of fever was not revealed. Complete remission was confirmed on bone marrow examination. A chest computed tomography (CT) scan (22nd febrile day) revealed multiple lung nodules, 5 mm in diameter **(Graph 1)**. In addition to slightly enlarged liver and spleen, abdominal CT scan showed no abnormalities. Voriconazole (VRC) was initiated. The patient`s WBC stabilized at around 3 G/l, while the CRP was high despite antifungal therapy. A week later, while continuously febrile, the boy stopped coughing. However, he was extremely ill and complained of severe pain in the legs. Ferritin was high (2433 ug/l). In the sixth week, the patient was given a course of parenteral methylprednisolone (1 mg/kg daily) for 2 weeks with VRC concomitantly. On day 2 of steroids, there was a complete defervescence, and the CRP level decreased. During a reduction in the dose of steroids, the boy became febrile once again with an increase in CRP. Anti-Candida and anti-Aspergillus antibodies of the IgM and IgG classes, Aspergillus galactomannan and Candida mannan antigens values were monitored during the illness **(Table 1)**. VRC (used for 30 days) was switched to liposomal amphotericin B (L-AMB) in the 10th week of illness. The patient remained febrile and exhaustion worsened over the next weeks. Procalcitonin level was high and antibiotic therapy was reintroduced. Abdominal US (52nd febrile day) revealed multiple liver and splenic hypoechoic lesions, 7.5 mm in diameter, associated with hepatosplenomegaly **(Graph 1)**. Repeated abdominal US showed an increase in the size and number of these lesions. A peripheral venous catheter was applied at that point. At week 12 of high temperature, caspofungin (CPFG) was added to L-AMB. At the same time, maintenance therapy for ALL was initiated. As the high temperature was still present, in the 13th week a diagnostic abdominal laparoscopy was performed. Scattered spots up to 2 mm in diameter were observed macroscopically on the surface of the liver and spleen. The fungi were not detected by direct microscopy in the obtained samples. The sample culture remained positive for Acinetobacter spp. (susceptible to ciprofloxacin only) and Fusarium spp. Histopathology has shown chronic portal and light lobular hepatitis. Our patient received CPFG for 19 days and L-AMB for 45 days. After

Table 1. Fungal biomarkers during and after the fever
Tabela 1. *Gljivični biomarkeri tokom i nakon febrilnosti*

Elisa immunodiffusion assay *Elisa imunodifuzijski test*	week 7 *7. nedelja*	week 10 *10. nedelja*	week 13 *13. nedelja*	week 25 *25. nedelja*	reference values *referentne vrednosti*
anti-Candida IgM antibody *anti-Candida IgM antitela*	> 500	440	> 500	420	≥ 80 U/ml positive *≥ 80 U/ml pozitivan*
anti-Candida IgG antibody *anti-Candida IgG antitetela*	300	330	225	230	≥ 100 U/ml positive *≥ 100 U/ml pozitivan*
Candida mannan antigen *Candida mannan antigen*	0,34	0,94	1,28	0,4	index ≥ 0,5 positive *indeks ≥ 0,5 pozitivan*
anti-Aspergillus IgM antibody *anti-Aspergillus IgM antitela*	112	130	200	66	≥ 70 U/ml positive *≥70 U/ml pozitivan*
anti-Aspergillus IgG antibody *anti-Aspergillus IgG antitela*	390	310	280	450	≥ 70 U/ml positive *≥ 70 U/ml pozitivan*
Aspergillus galactomannan antigen *Aspergillus galactomannan antigen*	0,25	3,33	0,3	0,28	index ≥ 0,5 positive *indeks ≥ 0,5 pozitivan*



Graph 1. Clinical, biological, radiographic evolution and drug therapy
Grafikon 1. *Klinički, biološki, radiografski tok i primenjeni lekovi*

Legend: CRP - C-reactive protein; WBC - white blood cells; FLU - fluconazole; VRC - voriconazole; l-AMB - liposomal amphotericin B; CPFG - caspofungin
Legenda: CRP - C-reaktivni protein; WBC - bela krvna zrnca; FLU - flukonazol; VRC - vorikonazol; l-AMB - lipozomalni amfotericin B; CPFG - kaspofungin

38 days of therapy with L-AMB and 3 days with ciprofloxacin (a total of 14 days) the patient became afebrile in the 15th week. Itraconazole (according to the antimycogram) was given during maintenance therapy. The CRP level was still high in the next 2 months (above 50 mg/l). The abdominal US was completely normal after 5 months of treatment with itraconazole. Six years after discontinuation of antifungal therapy, the patient remains in complete remission of his neoplastic disease without signs of clinical infection.

Discussion

Fusarium spp. are widespread in nature. This genus of fungal opportunists was first identified in 1958. Among immunocompromised patients, Fusarium spp. are second to Aspergillus spp. as the most common cause of invasive fungal infections [1, 3]. Only a few species cause disease in humans, most often F. solani complex, F. oxysporum complex and F. fujikuroi complex [1].

Disseminated fusariosis occurs only in conditions associated with immunosuppression and some risk factors. Our patient had most of them: acute leukemia, previous treatment with high doses of dexamethasone, prolonged and severe neutropenia and extended antibiotic treatment [4, 5].

The typical clinical presentation is neutropenic fever in patients with myalgia and sudden appearance of erythematous papular or nodal painful skin lesions with central necrosis. Cutaneous lesions were not observed in our patient, although they are seen in approximately 85% of patients with early stage disseminated fusarium infections [6]. Pneumonia may be the only manifestation of the disease or part of a disseminated disease. It is very similar to invasive pulmonary aspergillosis with angioinvasion, lung infarction and characteristic nodules with or without the halo sign [6, 7]. The clinical signs of hepatosplenic disease typically develop after neutrophil recovery [7].

Despite broad-spectrum antibiotic therapy, refractory fever, nausea, myalgia, pulmonary symptoms with pulmonary infiltrates, liver and spleen involvement indicated an invasive fungal infection.

Standard diagnostic procedures for fungal infections include microscopic examination of liquid and solid diagnostic specimens, blood cultures and all clinical cultures, non-culture assays for fungal antigens and imaging studies [8].

Direct microscopic examination of biological samples is the fastest way for obtaining the diagnosis [6]. The sensitivity of blood culture to detect fungemia ranges between 21 and 71% for Candida, but it is very low for the infection caused by Aspergillus spp. [9]. Our patient did not have positive blood cultures, although they are often positive with the infections caused by Fusarium spp.

Fusarium spp. may contaminate laboratory specimens. The growth of fusarium from non-sterile samples in high risk patients should be interpreted as a probable infection until proven otherwise [6]. Liver culture from our patient was positive for Acinetobacter and Fussarium spp. The genus Acinetobacter is a major cause of nosocomial infections. Its ability to develop multidrug resistance and to persist in any environmental conditions makes infections by Acinetobacter very dangerous [10]. Our patient received antibiotics from all groups except fluoroquinolones. In this case, Acinetobacter was susceptible to ciprofloxacin only.

Identification of the genus Fusarium is not difficult. However, species identification requires molecular methods (matrix-assisted laser desorption ionization-time of flight mass spectrometry, multiplex PCR assay) [2, 6].

Remarkable advances in the early detection of invasive fungal infections have been made by the development of non-culture assays for fungal antigens [8]. Routine antigen detection with the Aspergillus galactomannan index (GMI) enzyme-linked immunosorbent assay (ELISA) test should be considered in high-risk patients. Positive GMI tests should be interpreted as indicative of invasive aspergillosis or invasive fusariosis [6]. Cross-reactivity has been observed for a number of other fungi including Fusarium species and some beta-lactam antibiotics. Serial GM assessments can also be used to monitor the effectiveness of antifungal therapy [9]. Routine Candida antibody and antigen testing and routine testing for Aspergillus antibodies are not recommended for patients with hemato-oncological malignancies [7]. In our patient, positive test for mannan and GMI, as well as antibodies to Candida and Aspergillus were probably due to cross-reactivity.

Our patient developed a disseminated fusarium infection. He received a broad spectrum of antibiotics and FLU first. After detection of pulmonary nodes he received an empiric treatment with VRC and later L-AMB for suspected aspergillosis. In the meantime, the cough has stopped. Chest X-ray findings were still normal. Due to suspicion of hemophagocytic lymphohistiocytosis, he received a short course of corticosteroids. Later, CPFG was added due to positive anticandida antibody and mannan antigen. Considering that the infection spread to the liver and spleen, both fusarium and acinetobacter were isolated from the liver sample. The fungigram revealed that the pathogenic agent was resistant to antifungal polyene, FLU, VRC and was sensitive to itraconazole, which is inconsistent with literature data.

This boy was our first patient with a disseminated fusarium infection. At that time, the Fusarium spp. was detected in the hospital water system and in hospital air samples.

Conclusion

A timely diagnosis of invasive fungal disease in children is a big challenge. Over the past decade, survival from invasive fusariosis has increased. This is most likely the result of much more frequent use of voriconazole or combined antifungal therapy, although European Guidelines recommend only one week of combination therapy.

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Rad je primljen 9. VI 2018.
Recenziran 19. VII 2018.
Prihvaćen za štampu 7. VIII 2018.
BIBLID.0025-8105:(2018):LXXI:9-10:314-318.

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Case report
Prikaz slučaja
UDK 616.718-006.03-07/-08-053.5/.6:796
https://doi.org/10.2298/MPNS1810321P

OSTEOID OSTEOMA IN A YOUNG ATHLETE – A CASE REPORT FROM THE PERSPECTIVE OF A PRIMARY HEALTH CARE PEDIATRICIAN

OSTEOID OSTEOMA KOD MLADOG SPORTISTE – PRIKAZ SLUČAJA IZ UGLA PEDIJATRA U PRIMARNOJ ZDRAVSTVENOJ ZAŠTITI

Vesna PETROVIĆ, Tanja ROŽEK MITROVIĆ and Danilo VIŠNJEVAC

Summary
Introduction. Osteoid osteoma is a benign osteoblastic tumor, usually 1.5 - 2 cm in diameter, characterized by a well demarcated central area (osteoid nidus) surrounded by a sclerotic bone. It accounts for 5% of all primary bone tumors and most often affects the femur and tibia. The tumor is mostly seen in the second and third decades of life, more often in males, and the typical symptom is pain that worsens at night and responds well to analgesics. **Case Report**. We present a case of a young athlete, 11 years of age, who presented with characteristic symptoms and localization, as well as a typical radiographic finding. The timely action of a primary health care pediatrician and radiography reduced the time to definitive diagnosis and prompt surgical treatment was provided. The boy gradually started resuming his sports activities six months after the beginning of symptoms and 2.5 months after the surgery. **Conclusion.** In differential diagnosis of chronic leg pain in patients under the age of 18 years, primary health care pediatricians rely on medical history and physical examination, whereas after that plain radiography of the painful area should be considered. If the nidus is clearly seen in the X-ray, the possibility of diagnosing an osteoid osteoma increases, and timely and adequate treatment are provided.
Key words: Bone Neoplasms; Osteoma, Osteoid; Pain; Child; Radiography; Signs and Symptoms; Primary Health Care; Treatment Outcome

Sažetak
Uvod. Osteoid osteoma je benigni osteoblastni tumor, obično veličine 1,5 – 2 cm, koga karakteriše dobro ograničena centralna zona (nidus), oko koje se nalazi zona sklerotične kosti. Čini 5% svih primarnih tumora kosti, a najčešće zahvata femur i tibiju. Karakteristično se javlja u drugoj i trećoj dekadi života, češće kod muškaraca, a tipičan simptom je bol koji se pojačava noću i koji dobro reaguje na analgetike. **Prikaz slučaja.** Prikazujemo slučaj mladog sportiste uzrasta 11 godina kod koga se bolest javila karakterističnim simptomima i lokalizacijom, uz dobru radiografsku vidljivost tumora te je pedijatrijski pregled i pravovremeno radiografsko snimanje značajno skratilo vreme do postavljanja konačne dijagnoze bolesti i obezbeđeno je promptno hirurško lečenje. Dečak je 6 meseci od pojave prvih simptoma, a 2,5 meseca nakon operacije uključen postepeno u sportske aktivnosti. **Zaključak.** U slučaju bola u nozi kod pacijenata starih do 18 godina u primarnoj zdravstvenoj zaštiti, pedijatar se u diferencijalno-dijagnostičkoj razradi mora osloniti na anamnezu i fizikalni pregled, te doneti odluku o radiografiji bolnog područja. Tako se povećava mogućnost postavljanja dijagnoze osteoid osteoma ukoliko je nidus jasno radiografski izražen te se obezbeđuje pravovremeno i adekvatno lečenje.
Ključne reči: neoplazme kosti; osteoid osteoma; bol; dete; radiografija; znaci i simptomi; primarna zdravstvena zaštita; ishod lečenja

Introduction

Osteoid osteoma (OO) is a benign osteoblastic tumor that was first described as a separate entity by Jaffe in 1935 [1]. It is usually of 1.5 – 2 cm in diameter and characterized by well demarcated central area (osteoid nidus) in a highly loose, well vascularized connective tissue. Surrounding the nidus is the zone of sclerotic bone and the central part of the nidus is often calcified. This tumor does not have the potential for distant metastases but can lead to functional and esthetic deficits [2, 3]. About 50% of lesions occur in the proximal meta-diaphysis of the long bones, most commonly femur or tibia [4, 5]. OO accounts for 10% of all symptomatic benign bone tumors and 5% of all bone tumors. It mostly occurs between 10 and 30 years of age and affects males 2 to 4 times more often than females [2, 3]. The main symptom is pain that worsens at night and is relieved by non-steroidal anti-inflammatory drugs (NSAIDs). The exact mechanism of pain is unknown but increased production of prostaglandins and increased nerve fiber density are found in the nidus [6]. In particular, prostaglandin E2 (PGE 2) is increased up to 1.000 times in comparison with the normal bone and is presumed to be the cause of severe pain seen in pa-

Acknowledgement
The authors would like to thank Vesna Vujić-Aleksić, MD (The Republic of Srpska Agency for Certification, Accreditation and Quality Improvement in Health Care, Bosnia and Herzegovina; Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Banja Luka, Bosnia and Herzegovina) for her kind help and contribution in the preparation of this manuscript.

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Abbreviations

OO – osteoid osteoma
NSAIDs – non-steroidal anti-inflammatory drugs
PGE2 – prostaglandin E2
COX – cyclooxygenase
ECG – electrocardiogram
BMI – body mass index
CT – computed tomography

tients with lesions [7]. Strong expression of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) in the osteoblasts within OO suggests an important role of COX in PGE production, thus explaining good response to NSAIDs [8].

Case Report

We report a case of an 11-year-old boy who visited the Pediatric Outpatient Clinic, part of the Primary Health Care Center complaining about pain in the right thigh in the past 3 months. He started complaining about the pain after a basketball training which he practiced regularly for 3 years, 3 times a week for 1.5 hours. His medical history showed that he had an annual sports physical exam by a pediatrician a month before the first symptoms occurred, without abnormalities at physical examination, electrocardiogram (ECG) and routine laboratory at that time, normal height of 150 cm and weight of 39 kg, and body mass index (BMI) of 17.33 kg/m². Initially, the pain was nonspecific, occurred occasionally and lasted for a short time, but sometimes woke him up at night. During the day he felt pain only if he received an accidental kick in the right foot, even if it was of low intensity (unintentionally, passing by). One month after the pain started, he stopped training, and even walking became difficult. His appetite decreased. The NSAIDs relieved the pain, but for a short time, especially at night. Two weeks before seeking medical attention, the pain worsened at night and sometimes he had to take NSAIDs twice a night. He denied any history of trauma or fever. At the first examination by his pediatrician, he was cautious when walking, pale, and felt severe pain on right thigh palpation (pain was evaluated as 7/8 on the Wong Baker Faces Pain Rating Scale). There was no leg deformity, no local swelling, and the skin above the painful area was normal. The X-ray of the right femur in two directions was performed and showed a homogeneous shadow of 80 x 20 mm in the proximal and medial part of the right femur body. In the upper third part of the described shadow an illumination of 10 mm x 3 mm was seen **(Figure 1)**. OO was suspected and the patient was referred to an orthopedist.

The orthopedic physical exam revealed a muscle atrophy of the right thigh and a difference between the right and left thigh muscle volume of 1.5 cm. Hospitalization was indicated by the orthopedist. Computed tomography (CT) of the right thigh confirmed a nidus and open surgical resection under

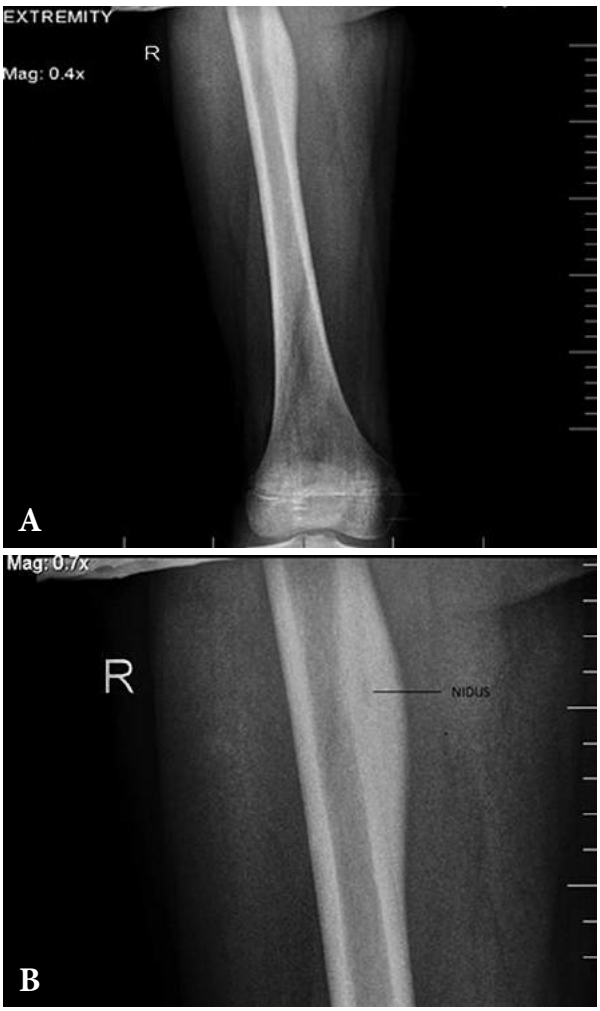

Figure 1. A) X-ray of the right femur with a homogeneous shadow in the proximal and medial part of the femoral shaft (standard image); B) Enlarged view shows a nidus in the upper third of the shadow (arrow)
Slika 1. A) Rendgenski snimak desnog femura s homogenom senkom u proksimalnom i medijalnom delu tela femura (standardni snimak); B) Uvećani prikaz nidusa u gornjoj trećini opisane senke (strelica)

general anesthesia was performed 2 weeks after the pediatrician's examination. The patient recovered rapidly and was discharged after 7 days of hospitalization. Weight bearing and physical activities were restricted. The patient was able to walk with crutches with the affected foot touching the ground. Five days after discharge, the pain was completely relieved and he stopped taking NSAIDs. Exercises for muscle strengthening progressively improved the range of motion through eight weeks and he was gradually involved in sports activities afterwards. The histopathological analysis confirmed the diagnosis of OO (dg. Neoplasm benignum ossium longorum extremitatis inferioris osteoid osteoma) barely 4 months after the onset of pain. After 12 months of follow up, no local recurrence

was observed and the difference between the right and left thigh volume was less than 1 cm. Nine months after surgery and 13 months since the routine pediatric visit, the patient had normal height and weight (155 cm, 41 kg, BMI 17.07 kg/m²).

Discussion

Osteoid osteoma is a benign tumor that commonly affects male adolescents and young adults. The proximal femur and tibia are the commonest locations of OO. The typical clinical presentation is nocturnal pain that responds well to NSAIDs. However, the pain is associated with various disorders which is one of the reasons why OO may be misdiagnosed and correct diagnosis delayed (median 16 months, range 8 – 36 months) [9, 10]. In children, OO may be mimicking different diseases such as infantile cortical hyperostosis, osteomyelitis, Perthes disease, leg length discrepancy, healing stress fractures, tuberculosis, neuromuscular conditions, as well as malignant tumors (osteosarcoma, Ewing sarcoma) [11]. The diagnosis of OO can be a challenging process especially in young athletes. The etiologic factors may be the overuse injury and muscle strain. Some reports present similar clinical cases in athletes with femoral neck stress fractures and intra-articular OO of the femoral neck that may further delay the correct diagnosis [12]. Other unusual localizations, such as metacarpal, hallux, talus or intra-articular are also associated with delayed diagnosis or misdiagnosis of OO [13–16]. Primary health care pediatricians always have a dilemma whether to perform an X-ray of the painful area or not. Radiography is sufficient for establishing the diagnosis of OO if the nidus is clearly expressed, as in this case, so the initial diagnosis was confirmed barely 4 months after the onset of pain. However, there are vague, more calcified cases where nidus overlaps with the surrounding bone sclerosis on the X-rays. Even in case of clear radiographic findings, CT is a gold standard in the preoperative diagnosis of OO [17]. The therapeutic approach depends on the localization, as well as on the severity of symptoms or a potential deformity. NSAIDs may sometimes lead to withdrawal of symptoms and resolution of the tumor [18]. Unsuccessful treatment with NSAIDs or intolerance to analgesics leads to surgical treatment. Open surgical resection is gold standard, but nowadays, minimally invasive surgical methods, such as percutaneous radiofrequency ablation and percutaneous excision, are also becoming important modalities [19, 20]. Marić et al. showed that modified percutaneous techniques provide fast recovery after the procedure and significantly decrease length of hospital stay compared with traditional surgical methods (2,43 ± 0,53 versus 10 ± 7.79 days) [21, 22]. However, even after complete nidus removal, anatomical changes and sequelae may progress and become symptomatic, so long-term follow-up is therefore essential [23]. Histopathological analysis sets the final diagnosis, and as in our case, confirming the clinical and imaging findings. Removal of nidus leads to resolution of pain, so except during the short post-operative period, analgesia is no longer needed, which was also the case in our patient [24].

Conclusion

In differential diagnosis of chronic leg pain in patients under the age of 18 years, primary health care pediatricians rely on medical history and physical examination, whereas after that plain radiography of the painful area should be considered. If the nidus is clearly expressed, the possibility of diagnosing osteoid osteoma timely and providing adequate treatment increases. In the presented case, the correct diagnosis was confirmed barely 4 months after the onset of pain.

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Rad je primljen 25. II 2018.
Recenziran 25. VI 2018.
Prihvaćen za štampu 3. X 2018.
BIBLID.0025-8105:(2018):LXXI:9-10:319-322.

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UDK 616.72-002-07/-08
https://doi.org/10.2298/MPNS1810325B

CURRENT PRINCIPLES OF DIAGNOSIS AND TREATMENT OF REACTIVE ARTHRITIS

SAVREMENI PRINCIPI DIJAGNOSTIKE I LEČENJA REAKTIVNOG ARTRITISA

Ksenija BOŠKOVIĆ

Summary

Introduction. Reactive arthritis is an autoimmune inflammatory rheumatic disease which develops as a reaction to urogenital or intestinal infections. **Clinical Signs and Symptoms.** It manifests as a peripheral asymmetrical monoarthritis or polyarthritis, mainly involving the lower extremities. Apart from joints, it can also affect the spinal cord, but also involve the muscle attachment sites, tendons, bursae, conjunctiva, anterior segment of the eye, damage to the skin and mucous membranes, causing typical asymmetrical sausage-like edema of fingers and/or toes. **Diagnosis.** The diagnosis is based on the Berlin Diagnostic Criteria including the characteristics of peripheral arthritis and evidence of previous infection. Approximately 65% - 85% of patients with reactive arthritis are positive for human leukocyte antigen - B27. **Treatment of Reactive Arthritis.** The therapy includes antibiotics chosen according to the antibiogram for the causative agent of the infection. The therapy is aimed at pain management and control of the autoimmune response of synovial lining of the joints, i.e. at prevention of articular damage. Drug therapy includes non-steroidal anti-inflammatory drugs, analgesics, steroids, immunosuppressive agents and biological drugs. Other methods of treatment are also recommended, such as rest in the acute phase of the disease, physical therapy and patient's education. **Conclusion.** Development of new diagnostic methods, particularly molecular diagnostics, and new therapeutic modalities using new generation drugs, has created conditions for more efficient treatment of reactive arthritis.

Key words: Arthritis, Reactive; HLA-B27 Antigen; Diagnosis; Signs and Symptoms; Pain; Bacterial Infections; Anti-Inflammatory Agents, Non-Steroidal

Sažetak

Uvod. Reaktivni artritis predstavlja autoimuno zapaljensko reumatsko oboljenje koje nastaje najčešće kao odgovor na urogenitalnu ili crevnu infekciju. **Klinička slika** se manifestuje kao periferni asimetrični monoartritis ili oligoartritis, pretežno lokalizovan na donjim ekstremitetima. Može biti zahvaćen i kičmeni stub uz vanzglobne manifestacije bolesti kao što su upala pripoja mišića, tetiva, burzi, konjuktiva, prednjeg segmenta oka, oštećenja kože i sluznica, karakterističnog asimetričnog kobasičastog otoka prstiju šaka i/ili stopala. **Dijagnostika** se postavlja na osnovu *Berlinskih dijagnostičkih kriterijuma* koji se zasnivaju na karakteristikama perifernih artritisa i dokazu prethodne infekcije. Kod 65–85% bolesnika nalazimo pozitivan nalaz humanog leukocitnog antigena-B27. **Lečenje reaktivnog artritisa.** U terapiju se uključuju antibiotici prema antibiogramu za dokazanog uzročnika infekcije. Terapija je usmerena na kontrolu bola i kontrolu autoimunog odgovora sinovijalne ovojnice zgloba, odnosno na prevenciju zglobnih oštećenja. Od medikamenata daju se nesteroidni antireumatici, analgetici, glikokortikoidi, imunosupresivi, biološki lekovi, a od drugih metoda lečenja preporučuje se mirovanje u akutnoj fazi oboljenja, fizikalna terapija i edukacija bolesnika. **Zaključak.** Razvojem novih dijagnostičkih metoda, posebno molekularne dijagnostike, kao i novih terapijskih mogućnosti, tj. primene novih generacija lekova, stvoreni su uslovi za efikasnije lečenje reaktivnog artritisa.

Ključne reči: reaktivni artritis; HLA-B27 antigen; dijagnoza; znaci i simptomi; bol; bakterijske infekcije; nesteroidni antiinflamatorni lekovi

Introduction

Reactive arthritis (ReA) is an inflammatory rheumatic disease of the joints, which usually follows a urogenital or intestinal infection, and less frequently is a reaction to an infection of the upper respiratory tract after a certain period of time. It belongs to the group of seronegative spondyloarthropathies [1]. ReA is usually a peripheral, asymmetrical monoarthritis or polyarthritis, predominately localized on the lower extremities. The human leukocyte antigen (HLA) finding is positive in 65% – 85% of patients. ReA develops in 30 – 40 of the diseased per 100,000 inhabitants, and after nonspecific urethritis or enteritis in 1 – 3% of patients [2]. ReA incidence is high (about 75%) in patients with human immunodeficiency virus (HIV) - positive infection and those positive for HLA-B27. Its

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Abbreviations

ReA – reactive arthritis
PCR – polymerase chain reaction
NSAIDs – non-steroidal anti-inflammatory drugs
TNF-α – tumor necrosis factor alpha
DMDs – disease-modifying drugs
HLA – human leukocyte antigen

incidence is highest in persons from 20 to 40 years of age, although it may also be found in children as a consequence of post-dysenteric infection. The primary infection remains unrecognized in 25% of patients, whereas about 10% of patients had no previous symptomatic infection.

The etiopathogenesis of ReA has not been fully explained. Genetic factors may play a very important role, although it is not clear how the association between HLA-B27 antigen and bacterial antigens affects the onset of the inflammatory process [3]. HLA-B27 antigen is positive in 80 – 90% of patients with Shigella infection, in 70 – 80% of those with Yersinia infection, in 40 – 50% of those with Chlamydia infection and only in 20 – 33% of patients with Salmonella infection. More than 40 subtypes of HLA-B27 are known, and some of them (HLA-B2702, B2704, B2705) are related to spondyloarthropathies. The role of antigen modulation, interaction with immunogenic characteristics of the organism, hypothesis of "molecular mimicry" – specific immune tolerance and "cross-reactivity", are only some of the theories attempting to clarify the etiopathogenesis of ReA [3]. The most frequent causes of ReA among enterobacteria are Salmonella (different species); Shigella flexneri, Shigella dysenteriae, Shigella sonnei; Yersinia enterocolitica, Yersinia pseudotuberculosis; Campylobacter jejuni, Campylobacter coli; Clostridium difficile and Escherichia coli [4]. Chlamydia trachomatis, Mycoplasma genitalium and Ureaplasma urealyticum are the most common pathogens causing urogenital infections [5, 6]. Group A ß-hemolytic streptococcus and Chlamydophila pneumoniae, bacteria causing upper respiratory tract infections, may cause ReA as well [7].

Clinical Picture

Patients with ReA usually present with asymmetrical monoarthritis, mostly of large joints of the lower extremities, but may also present with enthesitis, bursitis, conjunctivitis, anterior acute uveitis as well as various skin changes [8]. Spondylitis and sacroiliitis develop in about 30% and 14 – 49% of patients, respectively. The disease onset is most frequently acute, and rarely sub-acute. The first sign is usually urethritis or diarrhea, though it can be bilateral conjunctivitis i. e. arthritis as well. Iridocyclitis, unilateral at first and bilateral later, develops in 8% of the patients during the first attack, and in 30% of recurrent cases. Urogenital infection precedes the development of ReA usually two to three weeks after a sexual intercourse. Urinary symptoms (polyuria, dysuria) may not be present in 70 - 80% of female patients, though some of them develop unspecific mucopurulent cervicitis, salpingitis and vulvovaginitis. Prostatitis is often found in male patients (80%) and it usually occurs several weeks after the onset of disease. Nonbacterial hemorrhagic cystitis may develop as a complication of severe forms of urethritis. Both men and women are at similar risk of developing ReA if it is caused by a gastrointestinal infection. Skin changes and damaged mucous in ReA are seen as keratoderma changes i. e. pustular palmoplantar changes in 5 – 30% of cases. Balanitis and painless ulcerations of the oral cavity are present in 20 – 40% and 5 – 10% of cases, respectively. Erythema nodosum usually develops along with Yersinia infection, whereas balanits is often associated with Chlamydia infection. As for the manifestations not including the joints, changes in the eyes are the most frequent: conjunctivitis (35%), iritis (5%), acute anterior uveitis, corneal ulcers, episcleritis and retrobulbar neuritis. Cardiovascular changes are rare and if present, they present as conduction disorders or as asymptomatic myocarditis [9]. The central nervous system is rarely affected, and if it is, peripheral neuritis and transient hemiplegia may occur. Pleuropulmonary complications occur very rarely; however, renal changes manifest as proteinuria, microhematuria, sterile pyuria, glomerulonephritis or IgA-nephropathy [10].

The disease onset is usually acute and it is commonly associated with asymmetrical monoarthritis or polyarthritis of large joints, predominately of lower extremities. The most often affected joints are knees (70%), ankles (57%), and the big toe (35%) [11]. Heel pain is a characteristic sign. ReA is not limited only to the joints, and it develops as tendinitis, tenosynovitis and peritendinitis. Spondylitis and unilateral sacroiliitis occur in about 50% of the patients, usually along with HLA-B27. Night back pain is not alleviated by rest, but by exercise. Unilateral sacroiliitis may develop and is bilateral in 50% of patients [12]. Hands and wrists are involved in about 50% of patients. Arthritis becomes chronic in about one third of patients. Some fingers and toes are diffusely swollen in 16% of cases and have a sausage-like appearance [13]. Moderate muscle pain may last for more than a year in two thirds of patients. The ReA of urogenital origin tends to recur and 15 – 30% of patients develop chronic, recurrent peripheral arthritis, sacroiliitis and/or spondylitis [14].

Diagnosis

Diagnosis is made according to the diagnostic criteria presented at the Fourth International Workshop on Reactive Arthritis in Berlin in 1999 **(Table 1)** [15]. These criteria are based on clinical examinations, characteristic peripheral arthritis and confirmation of the previous infection. The presence of HLA-B27 antigen, laboratory analyses and radiologic processing of the involved segment are important when establishing the diagnosis.

Table 1. Diagnostic criteria for reactive arthritis (ReA)
Tabela 1. *Dijagnostički kriterijimi za reaktivnni artritis (ReA)*

Major criteria/*Veliki kriterijumi*	
1. arthritis + 2 of 3 criteria *1. artritis + 2 od 3 kriterijuma:*	a. asymmetry/*asimetrija* b. monoarthritis/oligoarthritis/*monoartritis/oligoartritis* c. lower extremities are predominately affected/*zahvaćeni pretežno donji ekstremiteti*
2. previous symptomatic infection with one or two of these criteria: 2. *prethodna simptomatska infekcija s jednim ili dva ova kriterijuma:*	a. enteritis (diarrhea which happened from 3 days to 6 weeks before the onset of arthritis and lasted more than 1 day)/*enteritis (diareja koja se desila 3 dana do 6 nedelja pre pojave artritisa i trajala je duže od 1 dana)* b. urethritis (dysuria which happened from 3 days to 6 weeks before the onset of arthritis and lasted more than 1 day)/*uretritis (dizurija koja se desila 3 dana do 6 nedelja pre pojave artritisa i trajala je duže od 1 dana)*
Minor criteria (one of these)/*Mali kriterijumi (jedno od ovoga):*	
1. confirmed previous infection *1. dokazana prethodna infekcija*	a. a positive morning urine or cervical/urethra test for Chlamydia trachomatis *pozitivan test u jutarnjem urinu ili brisu cerviksa/uretre za hlamidiju trahomatis* b. positive coproculture for enteropathogenic causes associated with ReA *pozitivna koprokultura za enteropatogene uzročnike povezane s ReA*
2. acute or persistent synovitis 2. *aktuelni ili perzistentni sinovitis*	Positive immunohistology or PCR for Chlamydia trachomatis/*Pozitivna imunohistologija ili polimerazna lančana reakcija za hlamidiju trahomatis*
ReA can be defined as:/*ReA definišemo kao:* • Confirmed ReA: two major and one relevant minor criteria are met/*sigurni ReA: kad su ispunjena dva velika kriterijuma i relevantni mali kriterijum* • Probable ReA: when one of the following is fulfilled: two major criteria but no relevant minor criteria are met, or one major and one or more minor criteria are met/*verovatni ReA: kada je ispunjeno jedno od ovoga: dva velika kriterijuma, ali bez relevantnih malih kriterijuma ili jedan veliki kriterijum i jedan ili više malih kriterijuma*	

Laboratory diagnosis is not specific and it is based on the confirmation of infection preceding the onset of disease – isolation of a microorganism or confirmation of a high titer of antibodies to intestinal bacteria or Chlamydia [16]. Serologic diagnostics is performed if it is possible, although positive serology is not sufficient for making the diagnosis [14]. The following microbiological analyses are performed: urinoculture, coproculture, swabs taken from the cervix, urethra, rectum, conjunctiva, nose and throat as well as antibodies to intestinal bacteria and Chlamydia when suspected, as well as methods for confirmation of the genomic causative factors. Arthrogenicity of the bacterial deoxyribonucleic acid and ribonucleic acid from Chlamydia trachomatis, Chlamydophila pneumoniae and Yersinia pseudotuberculosis can be confirmed by polymerase chain reaction (PCR) test of the synovial fluid of patients with ReA [14]. Microorganisms or their components cannot always be cultivated from the joint which suggests that the inflammatory process in the joints may develop as a response to bacterial antigen [7]. About 90% of patients with ankylosing spondylitis test positive to HLA-B27 antigen, whereas HLA-B27 is found in 60 – 80% of patients with ReA [12]. Epidemiological studies have not shown a significant frequency of HLA-B27 antigen in ReA, so it is not necessary to determine HLA-B27 antigen in order to make the diagnosis. However, a more severe form of the disease can be expected in patients positive to HLA-B27 antigen. An increased erythrocyte sedimentation rate and C-reactive protein may be found in the acute phase of the disease. The analysis of the synovial fluid shows a leukocyte count from 2,000 to 64,000 mm^3, and the analysis of synovial fluid does not offer a specific finding, but one similar to that found in early ReA.

Radiological imaging is not crucial for making the diagnosis; however, the choice of radiologic method is important because of the lesions and tissues found in ReA. Nowadays, ultrasound imaging of the affected joints is a mandatory diagnostic method particularly in young men [11]. Edema of the affected soft tissues (ankles, heels, feet, knees, and fingers) is seen at an early stage. Some of the characteristics are asymmetrical proliferations or periosteal reactions, but less frequently erosions on tendons and their attachments at the site of inflammation. Joint space narrowing, discrete erosions and rarely ankylosis of the affected joints occur after longer duration of the disease. Magnetic resonance imaging is obligatory when sacroiliitis is suspected and therefore it is included into diagnostic protocols.

In the early phase of the disease, particularly in case of unrecognized infection, there is a differential diagnostic dilemma regarding the early form of rheumatoid arthritis [12]. Urologic and gynecologic symptoms of the disease may resemble those in gonorrhea or syphilis. Acute joint changes may be mistaken for gout, pseudogout, or rheumatic fever in children. Diarrhea occurs in inflammatory bowel diseases as well [4].

Treatment of Reactive Arthritis

Once the diagnosis is made, the acute urogenital or intestinal infection is to be treated first. Urogenital acute infection caused by Clostridium trachomatis is treated by azithromycin (a single dose of 1

g) or doxycycline (200 mg per day for 7 days). The sexual partner must be treated at the same time [16]. Antimicrobial therapy is not administered to treat enteric infections associated with ReA. Although antibiotic therapy has no direct effects on arthritis, it should be given to prevent the recurring form of ReA. Acute enteritis is treated by fluoroquinolones and macrolides [17]. The drugs of choice for treating infections caused by Clostridium pneumonia are tetracyclines and erythromycin as well as the new generation of macrolides and fluoroquinolones. Today, a combination of several antibiotics is also applied and it is more efficient than monotherapy. The most frequently recommended combination is doxycycline + rifampin, which improves the disease symptoms significantly, particularly in case of Chlamydia trachomatis infections [17].

In the acute phase of ReA, high doses and long-term non-steroidal anti-inflammatory drugs (NSAIDs) are given along with physical therapy to alleviate pain, edema and functional incapacity. NSAIDs are the first line therapy for elimination of pain and joint edema. When choosing NSAIDs, special attention must be paid to cardiovascular and gastrointestinal risk of developing side effects. Cyclooxygenase 2 inhibitors and non-selective NSAIDs are also efficient in the treatment of ReA. Rest adn inactivity are recommended in the acute phase of arthritis. Glucocorticoids applied locally or intra-articularly are administered to treat monoarthritis, less frequently in polyarthritis, enthesitis and dactylitis. Systemic glucocorticoids (1 mg/kg of body weight for 2 to 4 months) are indicated in severe forms of acute ReA and polyarthritis (usually 20 – 40 mg per day). Skin changes are treated with topical preparations of glucocorticoids and keratolytics when necessary.

Somewhat poorer prognosis is seen in a number of affected joints, as well as active inflammations, increased inflammatory reactants, and radiological changes. Therefore, it is justified to apply glucocorticoids, immunosuppressants - disease-modifying drugs (DMDs) (sulfasalazine in lower doses i. e. 2 – 3 mg per day; 15 – 25 mg of methotrexate per week or 100 – 150 mg of azathioprine per day). If the treatment of ReA starts with sulfasalazine during the first three months, faster remission is achieved than with placebo, as well as in the chronic form of the disease. The DMDs have not proved to be efficient in the treatment of out of joint manifestations of the disease nor in case of axial skeleton joints involvement. In these cases, better therapeutic efficiency is expected with azathioprine or biological therapy i. e. tumor necrosis factor alpha inhibitors (TNF-α inhibitors). TNF-α inhibitors are also applied in chronic cases where the applied therapy has failed. Anti-TNF-α (etanercept) and other biological drugs (infliximab, adalimumab, ustekinumab) are used to treat chronic ReA and when it is not possible to apply DMD; however, controlled studies have proved that only etanercept is efficient [18, 19].

Conclusion

Nowadays, reactive arthritis is associated with an increasing number of various infections. It is of utmost importance to make an early diagnosis and start treatment as soon as possible, as well as to apply preventive measures in order to control spreading of the infection, particularly among young and sexually active people. Development of new diagnostic methods (particularly molecular diagnostics) and new therapeutic modalities, as well as new generations of drugs, has created conditions for more efficient treatment of reactive arthritis.

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Rad je primljen 7. VIII 2018.
Recenziran 21. VIII 2018.
Prihvaćen za štampu 25. IX 2018.
BIBLID.0025-8105:(2018):LXXI:9-10:323-327.

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Seminar for physicians
Seminar za lekare u praksi
UDK 616.24-005.6/.7-08
https://doi.org/10.2298/MPNS1810330L

MANAGEMENT OF PULMONARY EMBOLISM

LEČENJE PLUĆNE EMBOLIJE

Biljana LAZOVIĆ[1] and Vladimir ŽUGIĆ[2]

Summary

Introduction. Pulmonary embolism is a common condition with high morbidity and mortality, particularly if misdiagnosed or untreated. It has non-specific clinical manifestations, often presenting with symptoms similar to other cardiovascular or common respiratory diseases. Dyspnea is the most common symptom. The main goals of pulmonary embolism therapy are to stop blood clots from getting bigger and prevent formation of new clots. The aim of this article was to review the clinical presentation, incidence, diagnostic algorithms and prevention of pulmonary embolism. **Management of pulmonary embolism.** The management of pulmonary embolism depends on patients' hemodynamic stability (hemodynamically stabile and hemodynamically unstable patients), as well as on specific conditions (population who cannot receive the same therapy as the previously mentioned patients). The management is largely focused on medical therapy of pulmonary embolism, as the first line therapy (emergency) and then on medical options for this disease. Special attention was given to urgent intravenous thrombolytic therapy in hemodynamically unstable patients, considering that these patients are the most vitally compromised, in shock and with high mortality rate. The initial treatment in hemodynamically stable patients consists of low molecular weight heparin and unfractionated heparin, which is later replaced by long term oral anticoagulation therapy. Its duration depends on the nature of the basic disease. Some populations cannot receive any thrombolytic therapy (pregnant women, patients suffering from malignant diseases and heparin-induced thrombocytopenia). These patients may receive low molecular weight heparin, unfractionated heparin and warfarin; patients with malignant diseases receive life-long anticoagulation therapy; argatroban or lepirudin are used in the management of heparin-induced thrombocytopenia. **Conclusion.** Prevention of pulmonary embolism is lifesaving. It includes prophylactic medical regimens and "mechanical" supportive therapy (elastic graduated compression stockings, inferior vena cava filters).

Key words: Pulmonary Embolism; Thrombosis; Thrombolytic Therapy; Catheterization; Thrombectomy; Anticoagulants; Fibrinolytic Agents; Heparin

Sažetak

Uvod. Plućna tromboembolija je vrlo uobičajeno stanje sa visokim morbiditetom i mortalitetom, naročito ako je pogrešno dijagnostifikovana ili nelečena. Ima nespecifične kliničke manifestacije koje mogu ličiti na druga kardiovaskularna ili respiratorna oboljenja. Otežano disanje je obično vodeći simptom. Glavni ciljevi lečenja plućne tromboembolije su zaustavljanje nastanka krvnog ugruška (tromba) i zaustavljanje formiranja novih. Cilj ovog rada je sumacija kliničke prezentacije, incidencije, dijagnostičkih algoritama i naposletku prevencija plućne tromboembolije. Terapija plućne embolije. Terapiju za plućne tromboembolije podelili smo, na osnovu stanja pacijenta, na hemodinamički stabilne i one koji to nisu, kao i na specijalnu populaciju koja ne može da primi istu terapiju kao prethodno navedeni. Osnovni fokus u lečenju plućne tromboembolije stavljamo na terapiju prve linije, tzv. urgentnu terapiju kada se prepozna plućna tromboembolija, a zatim sumiramo sve terapijske (medikamentne) opcije u lečenju ovog oboljenja. Posebno smo obratili pažnju na intravensku trombolitičku terapiju, imajući u vidu da su u pitanju pacijenti koji su hemodinamički nestabilni i vitalno ugroženi (prevashodno za neodložnu trombolitičku terapiju) i oni koje treba inicijalno stabilizovati a nakon toga lečiti. Iako dva odvojena entiteta, oba predstavljaju urgentnu terapiju plućne tromboembolije. Inicijalni tretman kod hemodinamički stabilnih pacijenata obuhvata niskomolekularni heparin i nefrakcionisani heparin. Specijalan osvrt dat je i posebnoj populaciji koja nije u mogućnosti da primi standardnu, uobičajenu terapiju za plućnu tromboemboliju. To su trudnice, osobe sa malignitetom, kao i one kod kojih je heparinom indukovana trombocitopenija. Ovakvi pacijenti primaju heparin male molekulske težine, kod maligniteta doživotnu antikoagulantnu terapiju, a argatroban ili lepirudin se primenjuje kod bolesnika sa indukovanom trombocitopenijom. **Zaključak.** Sprečavanje nastanka plućne tromboembolije je ključno. Ono uključuje profilaktičke medicinske režime i mehaničku potporu u vidu elastičnih kompresionih čarapa i postavljanja filtera u *v. cava inferior.*

Ključne reči: plućna embolija; tromboza; trombolitička terapija; kateterizacija; trombektomija; antikoagulanti; fibrinolitici; heparin

Introduction

Acute pulmonary embolism (PE) is a common and sometimes fatal condition with a highly variable clinical presentation. It is critical for the therapy to be administered in a timely fashion so that recurrent thromboembolism and death can be prevented [1, 2].

The PE is a relatively common acute cardiovascular disorder associated with high early mortality rates that have not changed significantly despite advances in diagnosis and treatment over the past 30 years [3, 4]. Due to pulmonary bed obstruction, PE may result in acute right ventricular failure, a life-threatening condition. Due to the fact that most

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Abbreviations

PE – pulmonary embolism
DVT – deep vein thrombosis
BP – blood pressure
VKA – vitamin K antagonist
LMWH – low-molecular weight heparin
HIT – heparin-induced thrombocytopenia
UFH – unfractionated heparin
VTE – venous thromboembolism

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The PE is a relatively common acute cardiovascular disorder associated with high early mortality rates that have not changed significantly despite advances in diagnosis and treatment over the past 30 years [3, 4]. Due to pulmonary bed obstruction, PE may result in acute right ventricular failure, a life-threatening condition. Due to the fact that most patients ultimately die within the first hours after the onset of symptoms, early diagnosis is of paramount importance. Depending on PE presentation, the initial treatment is primarily focused on restoring adequate blood flow through the pulmonary bed and preventing PE recurrence. Appropriate therapy is best selected using risk stratification by assessing hemodynamic impact as the strongest marker of short-term prognosis, morphological extent of PE, the patient's cardiovascular and pulmonary system status, the degree of neurohumoral adaptation and potential risks of the therapy [5, 6].

Since no exact epidemiological data are available, the incidence of PE is estimated to be approximately 60 to 70 per 100.000, and that of venous thrombosis approximately 124 per 100,000 of the general population [7, 8]. The European guidelines for the diagnosis and management of PE report annual incidence rates of venous thrombosis and PE of approximately 0.5 to 1.0 per 1.000 inhabitants [8, 9]. However, the actual figures are likely to be substantially higher because silent PE can develop in up to 40% to 50% of patients with deep vein thrombosis (DVT) [10]. In addition, autopsy studies have shown that PE had been diagnosed before death in 30% to 45% of patients [9, 10]. After coronary artery disease and stroke, acute PE ranks third among the most common types of cardiovascular diseases. While clinical data indicate that most cases of PE occur at 60 to 70 years of age, autopsy data show the highest incidence among individuals aged 70 to 80 years. If untreated, acute PE is associated with a significant mortality rate (as high as 30%), whereas the death rate of diagnosed and treated PE is 8%. Up to 10% of acute PE patients die suddenly. Two of three patients dying from PE die within 2 hours after the onset of symptoms [11].

The accuracy of diagnosis decreases as the patient age increases. The diagnosis is difficult due to comorbidities, such as bronchopneumonia, chronic obstructive pulmonary disease, asthma or chronic fibrotizing pulmonary processes. In contrast, PE is easily diagnosed in patients with DVT. The most common sources of PE (up to 85% of cases) include DVT followed by thrombosis of iliac and renal veins, and the inferior vena cava. The upper limbs are not usually identified as a source of major PE [11, 12].

The risk of PE can be assessed based on hemodynamic stability (systolic blood pressure (BP) > 90 mmHg or systolic BP < 90 mmHg), and high (> 4 points) or low (< 4 points) probability according to the original modified Wells' criteria [12]. Due to high mortality in the early stages of PE, [13] aggressive treatment is necessary in high-risk patients (modified Wells' score > 4, systolic BP < 90 mmHg). Hypoxemia with systolic BP < 90 mmHg suggests massive PE with high mortality [13].

All patients being evaluated for PE should receive supportive therapies and empirical anticoagulation (unless contraindicated) should be initiated without delay [14]. The data on exclusive outpatient management of acute symptomatic PE are limited, but the existing evidence supports the feasibility and safety of this approach in carefully selected low-risk patients.

The first line PE therapy is supportive therapy including [15]:

Respiratory support

– Supplemental high-flow oxygen should be administered;

– Mechanical ventilation may be necessary for patients with severe hypoxemia/respiratory failure.

Intravenous fluids

– If systolic BP is < 90 mmHg, intravenous fluids should be given. Acute right ventricular failure with resulting low systemic output is the leading cause of death in patients with PE.

– Studies indicate that aggressive volume expansion is of no benefit, and may even impair right ventricular function by causing mechanical overstretch, or by reflex mechanisms that depress contractility. However, modest fluid challenge (i.e., 500 mL) may help to increase the cardiac index in patients with PE, low cardiac index, and normal BP.

– Local resuscitation protocols should be followed.

Vasopressors

– If systolic BP is < 90 mmHg, vasopressors should be given. They are often necessary in association with (or while waiting for) pharmacological, surgical, or interventional reperfusion treatment.

– Noradrenaline (norepinephrine) appears to improve right ventricular function via a direct positive inotropic effect, while also improving right ventricular coronary perfusion by peripheral vascular alpha-receptor stimulation and the increase in systemic BP. However, its use should probably be limited to hypotensive patients.

– Dobutamine may be considered for patients with PE, low cardiac index, and normal BP; however, raising the cardiac index above physiological values may aggravate the ventilation-perfusion mismatch by further redistributing flow from (partly) obstructed to unobstructed vessels.

– Adrenaline (epinephrine) combines the beneficial properties of noradrenaline and dobutamine, without the systemic vasodilatory effects of the latter.

– Bed rest - systematic recommendation of bed rest, as part of the early management of patients with DVT, PE, or both, is not supported by available evidence.

Initial anticoagulation

Anticoagulation should be initiated immediately in all patients who present with suspected PE, unless contraindicated [16]. If PE is subsequently excluded, anticoagulation can be discontinued. In patients with confirmed PE, anticoagulation should continue for at least 3 months [16].

Therapeutic anticoagulation can be achieved with dabigatran, rivaroxaban, apixaban, or edoxaban, which are recommended over vitamin K antagonist (VKA) therapy (usually warfarin), which is in turn recommended over low-molecular weight heparin (LMWH) [17]. Fondaparinux is generally reserved for patients with heparin-induced thrombocytopenia (HIT) or those with a history of this condition.

In hemodynamically stable patients, direct-acting oral anticoagulants (i.e., apixaban, edoxaban, rivaroxaban, dabigatran) are considered to be an acceptable therapeutic intervention. Dabigatran is a direct thrombin inhibitor, while apixaban, edoxaban, and rivaroxaban are selective factor Xa inhibitors. The advantage of these agents is that they require no monitoring, have a rapid onset of action, and are short-acting. They also do not interact with food; however, they do undergo drug interactions and have limited reversibility, although dabigatran can be reversed with idarucizumab. Randomized clinical trials have demonstrated non-inferiority of efficacy and safety in patients with hemodynamically stable PE [18]. Rivaroxaban and apixaban are used as monotherapy, whereas dabigatran and edoxaban require lead-in therapy with a parenteral anticoagulant for 5 to 10 days before they are initiated. Because of this, rivaroxaban and apixaban are often the preferred treatments in hemodynamically stable patients.

If a patient has started taking warfarin, it is usually appropriate to discontinue the parenteral anticoagulant once a therapeutic international normalized ratio of 2.0 to 3.0 has been established.

Fondaparinux is generally not recommended in hemodynamically unstable patients. LMWHs can be used interchangeably, in accordance with local protocols [19]. No difference in thromboembolism recurrence, hemorrhage, or overall mortality has been found between the different drugs in this class.

Unfractionated heparin (UFH) is recommended in cases where primary reperfusion is being considered, as well as in those with serious renal impairment (i.e., creatinine clearance < 30 mL/min), or severe obesity. The majority receives intravenous UFH administered as a bolus followed by continuous infusion titrated to a target activated partial thromboplastin time of 2 to 3 times the upper limit of normal (approximately 60 to 80 seconds). Weight-based nomograms may achieve therapeutic range faster. UFH, which can be rapidly reversed, is preferred in patients undergoing fibrinolysis or embolectomy. These recommendations are based on the short half-life of UFH, the ease of monitoring, and its rapid reversal by protamine [19, 20].

On the other hand, LMWHs, such as enoxaparin have been shown to be as safe and effective as intravenous UFH [20, 21]. LMWHs offer several advantages over UFH, including a longer half-life, increased bioavailability, and a more predictable dose response. In addition, LMWHs are dosed by weight, administered subcutaneously, and usually do not require dose adjustments or laboratory monitoring. Besides, UFH is largely hepatically cleared and LMWHs are renally cleared. Patients with chronic kidney disease, massive obesity, pregnancy, or unanticipated bleeding or thromboembolism despite correct weight-based dosing of LMWH may benefit from laboratory monitoring. However, the utility of anti-Xa testing continues to be the subject of debate because the correlation of anti-Xa levels to antithrombotic effect and risk of bleeding has been questioned [21, 22].

Thrombolytic therapy

In hemodynamically compromised patients (shock or systolic BP < 90 mmHg) or right-sided heart strain (assessed by transthoracic echocardiography), thrombolytic treatment is recommended, as these patients have a high mortality rate. Primary therapy with fibrinolysis or embolectomy is generally considered for patients presenting with either massive or submassive PE. However, because of a relative paucity of randomized controlled trials, the use of primary therapy in the treatment of massive and submassive PE remains controversial [21].

Choice of intervention varies depending on the local provision: systemic thrombolysis [21] or catheter-directed thrombolysis [22], although current guidelines of the American College of Chest Physicians recommend systemic thrombolytic therapy using a peripheral vein over catheter-directed thrombolysis. Whichever technique is employed, a delay in treatment can be life-threatening [23].

Thrombolytic treatment of acute PE restores pulmonary perfusion more rapidly than anticoagulation with UFH alone. The Food and Drug Administration has approved t-PA (alteplase) 100 mg administered as a continuous infusion over 2 hours for the fibrinolysis of massive PE. Every patient being considered for fibrinolysis requires meticulous screening for contraindications, because the bleeding risk may be as high as 3.0% for intracranial hemorrhage [24, 25]. Although fibrinolysis is generally considered to be a lifesaving intervention in patients with massive PE, the extent of the clinical benefit remains unclear [26]. In a recent analysis of the International Cooperative Pulmonary Embolism Registry, fibrinolytics did not reduce the mortality rate or recurrent PE at 90 days. In submassive PE, the Management Strategies and Prognosis of Pulmonary Embolism-3 Trial demonstrated a reduction in the need for escalation of therapy among patients receiving alteplase [26, 27].

In patients with massive or submassive PE, in whom fibrinolysis is contraindicated or has failed, surgical embolectomy may be considered. Additional indications include paradoxical embolism, persistent right heart thrombi, and hemodynamic or respiratory compromise requiring cardiopulmonary resuscitation. In specialized centers caring for patients with massive PE, surgical embolectomy has been demonstrated to be a safe and effective treatment technique [28].

The early resolution of pulmonary obstruction leads to a prompt reduction in pulmonary artery pressure and resistance, with a concomitant improvement in right ventricular function. The hemodynamic benefits of thrombolysis are confined to the first few days; in survivors, differences are no longer apparent at 1 week after treatment [21, 22]. With thrombolysis, risk of major bleeding is 22%. In the case of thrombolysis, intracranial hemorrhage risk is 1% to 3% compared to 0.3% with heparin alone [21].

Absolute contraindications for thrombolysis include [22, 23]:

– Hemorrhagic stroke or stroke of unknown origin at any time
– Ischemic stroke in the preceding 6 months
– Central nervous system damage or neoplasms
– Recent major trauma/surgery/head injury (in the preceding 3 weeks)
– Gastrointestinal bleeding within the last month
– Known bleeding risk.

Relative contraindications for thrombolysis include [24]:

– Transient ischemic attack in the preceding 6 months
– Oral anticoagulant therapy
– Pregnancy, or within 1 week postpartum
– Traumatic resuscitation (in relation to this episode of PE)
– Refractory hypertension (systolic BP >180 mmHg)
– Advanced liver disease
– Infective endocarditis
– Active peptic ulcer.

When patients are excluded based on these criteria, the incidence of intracranial hemorrhage in the remaining treated patients has been shown to be negligible [24, 25]. In patients with confirmed PE, deciding whether to initiate thrombolysis or to continue with anticoagulation should be made on a case-by-case basis according to clinical presentation and pre-existing morbidity. This tends to vary according to local expertise and center provision.

Treatment of pulmonary embolism in special populations

– In general, the initial approach to the treatment of PE as well as the treatment of life-threatening PE in special populations is similar to that in the general population. However, definitive therapy may differ in hemodynamically stable patients with malignancy, pregnancy, and heparin-induced thrombocytopenia. Patients with malignancy: in hemodynamically stable patients with malignancy and PE, LMW heparin is the preferred agent for all phases of anticoagulation [29, 30];

– Pregnant patients with hemodynamically stable PE: adjusted-dose subcutaneous LMW heparin is the preferred agent for initial and long-term anticoagulation due to its favorable fetal safety profile [31];

– Patients with heparin-induced thrombocytopenia: although the risk is lower with LMWH, the use of both UFH and LMWH is associated with the development of HIT. It results from heparin-dependent immunoglobulin G antibodies directed against heparin-platelet factor 4 complex and may lead to devastating arterial and venous thromboembolism. Although a benign transient decrease in platelets may be seen within the first few days of heparin administration, a decline in platelet count over 50% from baseline or a new thromboembolic event in the setting of any heparin product including heparin flushes should raise concern about possible HIT and lead to discontinuation of all heparin. Even though it typically occurs within 4 to 14 days of heparin exposure, HIT may occur earlier if the patient has been previously exposed to heparin. Delayed-onset HIT should be considered in patients recently exposed to heparin who present with thromboembolism and experience thrombocytopenia on re-exposure [23]. If HIT is suspected or confirmed, a direct thrombin inhibitor, such as argatroban or lepirudin, should be considered [23].

Prevention of pulmonary embolism

Prophylaxis regimens include mechanical and pharmacological modalities. Mechanical prophylactic devices include graduated compression stockings and intermittent pneumatic compression which increase venous blood flow and may enhance endogenous fibrinolysis, reducing the risk of venous thromboembolism (VTE) [32]. Pharmacological prophylaxis includes subcutaneously administered UFH, LMWH, warfarin, and fondaparinux. Certain high-risk populations, such as neurosurgical patients may benefit from a combination of mechanical and pharmacological prophylaxis [33]. Several studies have evaluated the safety and efficacy of various VTE prophylaxis regimens in medical patients. Daily subcutaneously administered enoxaparin has been shown to safely reduce the risk of VTE among patients admitted with acute medical illnesses [34]. In a large, randomized, placebo-controlled trial of acutely ill medical patients, the LMWH dalteparin (5000 IU subcutaneously once daily) halved the rate of VTE, with a low risk of bleeding [35]. The ARixtra for Thromboembolism Prevention in a Medical Indications Study found that fondaparinux (2.5 mg subcutaneously once daily) reduced the risk of VTE among medical patients by 47% [36].

Orthopedic patients are at a significantly higher risk of VTE even after discharge from the hospital. Several studies have validated extended out-of-hospital prophylaxis with warfarin or LMWH in prevention of VTE among orthopedic patients. Fondaparinux (2.5 mg subcutaneously once daily) safely and effectively reduces

the risk of VTE in patients undergoing hip replacement, major knee surgery, and hip fracture repair [37, 38].

Abdominal or pelvic surgery for malignancy is associated with an elevated risk of postoperative VTE. The Enoxaparin and Cancer II study demonstrated that extended prophylaxis with enoxaparin reduced the risk of VTE in those patients [31].

Elastic graduated compression stockings

Elastic graduated compression stockings are not routinely used in patients with DVT to prevent postthrombotic syndrome [38].

Inferior vena cava filters

The primary indication for inferior vena cava filter placement is contraindicated anticoagulation and recurrent PE despite anticoagulation therapy. However, it may be appropriate as an adjunct to anticoagulation in patients in whom another embolic event would be poorly tolerated (i. e. poor cardiopulmonary reserve, or severe hemodynamic or respiratory compromise), although clinical data are lacking. Filters are not routinely placed as an adjunct in patients with PE. Filter placement is also sometimes used in patients at high risk of recurrence in whom it is anticipated that anticoagulation may need to be discontinued because of bleeding. Examples include patients at moderate risk of bleeding who cannot receive fresh frozen plasma or red blood cells (i. e. due to religious preference), and patients with metastatic malignancy who are at a high risk for both recurrence and bleeding. Although filters are not routinely placed as an adjunct in patients with PE, some experts place them in patients at risk for decompensation due to cardiopulmonary compromise. We agree that the adjunct use of filters should not be a routine and placement should be individualized taking into consideration the risk of recurrence and bleeding, patient preferences, institutional expertise, medical morbidities, and surgical complications [31, 39].

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Rad je primljen 28. V 2018.
Recenziran 26. VI 2018.
Prihvaćen za štampu 28. VI 2018.
BIBLID.0025-8105:(2018):LXXI:9-10:329-334.

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HISTORY OF MEDICINE
ISTORIJA MEDICINE

Clinical Center of Montenegro, Podgorica, Montenegro
Psychiatric Clinic

History of medicine
Istorija medicine
UDK 616.89-056.34:614.253]:608(091
https://doi.org/10.2298/MPNS1810337D

HISTORY OF RESEARCH INVOLVING MENTALLY DISABLED PERSONS - FROM EXPLOITATION THROUGH EXCLUSION TO APPROPRIATE INCLUSION

ISTORIJA ISTRAŽIVANJA NA MENTALNO OBOLELIM LICIMA – OD EKSPLOATACIJE PREKO ZABRANE DO ADEKVATNE INKLUZIJE

Tea DAKIĆ

Summary

Introduction. The inability to protect their own interests makes mentally disabled subjects particularly vulnerable; they face an increased likelihood of being wronged or harmed in the context of research. Therefore, they are due to having extra protection and safeguarding. **History of research misconduct and abuse of mentally ill patients.** The 20th century abounds with examples of ethically inadmissible experiments conducted on decisionally impaired patients. The most infamous among them are surely the atrocities of the Nazi doctors, whose fraudulent experiments resulted in death of hundreds of thousands of imprisoned innocent and mentally ill individuals. **Current and previous regulations and recommendations on research involving the mentally ill.** Extreme use of potentially vulnerable mentally ill persons in research has led to a set of policies and practices for protection from exploitation and abuse of human research participants. While the regulations initially protected these vulnerable patients by prohibiting research including the mentally disabled, current guidelines propose appropriate safeguarding so that they may be involved in appropriate research. **Conclusion.** Protection measures for the mentally disabled persons who are unable to consent to their involvement in research, by banning all biomedical research including the mentally ill are restrictive and unnecessary. Even if well-intended, such overprotection is discriminatory and implies that new treatments for conditions that directly affect the incapacitated subjects will not be developed. Providing that they are properly protected from unnecessary harms, appropriate inclusion of vulnerable mentally ill patients in research is necessary in order to meet their health needs in a safe manner.

Key words: History of Medicine; Mentally Ill Persons; Codes of Ethics; Human Experimentation; Patient Rights; Informed Consent; Bioethics; Psychiatry

Sažetak

Uvod. Nemogućnost da zaštite sopstvene interese čini mentalno obolela lica posebno vulnerabilnim. Oni su, stoga, podložni većem riziku da im se u kontekstu biomedicinskih istraživanja naškodi, zbog čega im se moraju osigurati dodatne mere zaštite. **Istorija zloupotrebe mentalno obolelih pacijenata u istraživanjima.** Dvadeseti vek obiluje primerima etički nedopustivih eksperimenata sprovedenih na osobama koje nisu bile u stanju da ispravno rasuđuju i donose odluke za sebe. Verovatno među ovim primerima najstrašnija su zlodela koja su sprovodili nacistički doktori, čiji su eksperimenti rezultirali smrću stotina hiljada nevoljnih i prisilno zatvorenih mentalno obolelih osoba. **Dosadašnji i aktuelni propisi o istraživanjima na mentalno obolelim osobama.** Preterana upotreba potencijalno vulnerabilnih mentalno obolelih osoba u istraživanjima dovela je do uspostavljanja zakonodavnih politika i praksi koje služe zaštiti ovih lica od eksploatacije i zloupotrebe u istraživanjima. Dok je zakonska regulativa inicijalno štitila ove vulnerabilne pacijente tako što je potpuno zabranjivala istraživanja na njima, aktuelne smernice se okreću ka uspostavljanju adekvatnih mera zaštite tako da se ova lica mogu uključiti u odgovarajuća istraživanja. **Zaključak.** Zaštita mentalno obolelih lica koja nisu sposobna da daju svoj pristanak za učešće u istraživanjima tako što će se zabraniti svako istraživanje na njima suviše je restriktivna i nepotrebna. Čak iako je dobronamerna, ovakva prezaštićujuća politika je diskriminatorna i onemogućava da se otkriju nove terapije za stanja koja direktno pogađaju ova lica. Pod uslovom da su valjano zaštićeni od nepotrebnih rizika, neophodno je omogućiti vulnerabilnim mentalno obolelim pacijenatima da učestvuju u biomedicinskim istaživanjima kako bi se na adekvatan način izašlo u susret njihovim zdravstvenim potrebama.

Ključne reči: istorija medicine; mentalno obolele osobe; etički kodovi; eksperimenti na ljudima; prava pacijenata; informisani pristanak; bioetika; psihijatrija

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Acknowledgement
This research was partially supported by NIH Research Grant, funded by the Fogarty International Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Abbreviations
WW II – World War II
UCLA – University of California, Los Angeles
CIOMS – Council for International Organizations of Medical Sciences

Introduction

Mental health care has a well-documented history of patient neglect, patient-subjects abuse, and stigmatization [1]. Since the World War II (WW II), through dubious experiments on humans that earned the investigators Nobel Prizes, to various misconducts in the developed world up until the late 20th century, mentally ill patients have suffered from being inappropriately included in medical research. Inclusion of potentially vulnerable mentally ill individuals in research, especially in research they were unlikely to benefit from, has led to a set of policies and practices to protect human subjects from exploitation and abuse in research [2]. The main goal of this study is to provide a comprehensive historical overview of abuse of mentally ill patients in research, demonstrate how reactions to research misconduct shaped previous and current regulations and recommendations on research including the mentally ill, and outline how and why those regulatory documents have shifted their focus from protective exclusion to appropriate inclusion of these vulnerable patient-subjects into research.

History of research misconduct and abuse of mentally ill patients

Like ghosts from a dark past, the victims of research haunt the dream of biomedical progress, returning again and again to raise the harsh reality of dignity violated, integrity invaded, and lives destroyed [3, p. 25].

When the horrors of the Nazi doctors were revealed after the WW II, it was realized that the atrocities of the Holocaust were preceded by a systematic program designed to eradicate the disabled and others whose lives were "unworthy of life" [4]. The emphasis was placed on the burden that the mentally ill and their facilities represented for the healthy who supported them. "Selective breeding" and "special treatment" were cover-names for forced sterilization of the mentally and physically disabled. Once the war began, efforts were made to realize the T4 euthanasia program, in which 70,000 were killed in "hospitals of death" by 1941 in the name of "racial health" and in order to eliminate the financial burden of caring for those deemed unworthy of living [5]. There is evidence that, under the Action T4, more than 200,000 children and adults who were identified as "defective" were killed at the institutions that once provided care for them [4].

Moreover, experiments were conducted on mentally ill patients primarily to improve mass-murder techniques, as a rehearsal for the subsequent persecution of Jews, Roma, homosexuals and others demonized by the ruling Nazi party. This included improving the effectiveness of the gas chambers from the T4 program, which were later shipped to concentration camps and used for the extermination of ethnic and social minorities. As the mentally ill have already been sentenced to death, medical professionals of the time "put them to use" justifying their inclusion in all kinds of tortuous, unethical and unscientific experiments [5].

Unfortunately, the dark times of the Nazi-era were not the first time that the unwilling and unaware mentally ill patients were abused under the veil of medical research. Their ready availability, powerlessness, and lack of autonomy made them extremely vulnerable to exploitation. Many praiseworthy discoveries used unwitting and non-consenting patients, such as the discovery of malaria-induced fever treatment for paralytic dementia caused by tertiary syphilis fever that earned Julius Wagner von Jauregg a Nobel Prize in Medicine or Physiology in 1927. Another psychiatrist won the Nobel Prize for his "discovery of the therapeutic value of leucotomy in certain psychoses" [6]. In 1949, António Egas Moniz was so rewarded for his experiments on severely mentally ill patients using frontal lobotomy. The use of non-consenting patients as research subjects by von Jauregg, Moniz and others was a common practice at that time [7].

Unlike these two Nobel Prize winners, whose research included non-consenting patients with the goal of benefitting the subjects or address the conditions of disability, there were others who took advantage of institutionalized and decisionally impaired individuals for research on conditions not related to mental disorders. Two prominent examples of such non-therapeutic research experiments were conducted at two Massachusetts schools for "mentally retarded" children and adolescents. In the 1940s and 1950s, for instance, residents of the Walter E. Fernald School were given oatmeal containing minute amounts of radioactive material, as part of a study that was designed to establish how the body absorbed minerals from dietary sources and explore the effects of different compounds on mineral absorption. Similarly, in 1961, residents of the Wrentham State School were administered small amounts of radioactive iodine, with the goal of determining the amount of nonradioactive iodine that could block the uptake of radioactive iodine in the event of a nuclear war [8]. In 1995, the Final Report to the President by the Advisory Committee on Human Radiation Experiments found that:

Even at the time, government officials and biomedical professionals should have recognized that when research offers no prospect of medical benefit, whether subjects are healthy or sick, research should not proceed without the person's consent. It should have been recognized that despite the significant decision-making authority ceded to the physician within the doctor-patient relationship, this authority did not extend to procedures conducted solely to advance science without a prospect of offsetting benefit to the person. This finding is supported by the moral principle, deeply embedded in the American

experience, that individuals may not be used as mere means toward the ends of others [8, p. 788].

Unfortunately, these were not the only examples of ethically egregious experiments conducted on decisionally impaired patients during the 20th century. There are numerous studies that did not obtain valid informed consent, used deceit, and subjected patients to unjustified risks in poorly designed scientific studies. One was the 1963 experiment performed on chronically ill, mostly demented patients at the Jewish Chronic Disease Hospital in New York. Live cancer cells were injected to unsuspecting and incapacitated patients, for the purpose of determining if the immunologic systems of debilitated individuals reacted any differently to the introduction of cancerous cells than those who were healthy, and also exploring how a chronically ill and weakened immune system affected the spread of cancer. There was no ethical review of the study, no prospect of benefit for the subjects, these individuals of diminished autonomy were not given adequate information on the research, did not consent to it, nor could they leave the study, and the investigators had no evidence that the subjects will not, in fact, develop cancer [9].

Another controversial experiment took place at the University of California at Los Angeles (UCLA), from 1983 into the 1990s, in which patients with schizophrenia were taken off their medication. Half of them suffered severe relapses, with symptoms that included hallucinations, paranoia, and severe decrease in functioning. One of the subjects consequently committed suicide, and another dropped out of college and attempted to kill his parents [10]. A United States federal agency later ruled that the UCLA failed to get a valid consent from the patient-subjects, by failing to inform them of the extent of risks they will be exposed to and that ordinary treatment would be safer for most of them [10]. At the time, federal ethics officials estimated that there were anywhere from 100 to 300 experiments in which psychiatric patients were taken off their medicines for the purpose of observation of their illness once they relapsed; these studies involved high levels of risk and no prospect of direct benefit to the participants, while providing subjects and their families little information about the true nature of the research and the foreseeable harms resulting from it [11].

The historical abuse of mentally ill patients who lack the ability to consent thus emphasizes the need for careful scrutiny and protection of study subjects who are unable to protect their own interests. However, despite this ethical imperative, they should not be "protected" to the degree that they are completely excluded from the research. Understanding the very conditions that deem them incapacitated requires research [12].

Current and previous regulations and recommendations on research including the mentally ill

The Nuremberg trial's revelations about the horrors performed on unwilling imprisoned individuals, including experiments that killed hundreds of thousands of mentally ill and "racially and cognitively compromised" individuals in psychiatric hospitals, prisons and death camps during the WW II [13], resulted in the first international code of ethics principles for research on human subjects. Of the key principles of the resulting Nuremberg Code [14], promulgated in 1947, the very first requires the informed and voluntary consent of the subject, regardless of their specific attributes:

This means that the person involved should have legal capacity to give consent; should be so situated as to be able to exercise free power of choice, without the intervention of any element of force, fraud, deceit, duress, overreaching, or other ulterior form of constraint or coercion; and should have sufficient knowledge and comprehension of the elements of the subject matter involved as to enable him to make an understanding and enlightened decision [14, p. 181].

This formulation, however, completely prohibits research on children, the decisionally impaired, or emergency room patients, who lack the legal, mental or physical capacity to consent, respectively [7]. Although there is evidence to suggest that the chief medical advisor to the Nuremberg Tribunal insisted that the mentally ill should be excluded and given special consideration and protections, the judges omitted this reference from their final ruling. This seems to be justified by their intention not to interfere with potential medical advancements, but only to address highly risky non-therapeutic research on the easily coerced population [7]. Since research on human subjects should "yield results for the good of society that are unprocurable by other methods or means of study" [14], such a wide-reaching policy of exclusion would have negative consequences for society. It could, for example, deprive it of important knowledge related to those whose conditions result in vulnerability or loss of competence [15]. This concern is reflected in well-known contemporary codes and regulations that do allow research on persons unable to provide consent, as long as there is justification and additional safeguarding for them. It is at present widely agreed that research on persons whose capacity to consent is compromised may be undertaken if research of comparable effectiveness cannot be carried out on individuals capable of providing consent, the research is likely to benefit the subjects themselves or others with the same competence-undermining condition, and that the risks are minimized. It is additionally required that permission for participation is acquired from a legally authorized representative of the subject, and that the subjects themselves assent to participation in research [16].

According to the Additional Protocol to the European Convention on Human Rights and Biomedicine, concerning Biomedical Research [17], research including a person whose capacity to consent is compromised may be undertaken only if there is a likely benefit to the subject and if the research cannot be performed on persons capable of providing informed consent. Alternatively, if the research does not have

the potential to directly benefit the subject, it needs to be intended to promote the health of the group the subject belongs to, and should entail only minimal risk and minimal burden. It means that the experimental interventions will inflict only a very slight and temporarily discomfort, or have a slight and temporary negative impact on the health of the person concerned. Other necessary preconditions for research on subjects who lack the capacity to provide consent include: 1) a necessary authorization given in writing by the legal representative or an authority, person or body provided for by law, taking into account the person's previously expressed wishes or objections, 2) the prospective subjects shall be informed of their rights, 3) the subject shall take part in the authorization procedure as far as possible, and 4) their dissent to participate shall be respected.

The 2013 revision of the Declaration of Helsinki [18] reaffirms these agreed upon recommendations for research subjects who are incapable of giving informed consent, and proposes an additional requirement that research involving these subjects may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population.

Council for International Organizations of Medical Sciences (CIOMS), in collaboration with the World Health Organization, recently issued the International Ethical Guidelines for Health-related Research Involving Humans [19]. These 2016 guidelines seek to avoid characterizing entire groups of individuals (i. e. the mentally ill) as vulnerable, and instead emphasize the importance of including adults who are not capable of giving informed consent in health-related research. Similarly, the newly-revised CIOMS guidelines recognize the need for specific protections to safeguard the rights and welfare of persons who, due to a lack of capacity, may not be able to protect their own interests. Like other modern-day recommendations, the CIOMS guidelines require that researchers must obtain permission from the legally authorized representative of the prospective subject, and that this permission takes into account the participant's previously expressed preferences and values, if known. CIOMS also recommends that the subject's assent to participate should also be obtained, if possible, and that a potential participant's refusal to take part in the research should be respected. It does, however, allow overriding the subject's dissent in exceptional circumstances, for example, in cases where the incapacitated person needs treatment that is not available outside the context of research. In these circumstances, research participation would be the best available medical option and thus in the person's best interest. CIOMS guidelines incorporate the usual requirement to maximize benefits and minimize risks for the research participants, or, for research interventions that have no potential individual benefits for participants, to ensure that the risks must be no more than minimal. Additionally, the guidelines allow for research ethics committees to permit a minor increase above minimal risk, in cases when the social value of the research is compelling.

Conclusion

The not-so-distant past of the modern world offers numerous examples that reaffirm the concern that individuals who lack the understanding or power to refuse participation may be overrepresented in research. Given that persons who suffer from mental disorders that render them unable to protect their own interests are easily abused and exploited, it is not difficult to understand how the need to protect them from harms of research reflected across the regulations in the form of complete prohibition of research including these subjects. However, the concern that their exclusion from research altogether also entails their exclusion from the benefits of research and access to treatments for which research evidence is needed prevailed at some point. It is now recognized that while there is a moral obligation to safeguard vulnerable individuals and groups in research, this obligation must not stand in the way of their right to the best available treatments and the highest attainable standard of health. Based on this premise, their exclusion from research was perceived as unjust, and their inclusion in research recognized as realization of their entitlement to proper, effective, scientifically researched, evidence-based care.

Mentally-ill subjects should neither be inappropriately included nor automatically excluded from participation in research on the basis of their vulnerability. Instead, they should be appropriately included, especially in research on those conditions that render them vulnerable. Contemporary legislation, regulations and guidelines on research including mentally-ill subjects reflect this requirement to adequately address the health needs of those suffering from mental disorders and enable them to benefit from research, while ensuring that appropriate safeguards for their wellbeing are in place.

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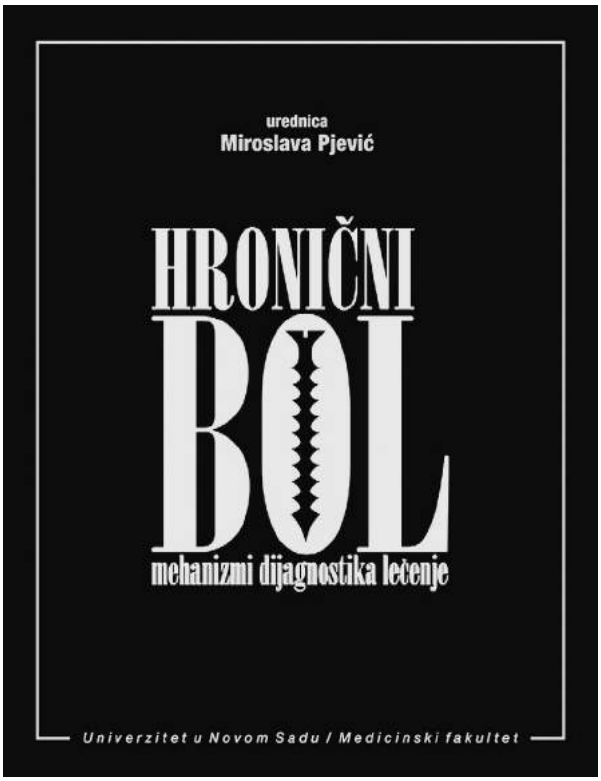
Rad je primljen 14. VI 2018.
Recenziran 1. VIII 2018.
Prihvaćen za štampu 27. VIII 2018.
BIBLID.0025-8105:(2018):LXXI:9-10:335-339.

BOOK REVIEWS
PRIKAZI KNJIGA

Knjiga HRONIČNI BOL – MEHANIZMI, DIJAGNOSTIKA I LEČENJE, edicija Medicinskog fakulteta Univerziteta u Novom Sadu, objavljena u decembru 2017. godine, nova je i jedinstvena publikacija iz oblasti medicine bola u Srbiji. Urednica, prof. dr Miroslava Pjević, anesteziolog i pionir medicine bola u Srbiji, okupila je multidisciplinarni tim od 32 pasionirana, ugledna stručnjaka sa medicinskih fakulteta i kliničkih ustanova u Novom Sadu, Beogradu, Nišu, iz regiona (Hrvatska, Slovenija), Italije i Kanade, koji su obradili hronični bol kao poseban zdravstveni entitet i bolest samu po sebi.

Hronični bol nije samo senzorni odgovor već i afektivni doživljaj i kompleksni biopsihosocijalni fenomen. Iz toga proizilazi da hronični bol često prate anksioznost, strah, depresija, pogoršanje ili izazivanje drugih simptoma bolesti i narušavanje svih aspekata kvaliteta života. Tako, integrisani farmakološki i nefarmakološki pristupi uključuju ne samo otklanjanje bola, već i funkcionalnu (fizičku, psihološku, socijalnu) nadoknadu i poboljšanje kvaliteta života. U tom kontekstu, domeni različitih hroničnih bolnih stanja u organizaciji Udruženja za istraživanje i tretman bola Srbije (UITBS) prethodnih godina, rasvetljavani su kroz brojne edukativne skupove i naučne simpozijume. Stalna potreba za sticanjem znanja kao i uža zdravstvena specijalizacija iz medicine bola usmerili su multidisciplinarni tim autora da ovom knjigom predstave mehanizme, dijagnostiku i lečenje najprevalentnijih hroničnih bolnih stanja i integrišu znanja iz različitih disciplina u jedinstvenu sliku o hroničnom bolu. Ovaj tim autora imao je cilj da svoja znanja i veštine prevede u savremenu kliničku praksu, a sve u korist pacijenata koji pate zbog bola.

Knjiga se sastoji iz 40 poglavlja podeljenih u tri odeljka. *Odeljak I, Bazični aspekti hroničnog bola,* sadrži osam poglavlja koja su posvećena evoluciji medicine bola, anatomiji, fiziologiji, mehanizmima hronifikacije bola, epidemiologiji, etici, psihološkim aspektima, proceni i merenju bola. *Odeljak II, Hronična bolna stanja,* obuhvata seriju od osamnaest poglavlja fokusiranih na mehanizme, dijagnostiku i lečenje (bol povezan sa artritisima, bol u donjem delu leđa i donjim ekstremitetima, glavobolje, hronični bol lica, postherpetička neuralgija, bolna dijabetesna neuropatija, kancerski bol i dr.). *Odeljak III, Terapijski aspekti hroničnog bola,* sadrži četrnaest poglavlja, koja pored farmakoterapije pružaju dokaze o značaju i drugih terapijskih pristupa i ističu važnost interdidisciplinarne terapije.



Knjiga je namenjena lekarima na specijalizaciji iz medicine bola, anesteziolozima, neurolozima, fizijatrima, lekarima opšte medicine i svim lekarima čiji je interes da unaprede razumevanje mehanizama i dijagnostike hroničnog bola i poboljšaju njegovo zbrinjavanje.

Doc. dr Gordana Jovanović

UPUTSTVO ZA AUTORE

Časopis ***Medicinski pregled*** objavljuje radove koji prethodno nisu objavljeni niti poslati u drugi časopis. U Časopisu mogu biti objavljeni radovi iz različitih oblasti biomedicine, koji su namenjeni lekarima različitih specijalnosti.

Od 1. januara 2013. godine *Medicinski pregled* je počeo da koristi usluge *e-Ur* − Elektronskog uređivanja časopisa. Svi korisnici sistema − autori, recenzenti i urednici, moraju biti registrovani korisnici sa jednom elektronskom adresom.

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U postupku prijave neophodno je da se pošalje saglasnost i izjava autora i svih koautora da rad nije delimično ili u celini objavljen ili prihvaćen za štampu u drugom časopisu.

Elektronsko uređivanje časopisa obezbeđuje korišćenje sistema *CrossCheck*, koji prijavljene radove automatski proverava na plagijarizam i autoplagijarizam. Autori ne bi smeli da pošalju isti rad u više časopisa istovremeno. Ukoliko se to desi, glavni urednik časopisa *Medicinski pregled* ima pravo da rad vrati autorima bez prethodnog slanja rada na recenziju; da odbije štampanje rada; da se obrati urednicima drugih časopisa u koje je rad poslat ili da se obrati direktoru ustanove u kojoj su autori rada zaposleni.

Primaju se samo radovi koji su napisani na engleskom jeziku, uz sažetak rada i naslov rada koji treba da budu napisani na engleskom i srpskom jeziku.

Radove koji su pristigli u časopis *Medicinski pregled* pregleda jedan ili više članova Uređivačkog odbora Časopisa. Oni radovi koji su napisani prema pravilima Časopisa šalju se na anonimnu recenziju kod najmanje dva recenzenta, stručnjaka iz odgovarajuće oblasti biomedicine. Načinjene recenzije radova pregleda glavni urednik ili članovi Uređivačkog odbora i one nisu garancija da će rad biti prihvaćen za štampu. Materijal koji je pristigao u časopis ostaje poverljiv dok se rad nalazi na recenziji, a identitet autora i recenzenata su zaštićeni, osim u slučaju ako oni odluče drugačije.

U časopisu *Medicinski pregled* objavljuju se: uvodnici, originalni članci, prethodna ili kratka saopštenja, pregledni članci, stručni članci, prikazi slučajeva, članci iz istorije medicine i drugi članci.

1. Uvodnici – do 5 strana. Sadrže mišljenja ili diskusiju o posebno značajnoj temi za Časopis, kao i o podacima koji su štampani u ovom ili nekom drugom časopisu. Obično ih piše jedan autor po pozivu.

2. Originalni članci – do 12 strana. Predstavljaju rezultate istraživanja autora rada i njihovo tumačenje. Istraživanje treba da bude obrađeno i izloženo na način da se može ponoviti, a analiza rezultata i zaključci jasni da bi se mogli proveriti.

3. Pregledni članci – do 10 strana. Predstavljaju sistematsko, sveobuhvatno i kritičko izlaganje problema na osnovu analiziranih i diskutovanih podataka iz literature, a koji oslikavaju postojeću situaciju u određenom području istraživanja. Literatura koja se koristi u radu mora da sadrži najmanje 5 radova autora članka iz uže naučne oblasti koja je opisana u radu.

4. Prethodna ili kratka saopštenja – do 4 strane. Sadrže izuzetno važne naučne rezultate koje bi trebalo objaviti u što kraćem vremenu. Ne moraju da sadrže detaljan opis metodologije rada i rezultata, ali moraju da imaju sva poglavlja kao originalni članci u sažetoj formi.

5. Stručni članci – do 10 strana. Odnose se na proveru ili prikaz prethodnog istraživanja i predstavljaju koristan izvor za širenje znanja i prilagođavanja originalnog istraživanja potrebama postojeće nauke i prakse.

6. Prikazi slučajeva – do 6 strana. Opisuju retke slučajeve iz prakse. Slični su stručnim člancima. U ovim radovima prikazuju se neuobičajeni oblici i tokovi oboljenja, neočekivane reakcije na primenjenu terapiju, primene novih dijagnostičkih procedura ili retke i nove bolesti.

7. Članci iz istorije medicine – do 10 strana. Ovi članci opisuju događaje iz prošlosti sa ciljem da omoguće očuvanje medicinske i zdravstvene kulture. Imaju karakter stručnih članaka.

8. Ostali članci – U časopisu Medicinski pregled objavljuju se feljtoni, prikazi knjiga, izvodi iz strane literature, izveštaji sa kongresa i stručnih sastanaka, saopštenja o radu pojedinih zdravstvenih organizacija, podružnica i sekcija, saopštenja Uredništva, pisma Uredništvu, novosti u medicini, pitanja i odgovori, stručne i staleške vesti i članci napisani u znak sećanja (*In memoriam).*

Priprema rukopisa

Kompletan rukopis, uključujući tekst rada, sve priloge i propratno pismo, treba poslati na elektronsku adresu koja je prethodno navedena.

Propratno pismo:

– mora da sadrži izjavu svih autora da se radi o originalnom radu koji prethodno nije objavljen niti prihvaćen za štampu u drugim časopisima;

– autori svojim potpisom preuzimaju odgovornost da rad ispunjava sve postavljene uslove i da ne postoji sukob interesa i

– autor mora navesti kategoriju članka (originalni rad, pregleni rad, prethodno saopštenje, stručni rad, prikaz slučaja, rad iz istorije medicine, itd.).

Rukopis

Opšta uputstva

Tekst rada treba da bude napisan u programu *Microsoft Word* za *Windows*, na A4 formatu stranice (sve četiri margine 2,5 cm), proreda 1,5 (isto važi i za tabele), fontom *Times New Roman*, veličinom slova 12 *pt*. Neophodno je koristiti međunarodni sistem mernih jedinica (*SI*), uz izuzetak temperature (° *C*) i krvnog pritiska (*mmHg*).

Rukopis treba da sadrži sledeće elemente:

1. Naslovna strana

Naslovna strana treba da sadrži: kratak i sažet naslov rada, bez skraćenica, skraćeni naslov rada (do 40 karaktera), imena i prezimena autora (ne više od 6) i afilijacije svih autora. Na dnu strane treba da piše ime, prezime i titula autora zaduženog za korespondenciju, njena/njegova adresa, elektronska adresa, broj telefona i faksa.

2. Sažetak

Sažetak ne može da sadrži više od 250 reči niti skraćenice. Treba da bude strukturisan, kratak i sažet, sa jasnim pregledom problema istraživanja, ciljevima, metodama, značajnim rezultatima i zaključcima.

Sažetak originalnih i stručnih članaka treba da sadrži uvod (sa ciljevima istraživanja), materijale i metode, rezultate i zaključak.

Sažetak prikaza slučaja treba da sadrži uvod, prikaz slučaja i zaključak.

Sažetak preglednih članaka treba da sadrži Uvod, podnaslove koji odgovaraju istima u tekstu i Zaključak.

Navesti do 10 ključnih reči ispod sažetka. One su pomoć prilikom indeksiranja, ali autorove ključne reči mogu biti izmenjene u skladu sa odgovarajućim deskriptorima, odnosno terminima iz *Medical Subject Headings*, *MeSH*.

Sažetak treba da bude napisan na srpskom i engleskom jeziku. Sažetak na srpskom jeziku trebalo bi da predstavlja prevod sažetka na engleskom, što podrazumeva da sadrži jednake delove.

3. Tekst članka

Originalni rad treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima istraživanja), Materijal i metode, Rezultati, Diskusija, Zaključak, spisak skraćenica (ukoliko su

korišćene u tekstu). Nije neophodno da se u posebnom poglavlju rada napiše zahvalnica onima koji su pomogli da se istraživanje uradi, kao i da se rad napiše.

Prikaz slučaja treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima), Prikaz slučaja, Diskusija i Zaključak.

Uvod

U poglavlju Uvod potrebno je jasno definisati predmet istraživanja (prirodu i značaj istraživanja), navesti značajne navode literature i jasno definisati ciljeve istraživanja i hipoteze.

Materijal i metode

Materijal i metode rada treba da sadrže podatke o vrsti studije (prospektivna/retrospektivna, uslove za uključivanje i ograničenja studije, trajanje istraživanja, demografske podatke, period praćenja). Detaljno treba opisati statističke metode da bi čitaoci rada mogli da provere iznesene rezultate.

Rezultati

Rezultati predstavljaju detaljan prikaz podataka koji su dobijeni istraživanjem. Sve tabele, grafikoni, sheme i slike moraju biti citirani u tekstu rada i označeni brojevima po redosledu njihovog navođenja.

Diskusija

Diskusija treba da bude koncizna, jasna i da predstavlja tumačenje i poređenje rezultata studije sa relevantnim studijama koje su objavljene u domaćoj i međunarodnoj literaturi. U poglavlju Diskusija potrebno je naglasiti da li su postavljene hipoteze potvrđene ili nisu, kao i istaknuti značaj i nedostatke istraživanja.

Zaključak

Zaključci moraju proisteći isključivo iz rezultata istraživanja rada; treba izbegavati uopštene i nepotrebne zaključke. Zaključci koji su navedeni u tekstu rada moraju biti u saglasnosti sa zaključcima iz Sažetka.

4. Literatura

Potrebno je da se literatura numeriše arapskim brojevima redosledom kojim je u tekstu navedena u parentezama; izbegavati nepotrebno velik broj navoda literature. Časopise bi trebalo navoditi u skraćenom obliku koji se koristi u *Index Medicus* (*http://www.nlm.nih.gov/tsd/serials/lji.html*). Pri citiranju literature koristiti Vankuverski sistem. Potrebno je da se navedu svi autori rada, osim ukoliko je broj autora veći od šest. U tom slučaju napisati imena prvih šest autora praćeno sa *et al*.

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ Organizacija kao autor

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ Bez autora

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ Volumen sa suplementom

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ Sveska sa suplementom

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ Sažetak u časopisu

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Knjige i druge monografije

∗ Jedan ili više autora

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ Urednik (urednici) kao autor (autori)

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ Poglavlje u knjizi

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ Zbornik radova sa kongresa

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

∗ Disertacija

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ Monografija u elektronskom formatu

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ Kompjuterska datoteka

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Prilozi (tabele, grafikoni, sheme i slike)

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

Tabele, grafikoni, sheme i slike se postavljaju kao posebni dokumenti.

– Tabele i grafikone bi trebalo pripremiti u formatu koji je kompatibilan programu u kojem je napisan tekst rada. Slike bi trebalo poslati u jednom od sledećih oblika: *JPG*, *GIF*, *TIFF*, *EPS*.

– Svaki prilog mora biti obeležen arapskim brojem prema redosledu po kojem se navodi u tekstu rada.

– Naslovi, tekst u tabelama, grafikonima, shemama i legende slika bi trebalo da budu napisani na srpskom i engleskom jeziku.

– Nestandardne priloge označiti u fusnoti uz korišćenje sledećih simbola: *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– U legendi slika trebalo bi napisati korišćeno uveličanje okulara i objektiva mikroskopa. Svaka fotografija treba da ima vidljivu skalu.

– Ako su tabele, grafikoni, sheme ili slike već objavljene, navesti originalni izvor i priložiti pisano odobrenje autora za njihovo korišćenje.

– Svi prilozi će biti štampani kao crno-bele slike. Ukoliko autori žele da se prilozi štampaju u boji, obavezno treba da plate dodatne troškove.

6. Dodatne obaveze

AUTORI I SVI KOAUTORI RADA OBAVEZNO TREBA DA PLATE GODIŠNJU PRETPLATU ZA ČASOPIS *MEDICINSKI PREGLED*. U PROTIVNOM, RAD NEĆE BITI ŠTAMPAN U ČASOPISU.

INFORMATION FOR AUTHORS

Medical Review publishes papers (previously neither published in nor submitted to any other journals) from various fields of biomedicine intended for broad circles of doctors.

Since January 1th, 2013 the Medical Review has been using the service e-Ur: Electronic Journal Editing. All users of the Registration system, i.e. authors, reviewers, and editors have to be registered users with only one e-mail address. Registration should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/user/register.

Manuscript submission should be made on the web address:

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

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Papers should be written in English language, with an abstract and title page in English, as well as in Serbian language.

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Medical Review publishes the following types of articles: editorials, original studies, preliminary reports, review articles, professional articles, case reports, articles from history of medicine and other types of publications.

1. Editorials – up to 5 pages – convey opinions or discussions on a subject relevant for the Journal. Editorials are commonly written by one author by invitation.

2. Original studies – up to 12 pages – present the authors' own investigations and their interpretations. They should contain data which could be the basis to check the obtained results and reproduce the investigative procedure.

3. Review articles – up to 10 pages – provide a condensed, comprehensive and critical review of a problem on the basis of the published material being analyzed and discussed, reflecting the current situation in one area of research. Papers of this type will be accepted for publication provided that the authors confirm their expertise in the relevant area by citing at least 5 self-citations.

4. Preliminary reports – up to 4 pages – contain scientific results of significant importance requiring urgent publishing; however, it need not provide detailed description for repeating the obtained results. It presents new scientific data without a detailed explanation of methods and results. It contains all parts of an original study in an abridged form.

5. Professional articles – up to 10 pages – examine or reproduce previous investigation and represent a valuable source of knowledge and adaption of original investigations for the needs of current science and practice.

6. Case reports – up to 6 pages – deal with rare casuistry from practice important for doctors in direct charge of patients and are similar to professional articles. They emphasize unusual characteristics and course of a disease, unexpected reactions to a therapy, application of new diagnostic procedures and describe a rare or new disease.

7. History of medicine – up to 10 pages – deals with history with the aim of providing continuity of medical and health care culture. They have the character of professional articles.

8. Other types of publications – The journal also publishes feuilletons, book reviews, extracts from foreign literature, reports from congresses and professional meetings, communications on activities of certain medical institutions, branches and sections, announcements of the Editorial Board, letters to the Editorial Board, novelties in medicine, questions and answers, professional and vocational news and In memoriam.

Preparation of the manuscript

The complete manuscript, including the text, all supplementary material and covering letter, is to be sent to the web address above.

The covering letter:

– It must contain the proof given by the author that the paper represents an original work that it has neither been previously published in other journals nor is under consideration to be published in other journals.

– It must confirm that all the authors meet criteria set for the authorship of the paper, that they agree completely with the text and that there is no conflict of interest.

– It must state the type of the paper submitted (an original study, a review article, a preliminary report, a professional article, a case report, history of medicine).

The manuscript:

General instructions.

Use Microsoft Word for Windows to type the text. The text must be typed in font *Times New Roman*, page format A4, space 1.5 (for tables as well), margins set to 2.5 cm and font size 12pt. All measurements should be reported in the metric system of the International System of Units – SI. Temperature should be expressed in Celsius degrees (°C) and pressure in mmHg.

The manuscript should contain the following elements:

1. The title page.

The title page should contain a concise and clear title of the paper, without abbreviations, then a short title (up to 40 characters), full names and surnames of the authors (not more than 6) indexed by numbers corresponding to those given in the heading along with the full name and place of the institutions they work for. Contact information including the academic degree(s), full address, e-mail and number of phone or fax of the corresponding author (the author responsible for correspondence) are to be given at the bottom of this page.

2. Summary.

The summary should contain up to 250 words, without abbreviations, with the precise review of problems, objectives, methods, important results and conclusions. It should be structured into the paragraphs as follows:

– Original and professional papers should have the introduction (with the objective of the paper), materials and methods, results and conclusion

– Case reports should have the introduction, case report and conclusion

– Review papers should have the introduction, subtitles corresponding to those in the paper and conclusion.

The authors should provide up to 10 keywords below the summary. These keywords will assist indexers in cross-indexing the article and will be published with the summary, but the authors' keywords could be changed in accordance with the list of Medical Subject Headings, MeSH of the American National Medical Library.

The summary should be written in both languages, English as well as Serbian. The summary in Serbian language should be the translation of the summary in English; therefore, it has to contain the same paragraphs.

3. The text of the paper.

The text of original studies must contain the following: introduction (with the clearly defined objective of the study), materials and methods, results, discussion, conclusion, list of abbreviations (if used in the text) and not necessarily, the acknowledgment mentioning those who have helped in the investigation and preparation of the paper.

The text of a case report should contain the following: introduction (with clearly defined objective of the study), case report, discussion and conclusion.

Introduction contains clearly defined problem dealt with in the study (its nature and importance), with the relevant references and clearly defined objective of the investigation and hypothesis.

Materials and methods should contain data on design of the study (prospective/retrospective, eligibility and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

Results give a detailed review of data obtained during the study. All tables, graphs, schemes and figures must be cited in the text and numbered consecutively in the order of their first citation in the text.

Discussion should be concise and clear, interpreting the basic findings of the study in comparison with the results of relevant studies published in international and national literature. It should be stated whether the hypothesis has been confirmed or denied. Merits and demerits of the study should be mentioned.

Conclusion must deny or confirm the attitude towards the 0based solely on the author's own results, corroborating them. Avoid generalized and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

4. References are to be given in the text under Arabic numerals in parentheses consecutively in the order of their first citation. Avoid a large number of citations in the text. The title of journals should be abbreviated according to the style used in Index Medicus (http://www.nlm.nih.gov/tsd/serials/lji.html). Apply Vancouver Group's Criteria, which define the order of data and punctuation marks separating them. Examples of correct forms of references are given below. List all authors, but if the number exceeds six, give the names of six authors followed by 'et al'.

Articles in journals

∗ *A standard article*

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ *An organization as the author*

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ *No author given*

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ *A volume with supplement*

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ *An issue with supplement*

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ *A summary in a journal*

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Books and other monographs

∗ *One or more authors*

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ *Editor(s) as author(s)*

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ *A chapter in a book*

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ *A conference paper*

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

∗ *A dissertation and theses*

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Electronic material

∗ *A journal article in electronic format*

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ *Monographs in electronic format*

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ *A computer file*

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Attachments (tables, graphs, schemes and photographs).

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

– Tables, graphs, schemes and photographs are to be submitted as separate documents, on separate pages.

– Tables and graphs are to be prepared in the format compatible with Microsoft Word for Windows programme. Photographs are to be prepared in JPG, GIF, TIFF, EPS or similar format.

– Each attachment must be numbered by Arabic numerals consecutively in the order of their appearance in the text

– The title, text in tables, graphs, schemes and legends must be given in both Serbian and English languages.

– Explain all non-standard abbreviations in footnotes using the following symbols *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– State the type of color used and microscope magnification in the legends of photomicrographs. Photomicrographs should have internal scale markers.

– If a table, graph, scheme or figure has been previously published, acknowledge the original source and submit written permission from the copyright holder to reproduce it.

– All attachments will be printed in black and white. If the authors wish to have the attachments in color, they will have to pay additional cost.

6. Additional requirements

SHOULD THE AUTHOR AND ALL CO-AUTHORS FAIL TO PAY THE SUBSCRIPTION FOR MEDICAL REVIEW, THEIR PAPER WILL NOT BE PUBLISHED.