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

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S A D R Ž A J / C O N T E N T S

Deo opreme prve državne apoteke u Srbiji, osnovane 1836. godine u Kragujevcu (Narodni muzej u Kragujevcu, Istorijsko odeljenje – Zbirka istorijskih predmeta, I-549/131) (vidi str.)

A part of furniture (equipement) of the first state pharmacy in Serbia, founded in 1836 in the town of Kragujevac (National Museum in Kragujevac, Historical Department – The collection of historical objects, I-549/131) (see pages ...)

U V O D N I K / E D I T O R I A L

Prvi impakt faktor časopisa "Vojnosanitetski pregled"

The first impact factor ever of the *Vojnosanitetski pregled*

Silva Dobrić

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"Vojnosanitetski pregled" (VSP), časopis lekara, farmaceuta i stomatologa Vojske Srbije, dobio je svoj prvi impakt faktor (faktor uticaja) čija vrednost iznosi 0,199.

Vest o ovome objavljena je na sajtu Konzorcijuma biblioteka Srbije za objedinjenu nabavku (KoBSON) 30. juna 2011, dva dana posle zvaničnog proglašenja impakt faktora najpoznatijih naučnih časopisa sveta od strane instituta *Thomson Reuters* (bivši *Institute for Scientific Information* – ISI, iz Filadelfije, SAD) koji je prvi uveo impakt faktor kao pokazatelj vrednosti nekog časopisa. Zbog toga podatke o impakt faktoru, koji stručne službe ovog Instituta objavljuju sredinom svake godine za prethodnu godinu, naučni krugovi širom sveta prihvataju kao najmerodavnije prilikom vrednovanja važnosti nekog naučnog časopisa, kao i vrednovanja samih autora i njihovog ukupnog naučnog opusa.

Podsećanja radi, VSP je 2008. godine, kao prvi medicinski časopis iz Srbije, uvršten u *Science Citation Index Expanded* (SCIe), čuvenu citatnu bazu instituta *Thomson Reuters*, što je predstavljalo osnovni preduslov za ulazak u sistem određivanja impakt faktora [1]. Naime, ova citatna baza, kao i druge dve citatne baze ovog Instituta, *Social Science Ciation Index* (SSCI) i *Arts and Humanitis Citation Index* (AHCI), omogućava uvid u literaturu koja je prethodila objavljivanju nekog rada, kao i uvid u njegovu citiranost, odnosno uticaj tog rada na dalja istraživanja prezentovana u drugim publikacijama. Zbog toga je citranost radova iz nekog časopisa u drugim radovima od presudnog značaja za procenu njegovog uticaja ili impakta na oblast koju svojim sadržajem pokriva.

Sam impakt faktor predstavlja numerički podatak o citiranosti "prosečnog" članka objavljenog u nekom časopisu u određenoj godini. Njegova vrednost izračunava se svake godine tako što se broj citiranih radova iz nekog časopisa za dve poslednje godine podeli ukupnim brojem objavljenih radova u te dve godine [2]. Na primer, impakt faktor nekog časopisa za 2010. godinu predstavlja kvocijent (količnik) između broja citata u 2010. godini za članke objavljene u tom časopisu tokom 2008. i 2009. godine i ukupnog broja članaka objavljenih u tom časopisu u te dve godine.

Vojnosanitetski pregled, the journal for physicians, pharmacists and dentists of the Army of Serbia got its first ever impact factor of 0.199.

This news was posted on the website of the Serbian Library Consortium for the Coordinate Acquisition (KoBSON) on 30th June, 2011, two days after the Thomson Reuters Institute (the former Institute for Scientific Information – ISI, Philadelphia, USA) officially revealed impact factors of the most influential scientific journals. This Institute was the first to devise impact factor as an index for evaluating value of scientific journals. The Institute publicly reveals impact factors for the previous year in the middle of each year that is widely accepted in the scientific community worldwide as the most competent in assessing the influence of a scientific journal, the authors themselves and their complete scientific involvement.

To remind, in 2008, *Vojnosanitetski pregled* (VSP for short), as the first Serbian medical journal, was included in the Science Citation Index Expanded (SCIE), well-known Thomson Reuters Institute's citation database, that was the major precondition for the journal to enter the system for assigning impact factor [1]. Namely, this citation database as well as the two other citation databases of this Institute, Social Science Citation Index (SSCI) and Arts and Humanitis Citation Index (AHCI), provides an insight into the literature published prior to a given article, but also an insight into its own citation, that is the impact of an article on further research presented in other publications. Thus, citation of articles published in a certain journal by other articles has a crucial significance for evaluating its own influence or impact on the field covered by it.

The impact factor itself is a numerical data on citation of an "average" article published in a certain journal in a given year. Its value is calculated each year by dividing the total number of citations of articles published in a given journal over the previous 2-year-period by a total number of articles published within these two years [2]. Let us give an example: the impact factor of a journal for the year 2010 is a quotient of the number of citations in 2010 of articles published in that journal within 2008 and 2009 and the total number of articles published in that journal over these two years.

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Izračunate vrednosti ovih faktora objavljuju se u *Journal Citation Reports* (JCR), posebnoj publikaciji instituta *Thomson Reuters*, svake godine u junu mesecu za prethodnu godinu. Kada se impakt faktor izračunava prvi put, onda se u obzir uzima citiranost samo onih radova objavljenih tokom prve dve godine od ulaska časopisa u sistem praćenja citatnih baza instituta *Thomson Reuters*. U slučaju VSP to su bile 2008. i 2009. godine, tako da se njegov prvi impakt faktor odnosi na 2010. godinu.

Inače, JCR izlazi u dve edicije: JCR *Science Edition* i JCR *Social Science Edition* koje se delimično preklapaju. Ovogodišnji JCR (objavljen 28. juna 2011), u svoje dve edicije, doneo je podatke o impakt faktorima za 2010. godinu za ukupno 10 196 časopisa (8 005 u *Science Edition* i 2 678 u *Social Edition*, s tim da je 487 časopisa klasifikovano u obe edicije), od kojih je 1 075 dobilo svoj prvi impakt faktor. Među njima je i VSP, koji je u JCR *Science Edition* svrstan u kategoriju medicinskih časopisa, njih 151, koji pokrivaju oblast opšte i interne medicine. Po vrednosti impakt faktora nalazi se na 133. mestu, što nije loše, ako se uzme u obzir da mu je to prvi impakt faktor i da se nalazi u društvo najpoznatijih medicinskih časopisa na svetu, kao što su *New England Journal of Medicine, Lancet, Journal of American Medical Association* (JAMA), *Annals of Internal Medicine, PloS Medicine, British Medical Journal* i mnogi drugi s velikom tradicijom i ugledom u medicinskim naučnim krugovima (Tabela 1) [3]. Osim toga, ako se uzme u obzir način izračunavanja impakt faktora (vidi gore) i činjenica da VSP izlazi svaki mesec (jedini je medicinski časopis u zemlji sa takvom dinamikom izlaženja), onda je jasno da je ukupan broj radova u VSP na godišnjem nivou mnogo veći nego kod časopisa koji izlaze dvomesečno ili, pak, kvartalno, a samim tim veći je i delilac u formuli za izračunavnje impakt faktora, što mu smanjuje vrednost. Uprkos tome, nastavićemo sa ovakvom dinamikom izlaženja objavljujući samo kvalitetne radove, duboko uvereni da ćemo, ubuduće, impakt faktor časopisa povećavati, upravo objavljivanjem većeg broja što kvalitetnijih radova koji će se više i citirati, a ne smanjenjem njihovog ukupnog broja na godišnjem nivou.

U ostvarenju ovog cilja očekujemo, kao i do sada, veliku podršku naših recenzenata, čitalaca i autora, jer, verujem, svi imamo istu želju – što veći impakt faktor i što bolju poziciju na listi srodnih časopisa!

Calculated values of these factors are published in the Journal Citation Reports (JRC), a special publication of the Institute Thomson Reuters in June each year for the previous year. When calculating impact factor for the first time ever then the Institute considers the citation of only those articles published within the first two years counting from the moment of including the journal in the system for assessing citation databases of the Thomson Reuters Institute. Regarding the VSP, those were the years 2008 and 2009, so that its first impact factor pertains to the year 2010.

The JCR appears in two editions: JCR Science Edition and JCR Social Science Edition that overlap partially. This year JCR, published on June 28, 2011, in its two editions provided data on impact factors for the year 2010 regarding a total of 10,196 journals (8,005 in Science Edition, and 2,678 in Social Edition so that 487 magazines were classified in both editions), out of which 1,075 were assigned their first impact factor. The VSP is among them, classified in JCR Science Edition into the category of medical journals, totally 151 of them, covering the field of general and internal medicine. By the value of the impact factor the VSP ranks 133rd in the list, which is not bad since it is its first impact factor ever and that it stands in the group of the most eminent medical journals worldwide, such are the New English Journal of Medicine, Lancet, Journal of American Medical Association (JAMA), Annals of Internal Medicine, PloS Medicine, British Medical Journal and numerous other journals with a long tradition and reputation in the medical scientific community (Table 1). Besides, if we consider the way of calculating impact factor (see above) and the fact that the VSP is published monthly (the only monthly medical journal in the country), then it is clear that a total number of articles in the VSP annually is higher than in those journals that appear each two monts, or even quarterly, thus having a higher divisor in the formula for counting impact factor, resulting in its lower value.

Anyhow, we will go on with this period of publishing and accepting only high quality articles, deeply hoping in higher impact factor as a consequence of a higher number of quality articles that would be citated more, rather than reducing a total number of articles on annual basis.

To make this aim come true, we expect, as usual, a great support of our peer-reviewers, readers and authors, since we believe all of us have the same wish – as higher impact factor as possible, thus the better rank in the list of related journals!

Tabela 1 / Table 1

Deset prvih i poslednjih časopisa iz oblasti *Medicina, opšta i interna*, svrstanih prema vrednosti impakt faktora za 2010. godinu
The ten first and last journals in the category *Medicine, General & Interna*l sorted regarding the impact factor value in 2010

Redni broj/No.	Prvih 10 časopisa/ The first 10 journals		Poslednjih 10 časopisa/ The last 10 journals	
	naziv časopisa/ journal title	IF_{2010}	naziv časopisa/ journal title	IF_{2010}
1	New England Journal of Medicine	53.484	Zdravniski Vestnik/ Slovenian Medical Journal	0.147
2	Lancet	33.633	Terapevticheskii Arkhiv	0.098
3	JAMA	30.011	Journal of the Korean Medical Association	0.096
4	Annals of Internal Medicine	16.729	Trakya Universitesui Tip Fakultesi Dergisi	0.093
5	Plos Medicine	15.617	Kuwait Medical Journal	
6	British Medical Journal	13.471	Turkiye Klinikleri Tip Bilimleri Dergisi	0.082
7	Annual Review of Medicine	12.457	Medicinski Glasnik	0.081
8	Archives of Internal Medicine	10.639	Nobel Medicus	0.056
9	Canadian Medical Association Journal	9.015	Salud I Ciencia	0.028
10	Cochrane Database of Systematic Reviews	6.186	Acta Medica Mediterranea	0.000

L I T E R A T U R A / R E F E R E N C E S

1. Dobrić S. Inclusion of "Vojnosanitetski pregled" into the world of most influental scientific journals – *Web of Science* [editorial]. Vojnosanit Pregl 2008; 65(7): 505-6. (Serbian)
2. Story about IF. Available from: http://kobson.nb.rs/vrednovanje/vrednovanje/prica_o_if.129.html (Serbian)
3. Searching for journals. Available from: http://www.kobson.nb.rs.proxy.kobson.nb.rs:2048/pretraživanje.84.html?words=&issn=&cat=171 (Serbian)

Категоризација домаћих научних часописа за медицинске науке за 2011. годину

бр	*Наслов часописа*	*МПН 2011*
1	Acta veterinaria*	M23
2	Војносанитетски преглед*	M23
3	Српски архив за целокупно лекарство*	M23
4	Journal of Medical Biochemistry*	M24
5	Acta chirurgica iugoslavica	M51
6	Ветеринарски гласник	M51
7	Медицински преглед	M51
8	Acta facultatis medicae Naissensis	M52
9	Acta medica Medianae	M52
10	Acta stomatologica Naissi	M52
11	Archive of Oncology	M52
12	Balkan Journal of Stomatology	M52
13	Facta Universitatis. Series: Physical Education and Sport	M52
14	Serbian Journal of Experimental Clinical Research	M52
15	Serbian Journal of Sport Sciences	M52
16	Београдска дефектолошка школа	M52
17	Билтен за трансфузиологију	M52
18	Геронтологија	M52
19	Глас САНУ, Медицинске науке	M52
20	Енграми	M52
21	Медицински часопис	M52
22	Психијатрија данас	M52
23	Специјална едукација и рехабилитација	M52
24	Стоматолошки гласник Србије	M52
25	Физичка култура	M52
26	Acta clinica	M53
27	Apollineum medicum et aesculapium	M53
28	Materia medica	M53
29	АБЦ - часопис ургентне медицине	M53
30	Актуелности из неурологије, психијатрије и граничних подручја	M53
31	Архив за фармацију	M53
32	Гласник Антрополошког друштва Србије	M53
33	Годишњак Факултета спорта и физичког васпитања	M53
34	Здравствена заштита	M53
35	Лековите сировине	M53
36	Медицина данас	M53

бр	*Наслов часойиса*	*МПН 2011*
37	Медицинска истраживања	M53
38	Медицинска пракса	M53
39	"Медицински гласник Института за штитасту жлезду и метаболизам "Златибор"	M53
40	Научни часопис ургентне медицине - Хало 94	M53
41	Општа медицина	M53
42	Тимочки медицински гласник	M53

* **часопис реферисан у Web of Science**

ORIGINAL ARTICLES

UDC: 340.6:[616.89-008.441.3-036.88-091.5:616.24-091.8

Association of pulmonary histopathological findings with toxicological findings in forensic autopsies of illicit drugs users

Povezanost patohistološkog nalaza na plućima sa toksikološkim nalazom kod sudskomedicinski obdukovanih narkomana

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Abstract

Background/Aim. Drug abuse remains a significant social problem in many countries. The aim of the study was to estimate association between pulmonary histopathological changes and results of toxicological analyses in forensic autopsies of illicit drug users. **Methods.** This investigation was performed in the Institute of Forensic Medicine, Belgrade, and in the Clinical Center, Department of Forensic Medicine, Kragujevac, from 2000 to 2004, and included 63 medicolegal autopsies of heroin or other drug consumers who suddenly died. Autopsies, postmortem toxicological examination of drugs and serological analyses of anti-HIV/HBV/HCV antibodies were performed. **Results.** The deceased persons were mostly male, 46/63 (73.01%), ranged in age from 19 to 49 years (mean 31 years) and all were whites. Postmortem toxicological examination was performed on all of the deceased persons and drugs in the fatal range were identified in only eight of them (12.7%), in the toxic range in ten (15.87%), and in minimal concentrations in 35 (55.56%) of the deceased persons. Drugs identified in the fatal, toxic or minimal range included heroin-morphine (38/53), cocaine (4/53), tramadol (3/53), and lorazepam (1/53). In the 7 remaining subjects, ethanol in combination with heroin was found in 4 cases, and diazepam in combination with heroin in 3 cases. Dominant pathomorphological changes were findings in the lung tissue. Most common histological changes observed in drug users were pulmonary edema – 55/63 (87.3%), acute alveolar hemorrhages – 49/63 (77.78%), hemosiderin-laden macrophages (siderophages) – 52/63 (82,54%), and emphysematous changes – 51/63 (80,95%). **Conclusion.** Pulmonary edema is the frequent non-specific autopsy finding which is associated with virtually all routes of drug administration. The histopatological study is necessary to determinate a cause of death when a deceased person has the history of dependence or abouse of psychoactive drugs with negative toxicological results.

Key words:
autopsy; histological techniques; overdose; pulmonary edema; substance-related disorder; toxicology.

Apstrakt

Uvod/Cilj. Zloupotreba droga i dalje predstavlja značajan društveni problem u mnogim zemljama. Cilj istraživanja bio je da se ispita udruženost, patohistoloških promena i rezultata toksikoloških analiza kod sudskomedicinski obdukovanih narkomana. **Metode.** Istraživanje je obavljeno u Institutu za sudsku medicinu u Beogradu i Službi za sudsku medicinu Kliničkog centra Kragujevac u periodu od 2000. do 2004. godine, i obuhvatilo je 63 sudskomedicinske obdukcije iznenadno umrlih heroinskih zavisnika i korisnika drugih droga. Urađene su obdukcije, postmortalna toksikološka ispitivanja na prisustvo droga i serološke analize na prisustvo antiHIV/HBV/HCV antitela. **Rezultati.** Većina umrlih osoba bili su muškarci 46/63 (73,01%), najmlađi je imao 19, a najstariji 49 godina (prosečna starost 31 godina) i svi su bili bele rase. Postmortalno toksikološko istraživanje obavljeno je kod svih umrlih osoba i droge u letalnoj koncentraciji pronađene su samo kod njih 8 (12,7%) osoba, u toksičnoj koncentraciji kod 10 (15,87%) i u minimalnoj koncentraciji kod njih 35 (55,56 %). Droge koje su identifikovane u letalnoj, toksičnoj ili minimalnoj koncentraciji bile su: heroin-morfin (38/53), kokain (4/53), tramadol (3/53) i lorazepam (1/53). Od preostalih sedam leševa, kod četiri pronađen je etanol u kombinaciji sa heroinom, a kod tri diazepam u kombinaciji sa heroinom. Najizraženije patomorfološke promene pronađene su u plućnom parenhimu. Najčešće uočene histološke promene kod korisnika droga bili su: plućni

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edem, 55/63 (87,3%), akutne alveolarne hemoragije 49/63 (77,78%), hemosiderofagi 52/63 (82,54%) i emfizematozne promene 51/63 (80,95%). **Zaključak.** Plućni edem je najčešći nespecifični obdukcijski nalaz povezan sa svim načinima unošenja droga. Neophodno je uraditi patohistološko ispitivanje preminulih osoba sa negativnim toksikološkim nalazom, ali pozitivnom anamnezom o zavisnosti ili predoziranju psihoaktivnih lekova.

Ključne reči:
autopsija; histološke tehnike; predoziranost; pluća, edem; poremećaji izazvani supstancama; toksikologija.

Introduction

Drug abuse remains a significant social problem in many countries. Drugs and poisons are divided into four groups: group I – drugs listed in the Single Convention on Narcotic Drugs 1961, schedule I (cocaine, dextromoramide, heroin/morphine, ketobemidone, methadone, oxycodone, etc.), and schedule II (codeine, ethylmorphine, pholcodine, propoxyphene, etc.); group II – drugs listed in the International Convention on Psychotropic Substances 1971, schedules I and II (amphetamines, MDMA of ecstasy, tetrahydrocanabinol, etc.); group III – drugs listed in the International Convention on Psychotropic Substances 1971, schedules III and IV (most barbiturates, benzodiazepines, buprenorphine, meprobramate, ethaqualone, etc.); group IV – all other drugs and poisons, including ethanol and carbon monoxide. Drug addict is defined as "a person who according to information from the police and/or autopsy report is known to have abused drugs intravenously and/or abused drugs listed in the Single Convention on Narcotic Drugs 1961, shedule I and/or the International Convention on Psychotropic Substances 1971, shedules I and II" [1].

According to postmortem toxicological examination, concentration of illicit drugs is not always in fatal or toxic range. Therefore, histopathological study is nesessary for determination of a cause of death when a deceased person has the history of dependence or abuse of psychoactive drugs with negative toxicological results.

The respiratory system is unvariably exposed to these drugs and is affected by them, either directly or secondarily, on the temporary or permanent basis. Illicit drugs and psychoactive substances affect all anatomical lung compartments producing diverse morphological changes. The major classes of drugs which cause respiratory manifestations are opiates, stimulants and cannabinoids [2]. Intrapulmonary site of injury and histological pattern of response depend not only on the pharmacological agents, but also on the dose, duration of abuse, route of delivery, and the presence of additives and adulterants [3]. Pulmonary pathohistological findings most commonly include edema, pulmonary hemorrhage and the presence of siderophages, pulmonary artery medial hypertrophy, panacinar emphysema, bronchiolitis obliterans, interstitial pneumonia or fibrosis [4]. The aim of the study was to examine association of pulmonary histopathological changes with results of toxicological analyses in forensic autopsies of illicit drug users.

Methods

This investigation was performed in the Institute of Forensic Medicine in Belgrade and in the Clinical Center, Department of Forensic Medicine in Kragujevac, and included 63 medicolegal autopsies of heroin or other drug consumers, who suddenly died during the period 2000 – 2004. The sample consisted of illicit drugs related deaths where a psychoactive substance directly caused the fatal outcome, or where the person had a history of dependence or abuse of psychoactive drugs with negative toxicological results.

Autopsies, postmortem toxicological analyses of drugs and serological research of anti-HIV/HBV/HCV antibodies were carried out. Multiple lung sections from each case were taken, microscopic slides were prepared, routinely stained with hematoxylin and eosin staining, and (H&E) under a light microscope (Carl Zeiss, Axioskop 40) and CCD camera (Canon PC 1089). During autopsy blood from the femoral vein, urine, samples of renal and liver tissue and vitreous samples were collected and submitted to the Department of Toxicology, the Institute of Forensic Medicine, Belgrade, where all the samples were routinely screened for illicit substances, pharmaceutical drugs and ethanol by conventional methods.

Results

The experimental group consisted of 63 individuals ranging in age from 19 to 49 years. Individuals were mostly male, 46/63 (73.01%), and all were whites. Twenty-nine deceased persons had serological evidence of hepatitis C virus infection, 12 of them had hepatitis B virus surface antigen, and 4 were HIV positive. In the majority of cases the manner of death was "natural", with the pulmonary edema as the most frequent mode of death. The drugs in the fatal range were identified in only 8 (12.7%) of them, in the toxic range in ten (15.87%), and in minimal concentrations in 35 (55.56%) of the deceased persons. The most common histological features observed in drug users were pulmonary edema – 55/63 (87.3%), acute alveolar hemorrhages – 49/63 (77.78%), siderophages – 52/63 (82.54%), and emphysematous changes – 51/63 (80.95%). The above mentioned microscopic changes are presented in Figure 1, a–d. In 10 cases with the history of dependence or abuse of psychoactive drugs, the postmortem toxicological results were negative. Drugs identified in fatal, toxic or minimal ranges included: only heroin-morphine (38/53), only cocaine (4/53), only tramadol (3/53), and only lorazepam (1/53). In the 7 remaining subjects, ethanol in combination with heroin was found in 4 cases, and diazepam in combination with heroin in 3 cases.

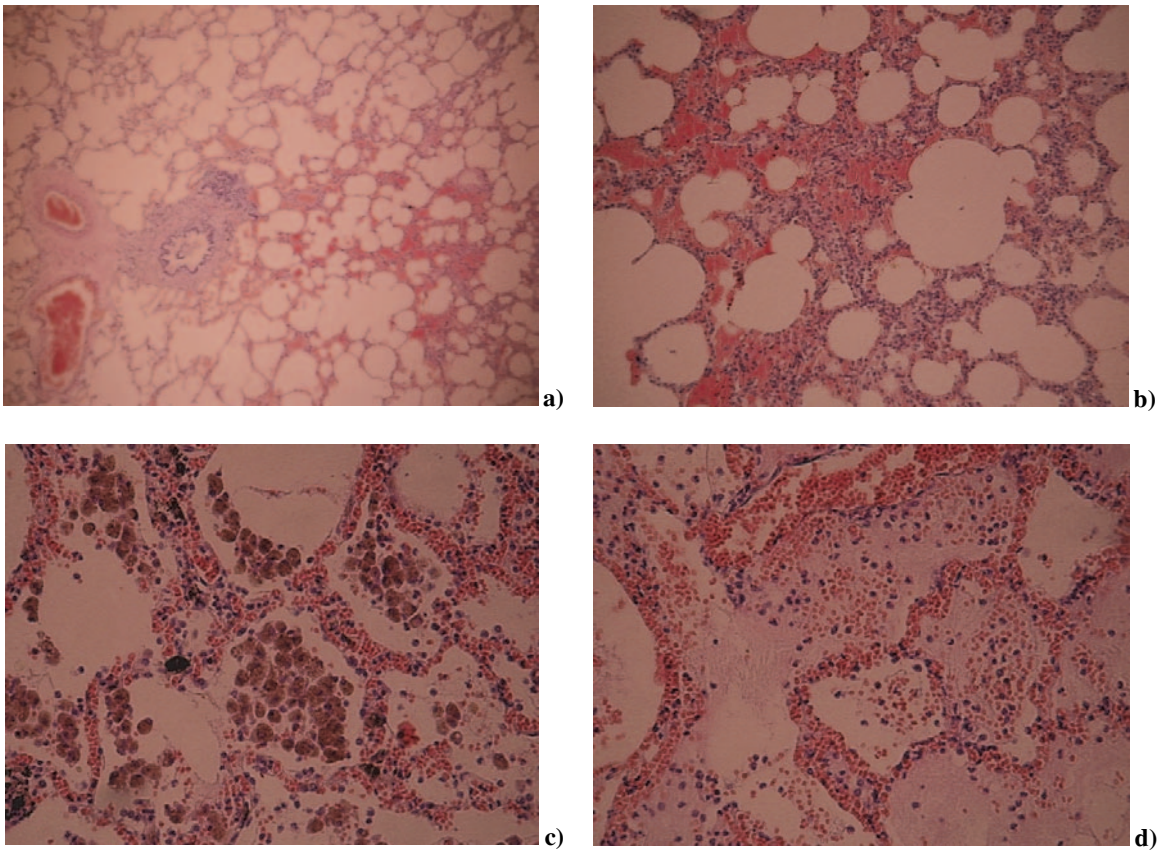

Fig. 1 – The most common pulmonary histopathological findings in forensic autopsies of illicit drugs users

a) pulmonary edema with erythrocytes and neutrophils in alveoli (H&E staining, ×40);
b) acute emphysematous changes and arterial medial hypertrophy (H&E staining, ×20);
c) siderophages in alveoli (H&E staining, ×40);
d) alveolar hemorrhages (H&E staining, ×20).

Discussion

The experimental group consisted of 63 individuals ranging in age from 19 to 49 years. Their median age was 31 years, while previous studies showed the median age of illicit drugs users of 25 and 26 years [5, 6]. Individuals were mostly male (73.01%), and all were whites. Twenty-nine deceased persons had serological evidence of hepatitis C virus infection, 12 of them had hepatitis B virus surface antigen, and 4 were HIV positive. In the majority of cases the manner of death was "natural", with pulmonary edema as the most frequent mode of death. The next most frequent manner of death was accident, predominantly heroine overdose. In other cases suicides (1/3 by gunshot, and 2/3 by hanging), and homicide by gunshot were registered.

A postmortem toxicological examination was performed in all cases, and drugs in the fatal range were identified in only 8 of them (12.7%), in the toxic range in ten (15.87%), and in minimal concentrations in 35 (55.56%) of the deceased persons. In 10 cases with the history of dependence or abuse of psychoactive drugs, the postmortem toxicological results were negative. The drugs identified in the fatal, toxic or minimal range included: only heroin-morphine (38/53), only cocaine (4/53), only tramadol (3/53), and only lorazepam (1/53). In the 7 remaining subjects, ethanol in combination with heroin was found in 4 cases, and diazepam in combination with heroin in 3 cases. In deceased drug addicts heroin is frequently detected. Cocaine was detected only in 4 fatalities, and is seemingly still not commonly used in Serbia. This pattern was confirmed in other countries [7].

The most common histological features observed in drug users were pulmonary edema (87.3%), acute alveolar hemorrhages (77.78%), siderophages (82.54%), and emphysematous changes (80.95%). Besides them, interstitial fibrosis, bronchiolitis obliterans, arterial medial hypertrophy, and inhaled particles were found in some cases. Pathologically, the lungs of individuals who die acutely are bilaterally heavy, voluminous, and congested [8]. In our research, widened, edematous interlobular septa and perivascular spaces were seen histologically in association with deeply eosinophilic alveolar edema. Pulmonary edema is a frequent non-specific autopsy finding which is associated with virtually all routes of administration of drugs [8, 9]. The prototype of drug-induced, non-cardiogenic pulmonary edema is that associated with heroin toxicity [9]. Proposed mechanisms of heroin-induced pulmonary edema include anoxic injury, direct toxicity or a hypersensitivity reaction involving the alveolocapillary membrane, neurogenic causes, or aspiration [9]. According to our study results margination and early exudation of neutrophils is often associated with proteinaceous alveolar edema. Trapped air bubbles cause focal airspace

ectasia and alveolar spaces expanded by trapped air bubbles (acute emphysematous change). The major parenchimal abnormality identified was the presence of numerous hemosiderin-laden macrophages (siderophages), and medial hypertrophy of medium-sized pulmonary artery. The usual findings are acute hemorrhages manifested as intact erythrocytes within the alveolar spaces. Previous investigators have suggested two mechanisms for alveolar hemorrhage: vasoconstriction of the pulmonary vascular bed after inhalation, resulting in ischemic damage of capillary endothelium, and a direct toxic effect of drugs on the capillary endothelium [10]. Our findings do not favor neither of these hypotheses. Although the heart was not specifically examined in our study, the frequent findings of capillary congestion and pulmonary edema in these individuals could indicate that alveolar hemorrhage is the result of passive congestion due to drug-related cardiac compromise [11].

Conclusion

Our study reports pulmonary vascular and parenchymal histopathologic changes associated with illicit drug use. Since a postmortem toxicological examination revealed the concentration of illicit drugs not always in the fatal or toxic range, the histopathological study is nesessary to determine a cause of death when a deceased person has the history of dependence or abuse of psychoactive drugs with the negative toxicological results. Pulmonary disease is a common cause of death in drug users. Pulmonary edema is a frequent autopsy finding which is associated with virtually all routes of narcotics administration. In addition, hemosiderin-laden macrophages were seen in 82.54% of cases, implying that alveolar hemorrhage may be a frequent complication of illicit drug abuse.

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Risk factors for brain metastases in surgically staged IIIA non-small cell lung cancer patients treated with surgery, radiotherapy and chemotherapy

Faktori rizika od pojave metastaza u mozgu kod bolesnika sa stadijumom IIIA nemikrocelularnog karcinoma pluća lečenih hirurški, zračenjem i hemioterapijom

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Abstract

Introduction/Aim. Lung cancer is a leading cause of mortality among patients with carcinomas. The aim of this study was to point out risk factors for brain metastases (BM) appearance in patients with IIIA (N2) stage of non-small cell lung cancer (NSCLC) treated with three-modal therapy. **Methods.** We analyzed data obtained from 107 patients with IIIA (N2) stage of NSCLC treated surgically with neoadjuvant therapy. The frequency of brain metastases was examined regarding age, sex, histological type and the size of tumor, nodal status, the sequence of radiotherapy and chemotherapy application and the type of chemotherapy. **Results.** Two and 3-year incidence rates of BM were 35% and 46%, respectively. Forty-six percent of the patients recurred in the brain as their first failure in the period of three years. Histologically, the patients with nonsquamous cell lung carcinoma had significantly higher frequency of metastases in the brain compared with the group of squamous cell lung carcinoma (46% : 30%; $p = 0.021$). Examining treatment-related parameters, treatment with taxane-platinum containing regimens was associated with a lower risk of brain metastases, than platinum-etoposide chemotherapy regimens (31% : 52%; $p = 0.011$). Preoperative radiotherapy, with or without postoperative treatment, showed lower rate of metastases in the brain compared with postoperative radiotherapy treatment only (33% : 48%; $p = 0.035$). **Conclusion.** Brain metastases are often site of recurrence in patients with NSCLC (IIIA-N2). Autonomous risk factors for brain metastases in this group of patients are non-squamous NSCLC, N1-N2 nodal status, postoperative radiotherapy without preoperative radiotherapy.

Key words:
carcinoma, non-small-cell lung; neoplasm metastasis; brain; antineoplastic combined chemotherapy protocols; radiotherapy; risk factors.

Apstrakt

Uvod/Cilj. Karcinom pluća vodeći je uzrok mortaliteta među obolelima od malignih bolesti. Cilj ove studije bio je da se ukaže na faktore rizika od pojave metastaza u mozgu kod bolesnika sa stadijumom IIIA (N2) nemikrocelularnog karcinoma (*non-small cell lung cancer* – NSCLC) lečenih trimodalitetnom terapijom. **Metode.** Analizirani su podaci 107 bolesnika sa stadijumom IIIA (N2) NSCLC lečenih hirurški uz dodatnu neoadjuvantnu terapiju. Učestalost metastaza u mozgu ispitivana je u zavisnosti od starosti, pola, histološkog tipa i veličine tumora, nodalnog statusa, redosleda primene zračne i hemioterapije i vrste hemioterapije. **Rezultati.** Dvogodišnja i 3-godišnja incidencija moždanih metastaza iznosila je 35% i 46%, respektivno. Četrdeset i šest procenata bolesnika imalo je metastaze u mozgu kao prvo mesto relapsa u trogodišnjem periodu. Histološki, bolesnici sa neskvamocelularnim karcinomom pluća imali su značajno veću učestalost metastaza u mozgu u odnosu na grupu skvamocelularnih karcinoma (46% : 31%; $p = 0,021$). Ispitivanjem terapijskih parametara, lečenje bolesnika primenom režima koji uključuju taksane i platinu bilo je povezano sa nižim rizikom od pojave metastaza u mozgu u

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odnosu na etoposid-cisplatin režim (31% : 52%; $p = 0{,}011$). Bolesnici koji su preoperativno zračeni, sa ili bez postoperativne zračne terapije, imali su nižu stopu metastaza u mozgu u odnosu na one koji su lečeni samo postoperativnom zračnom terapijom (33% : 48%; $p = 0{,}035$). **Zaključak.** Metastaze u mozgu često su mesto relapsa kod bolesnika sa NSCLC (IIIA-N2). Nezavisni faktori rizika od pojave metastaza u mozgu u ovoj grupi bolesnika su neskvamocelularni karcinom, nodalni status N1-N2 i postoperativna zračna terapija bez preoperativne zračne terapije.

Ključne reči:
pluća, nesitnoćelijski karcinom; neoplazme, metastaze; mozak; lečenje kombinovanjem antineoplastika, protokoli; radioterapija; faktori rizika.

Introduction

In patients with lung cancer metastases frequently occur in the brain. Around 20%–40% of patients with non-small cell lung cancer (NSCLC) develop brain metastases. The frequency of brain metastases in NSCLC is smaller than in small-cell lung cancer (SCLC), but their occurrence after treatment possess a big problem. Brain metastases in NSCLC attract attention because combined modalities of treatment bring progress in local control and overall survival rate among patients with NSCLC. With the increase of survival rate after combined modalities of treatment it can be noticed that the frequency of brain metastases is still increasing [1–3].

Big studies on NSCLC show increased risk from distant metastases, especially brain ones, after treatment has been completed. Andre et al. [2] in a retrospective study including clinical N2 patients, surgically treated with or without neoadjuvant therapy, have shown that overall frequency of brain metastases is 32% and 18%, respectively ($p < 0.05$). Ceresoli et al. [3] have shown that among patients with stage III NSCLC, treated with combined modalities of therapy, the brain has been the primary spot of relapse in 23% of the patients, and total of 50% of the patients have developed brain metastases in the further course of the disease.

Approximately 40,000 patients a year in the world are diagnosed with locally advanced, non-metastatic NSCLC (stage III). Patients with diagnosed IIIA stage of NSCLC belong to pretty wide varieties of different subgroups: some of them have favorable outcomes, such as patients with locally invasive tumor (T3) and hilar and/or peribronchial nodes (N1) or microscopically with N2 disease, and others groups have less than favorable outcomes, such as patients with bulky mediastinal adenopathy [4].

Nowadays priority treatment for the patients with stage IIIA is controversial. Some centers apply chemotherapy with full dose of radiation therapy without surgery, while others recommend surgery as an independent treatment or after neoadjuvant chemotherapy together with competitive radiation therapy or without it. Independent surgery is possible in 10%–40% patients with stage IIIA, depending on patient selection and the degree of lymph node involvement. Surgical treatment includes resection (lobectomy and pneumonectomy) and analysis of mediastinal lymph glands (mediastinal mapping). Complete dissection of lymph glands should be performed if the carcinoma is resectable and if mediastinal lymph glands are involved. However, even though carcinoma may be resectable in stage IIIA with N2 there is usually no use of surgical treatment. There is a clear biological difference when carcinoma is coinciding with N2 disease in comparison to N1 subcategory [5]. With patients in N2 substage of stage III only surgical treatment is considered (selected patients) and its expediency. In the last 30 years, many studies have been exploring the role of multi-modality approach in treatment of locally advanced NSCLC, that consist of combination of loco-regional surgical therapy and/or radiation therapy and systemic chemotherapy. Most of trials [6–10], but not all of them [11, 12], suggest preoperative induction chemotherapy due to better control of the disease and increase of survival rate. Combined chemotherapy, together with surgical and radiation therapy, can reduce the occurrence of brain metastases in patients with stage IIIA of NSCLC, and it can also improve the quality of life and survival expectancy.

The aim of this study was to present the risk factors for brain metastases occurrence in patients with stage IIIA of NSCLC, after completed surgical, radiation and chemotherapy.

Methods

The research involved 107 patients with NSCLC, IIIA stage of the disease, who underwent lung resection in the period from 1999 to 2008, in the Centre for Toracal Surgery of the Clinical Center Kragujevac, Institute for Pulmonary Disease of the Clinical Center of Serbia in Belgrade and the Clinic for Toracal Surgery of the Military Medical Academy, Belgrade. Histopathological confirmation of the disease was performed on tissue samples using standard hematoxylin and eosine (H&E) staining method. Histologically confirmed NSCLC was classified as stadium IIIA according to the TNM classification. The analysis included only the patients that had pulmonary resection and received preoperational therapy after mediastinoscopy. Most patients received neoadjuvant chemotherapy, radiation therapy or both modalities. The decision on the type of therapy depended on the size of the tumor, nodal status and patient's performance status. Preoperative chemotherapy was indicated in patients with IIIA (N2) stage of NSCLC, good performance status and minimal lymph nodes (< 1.5 cm), as well as in patients with bordering performance status (PS-2, ECOG scale) with lymph nodes size 1.5–3 cm. Patients in good physical state with N2 status (1.5–3 cm, bulky) received combined chemo and radiation therapy as induction therapy. Decision on the type of chemotherapy depended on the attitude of oncological consortium for lungs of the institution where the treatment was performed. Characteristics of tested patients (gen-

der, age, performance status, histological type of cancer) did not vary for different types of chemotherapy.

All patients that received preoperational therapy prior to surgical treatment of the lungs had to repeat CT of the chest and upper abdomen for the purpose of testing the progress of the disease. Computed tomography of central nervous system (CNS) was performed on each of the patients before the lung treatment.

Post-treatment screening was performed in all of the patients who had signs or symptoms of CNS ailment such as: defects of visual field, nausea, vomiting, motoric and sense dysfunctions, dysfunction of cranial nerves, new headaches, confusion or convulsions. Identification of brain metastases in most of the patients was performed by using computed tomography of CNS, only a few underwent magnetic resonance imaging. Other metastases were confirmed with CT of the chest or abdomen, *via* ultrasound of abdomen or scintigraphy of skeletal system. All patients with good performance status (0–2, ECOG scale) with confirmed metastases in CNS underwent radiation therapy of CNS. Patients with confirmed metastases of other localizations and good physical condition continued the treatment with secondary chemotherapy according to docetaxel-cisplatin protocol.

After the treatment of primary lung cancer had been completed, the risk of brain metastases was estimated, treating brain as the first place of relapse or relapse in further course of the disease.

Following factors were estimated using univariate analysis to determine the possible risk of appearance of brain metastases: age, gender, histological type, tumor size, nodal status, chronology of application of radiation therapy, chemotherapy and the type of chemotherapy. Log-rank test was used for univariate analysis. Proportional hazard regression model was used for multivariate analysis and identification of independent prognostic factors.

Pearson's chi-square test was used for statistical analysis, as well as univariate and multivariate analysis. Statistically significant value was $p < 0.05$.

Results

Demographic characteristics and variables analyzed in examined group of patients are shown in Table 1. There were 107 patients in examined group (64 men and 43 women); their average age was 61 (range from 37 to 73 year). Most of examined patients had squamous cell lung carcinoma (50.5%).

Table 1

Characteristics of examined patients with NSCLC

Characteristics	n	%
Total number of patients	107	100
Gender		
male	64	60
female	43	40
Average age (range), years	61 (37–73)	
Histological type		
squamous cell	54	50,5
adenocarcinoma	48	45
bronchialveolar	1	1
large cell	4	3,5
Tumor status, postoperative		
T0	10	9,5
T1	34	32
T2	45	42
T3	11	10
T4	7	6,5
Nodal status, postoperative		
N0	33	31
N1	24	22
N2	50	47

Most of primary lung tumor resections consisted of lobectomy (61.5%), and pneumonectomy (27%) (Table 2). Postoperative death rate was 2.8 % and determined number of deaths within 30 days since the operation. Chemotherapy according to the paclitaxel and cisplatin protocol, received 38% of the patients. Twenty nine (27%) patients received preoperative radiotherapy, 38% postoperative, while 12% of the patients received preoperative and postoperative radiotherapy (Table 2). Out of total number of patients, 72 (67%) received combined chemo and radiotherapy as a beginning of the disease initial treatment.

Out of 107 NSCLC treated patients, 44% had metastasis in the brain in the third year after the treatment was finished. Risk factors for appearance of metastases in the brain, examined with univariate analysis, are shown in Table 3. Patients with non-squamous cell lung carcinoma had significantly higher incidence of metastases in the brain within 3-year period compared to those with squamous cell carcinoma (46% : 31%; $p = 0.021$). The treatment with chemotherapy according to paclitaxel and cisplatin protocol was connected with lower brain metastases incidence within 3-year period, compared with that including chemotherapy according to the etoposide-cisplatin protocol (31% : 52%; $p = 0.011$) (Table 3).

Table 2

Treatment of examined patients with non-small cell lung cancer (NSCLC)

Treatment	n	%
Total number of patients	107	100
Surgical approach		
lobectomia	66	61,5
pneumonectomia	29	27
bilobectomia	6	5,5
segmentectomia	3	3
other	3	3
Chemotherapy		
etoposide and cisplatin	52	49
paclitaxel and cisplatin	41	38
other, postoperative	1	1
without therapy	13	12
Neoadjuvant and adjuvant therapy		
tri-modality	72	67
chemotherapy and surgery	15	14
radiation therapy and surgery	17	16
no therapy	3	3

Table 3

Risk factors for brain metastases

Variables	Risk factors (%)			*p*
	One year	Two-year	Three-year	
Patients (total number of patients 107; died 68)	20	33	49	
Gender				
male	19	35	48	0,652
female	22	39	52	
Age (years)				
≤ 60	18	37	39	0,535
> 60	16	48	49	
Tumor status, postoperative				
T0-T1	19	36	37	0,652
T2-T4	21	44	47	
Nodal status, postoperative				
N0	18	29	31	< 0,001
N1-N2	31	45	49	
Histological type				
squamous cell	15	28	35	0,021
non-squamous cell	32	43	46	
Chronology of radiotherapy				
pre ± postoperative	17	27	33	0,035
only postoperative	29	42	48	
Chemotherapy				
etoposide and cisplatin	19	26	31	0,011
paclitaxel and cisplatin	32	44	52	

Preoperative radiotherapy with postoperative therapy or without it, showed lower rate of metastases in the brain, compared with just postoperative therapy within period of three years (33% : 48%; $p = 0.035$) (Table 3).

Within 3-year period, incidence of metastases in the brain of patients with N0 status was significantly lower than that in the N1-N2 group (31% : 49%; $p < 0.001$) (Table 3).

Risk factors for occurrence of metastases in the brain, as a first site of relapse after the treatment was finished, examined by univariate analysis, were: histological type, nodal status, type of chemotherapy and radiotherapy order (table 4). Patients with squamous cell lung carcinoma compared to those with nonsquamous cell carcinoma (32% : 44%; $p = 0.033$), as well as patients treated with preoperative radiotherapy compared to those just postoperative treated with radiotherapy (33% : 48%; $p = 0.065$), have lower rate of brain metastases in the 3-years period (Table 4).

Multivariate analysis showed that autonomous risk factors for brain metastases after finished treatment were: nonsquamous cell lung carcinoma ($p < 0.01$), nodal status ($p < 0.001$) and the order of radiotherapy use ($p < 0.01$).

Analysis of metastases incidence in the brain of patients with NSCLC in relation to histological type and nodal status showed that non-squamous cell carcinoma with N1-N2 nodal status had significantly higher one-year (32%), two-year (51%) and three-year incidence compared to that in patients with squamous cell carcinoma and N1-N2 status ($p = 0.002$) (Table 5).

With univariate analysis we examined which of the risk factors for brain metastases affected the survival length of examined patients (Table 6). Age, tumor size, nodal status

Table 4

Incidence rate of meatastases in the brain as first relapse

Variables	Incidence of brain metastases (%)			*p*
	one year	two-year	three-year	
Patients (total number of patients 107; died 68)	17	35	46	
Gender				
male	17	32	45	0.405
female	21	35	49	
Age (years)				
≤ 60	17	34	33	0.462
> 60	16	41	42	
Tumor status, postoperative				
T0-T1	16	32	34	0.538
T2-T4	19	41	45	
Nodal status, postoperative				
N0	16	25	28	0.015
N1-N2	27	41	45	
Histological type				
squamous cell	12	24	32	0.033
non-squamous cell	28	39	44	
Chronology of radiotherapy				
pre ± postoperative	15	25	31	0.069
only postoperative	26	40	45	
Chemotherapy				
etoposide and cisplatin	17	24	29	0.023
paclitaxel and cisplatin	30	41	48	

Table 5

Incidence rate of metastases in the brain in relation to histological type and nodal (N) status

Histology and N status	Incidence of brain metastases (%)			*p*
	one year	two-year	three-year	
Squamous cell, N0	14	24	29	0.002
Non-squamous cell, N0	19	29	32	
Squamous cell, N1-N2	20	29	35	
Non-squamous cell, N1-N2	32	51	59	

Table 6

Prognostic factors for patients with stage IIIA of non-small cell lung cancer (NSCLC)

Variables	Average period of survival (months)	Survival (%)			*p*
		one year	two-year	three-year	
Patients (total number of patients 107; died 68)	25	74	52	41	
Gender					
male	30	76	58	46	0.231
female	27	72	54	43	
Age (years)					
≤ 60	23.6	73	58	36	< 0.001
> 60	15.3	47	33	24	
Tumor status, postoperative					
T0-T1	27.9	79	58	46	< 0.001
T2-T4	17.6	64	45	28	
Nodal status, postoperative					
N0	37.1	85	68	57	< 0.001
N1-N2	19.5	69	48	31	
Histological type					
squamous cell	28.2	80	54	43	0.351
non-squamous cell	23.7	70	49	36	
Chronology of radiotherapy					
pre ± postoperative	29.4	77	55	48	0.674
only postoperative	25.3	76	54	37	
Chemotherapy					
etoposide and cisplatin	36.6	88	70	49	< 0.001
paclitaxel and cisplatin	19.2	69	34	27	

and the type of chemotherapy were all significant factors in the group of patients with IIIA stage of NSCLC (Table 6).

Three-year survival rate was significantly higher in the group of patients aged ≤ 60 (36%) ($p < 0.001$), with tumor size T0-T1 (46%) ($p < 0.001$) and nodal status N0 (57%) ($p < 0.001$), compared to that in the group of patients older than 60 years, with tumor size T2-T4 (28%) and nodal status N1-N2 (31%) (Table 6).

In the group of patients who received chemotherapy according to the protocol paclitaxel-cisplatin, 2-year (70%) and 3-year survival rate (49%) was significantly higher compared with the patients received combination cisplatin- etoposid ($p < 0.001$) (Table 6).

Discussion

We analyzed the patients with IIIA (N2) stage of NSCLC treated with multimodal therapy, including surgery, radiotherapy and chemotherapy. All the patients in this study were surgically treated, and the biggest number of them were, before thoracotomy, subjected to mediastinoscopy and biopsy of lymph nodes. ESMO recommendations from 2009 suggest that preoperative chemotherapy based on cisplatin can be considered in patients with IIIA-N2 stage [13]. In the last 10–15 years most of phase II trials suggest that neoadjuvant chemotherapy with radiotherapy or without it, can improve surgical respectability, controlling the disease and survival rate at the stage IIIA N2 NSCLC [14–17]. Recent studies point out that aggressive chemo and radiotherapy can significantly reduce even the incidence of metastases in CNS in patients with IIIA stage, especially in three-modality therapy [18, 19]. Randomized studies and meta-analyses support the conclusion that combined treatment, which includes chemotherapy on the basis of platinum preparations, improves survival compared with radiotherapy only [19, 20].

Most common chemotherapy protocols at the end of 20th century implied the inclusion of the protocols based on platinum. Results of our analysis showed higher rate of 3-year survival in the group of patients who received protocols with taxane (46%) in comparison with that in the group of patients who received protocols without taxane (25%) ($p < 0.001$).

Aggressive approach to the primary tumor with three-modality therapy results also in a low rate of loco-regional recurrence with 14%. Although this result was better than the results that are achieved with only chemo or radiotherapy, most trials with definite chemo and radiotherapy are limited only to patients with non-resectable tumor and commonly to combined patients with IIIA i IIIB diseases [21–23]. Despite this, three-year survival in our analysis was just 40%, due to high percent of distant metastases, primarily in the brain. Forty-nine percent of our patients developed metastases in the brain in the three-year period (46% as the first site of relapse). The obtained results are compatible with the data from the literature [1, 2, 18, 23–25].

Moreover, results are consistent with the earlier reports, which have shown increased incidence of brain metastases in patients with non-squamous cell carcinoma [11, 19, 20, 25–27]. In univariate analysis, nodal status, after the induction therapy, and also a type of chemotherapy showed significant connection to decrease of brain metastases.

Robnett et al. [28] have shown that the delay of radio or chemotherapy, with surgery as the initial treatment, increases the incidence of brain metastases. Our results are similar to these data in which preoperative radiotherapy, compared with postoperative one is associated with a tendency to reduce the risk for metastases occurrence in the brain.

In SCLC incidence of metastases in the brain is higher than in NSCLC, and meta-analysis of seven randomized trials suggests prophylactic radiotherapy of endocranium as a progress in reducing brain metastases incidence and overall survival inprovement [29–32]. Some trials have shown that prophylactic brain radiotherapy in NSCLC affects decreasing the incidence of brain metastases, but not the overall survival [33]. Notwithstanding the fact that brain metastases are still a major problem of morbidity and death rate in patients with NSCLC, Radiation Therapy Oncology Group has now given a priority to randomized phase III with prophylactic radiotherapy in patients with IIIA stage of NSCLC. In our analysis, we identified a subgroup of patients with NSCLC (patients with residual nodal glands after neoadjuvant therapy and non-squamous cell histologic type) which have shown high risk for the occurrence of brain metastases. Results confirm other data that metastatic disease is, primarily of CNS, bigger and more significant problem than local disease in patients with IIIA stage of NSCLC. For that reason, improving the survival will require also the improvement of systematic therapy, in which prophylactic radiotherapy can be an integral part of, as an addition to systematic chemotherapy which lowers the risk of extracranial metastases, but not metastases in the brain [24].

Conclusion

Metastases in the brain are frequent site of relapse in patients with NSCLC (IIIA-N2). Autonomous risk factors for brain metastases in this group of patients are non-squamous cell lung carcinoma, N1-N2 nodal status and postoperative radiotherapy without preoperative radiotherapy.

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ORIGINAL ARTICLE

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Short-term use of continuous glucose monitoring system adds to glycemic control in young type 1 diabetes mellitus patients in the long run: a clinical trial

Kratkotrajna primjena kontinuiranog mjerenja glikemije poboljšava metaboličku kontrolu kod djece i adolescenata sa dijabetesom melitusom tip 1 na duži period: kliničko ispitivanje

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Abstract

Background/Aim. Balancing strict glycemic control with setting realistic goals for each individual child and family can optimize growth, ensure normal pubertal development and emotional maturation, and control long term complications in children with type 1 diabetes (T1DM). The aim of this study was to evaluate the efficacy of short-term continuous glucose monitoring system (CGMS) application in improvement of glycemic control in pediatric type 1 diabetes mellitus (T1DM) patients. **Methods.** A total of 80 pediatric T1DM patients were randomly assigned into the experimental and the control group. The experimental group wore CGMS sensor for 72 hours at the beginning of the study. Self-monitored blood glucose (SMBG) levels and hemoglobin A1c (HbA1c) levels were obtained for both groups at baseline, and at 3 and 6 months. **Results.** There was a significant improvement in HbA1c ($p < .001$), in both the experimental and the control group, without a significant difference between the groups. Nevertheless, after 6 months the improvement of mean glycemia was noticed only in the experimental group. This finding was accompanied with a decrease in the number of hyperglycemic events and no increase in the number of hypoglycemic events in the experimental group. **Conclusions.** The results suggest that the CGMS can be considered as a valuable tool in treating pediatric T1DM patients, however further research is needed to more accurately estimate to what extent, if any, it outperforms intensive self-monitoring of blood glucose.

Key words:
diabetes mellitus, type 1; adolescent; child; child, preschool; blood glucose self-monitoring.

Apstrakt

Uvod/Cilj. Balansiranjem stroge glikemijske kontrole sa postavljanjem realnih ciljeva za svako pojedinačno dete i porodicu može se optimizirati rast, odrediti normalan razvoj u pubertetu, kao i emocionalno sazrevanje i dugoročna kontrola komplikacija kod dece sa dijabetesom tip 1 (T1DM). Cilj ove studije bio je da se procijeni efikasnost metoda kontinuiranog supkutanog mjerenja glukoze u postizanju bolje glikemijske kontrole kod djece i adolescenata sa dijabetesom melitusom tip 1. **Metode.** Ukupno 80 djece sa dijabetesom melitusom tip 1 slučajnim odabirom određeno je u eksperimentalnu ili kontrolnu grupu. Ispitanici u eksperimentalnoj grupi nosili su aparat za kontinuirano praćenje glikemije (CGMS aparat) 72 sata na početku studije. Za ispitanike iz obje grupe evidentirani su podaci samokontrolisane glikemije (SMBG) i hemoglobina A1c (HbA1c) na početku studije, nakon tri i nakon šest mjeseci. **Rezultati.** Dobijeno je značajno poboljšanje koncentracije HbA1c na tri i šest mjeseci i u eksperimentalnoj i kontrolnoj grupi ($p < 0{,}001$), bez značajne razlike među grupama. Nasuprot tome, značajno sniženje srednje glikemije nakon šest mjeseci zabilježeno je samo u eksperimentalnoj grupi. Nadalje, u eksperimentalnoj grupi došlo je i do smanjenje broja hiperglikemijskih događaja, a, pri tom, nije evidentiran porast broja hipoglikemijskih događaja. **Zaključak.** Rezultati studije sugerišu da sistem kontinuiranog supkutanog praćenja glikemije može biti korisno sredstvo metaboličke kontrole kod djece sa dijabetesom melitusom tip 1, ali neophodna su dodatna istraživanja kako bi se preciznije utvrdilo u kojoj mjeri, ako je to uopšte slučaj, ovaj metod, u terapeutskom smislu, nadmašuje intenzivno samokontrolisanje glikemije.

Ključne reči:
dijabetes melitus, tip 1; adolescent; deca; deca predškolska; kontinuirani supkutani monitoring glikemije.

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Introduction

Balancing strict glycemic control with setting realistic goals for each individual child and family can optimize growth, ensure normal pubertal development and emotional maturation, and control long term complications in children with type 1 diabetes (T1DM) [1, 2]. The goal of the usual intensive therapy is to maintain near-normal glycemia, normalize hemoglobin A1c (HbA1c), control postprandial glycemic excursion and decrease the number of hypoglycemic events. Although very useful, multiple, four daily blood glucose measurements are still insufficient to provide and predict all relevant glycemic fluctuations. A newer method of continuous glucose monitoring (CGMS) seems to address the issue [3]. The CGMS provides the maximal amount of data about glycemic profile during activities of daily living, including physical activity, work, meals, and sleep. Thus, continuous monitoring have a great potential value to improve glycemic control while decreasing the incidence of hypoglycemia, especially in a patient with poorly controlled diabetes and those who are at the high risk of severe hypoglycemia. The benefits of CGMS use in T1DM patients have been shown in multiple studies [4–11].

Our study was conducted to analyze whether a three-day use of CGMS can significantly contribute to therapeutic decisions and thus to glycemic control over and above information provided by the standardized blood glucose self-monitoring in young T1DM patients.

Methods

The participants for this single-blinded randomized clinical trial were recruited from T1DM patients in the Children's Hospital in Banja Luka, Bosnia and Herzegovina. The study which lasted for 6 months was completed in 2007. It was approved by the Ethics Committee of Human Experimentation of Bosnia and Herzegovina. A total of 80 T1DM patients were randomly allocated into the experimental (CGMS and self-monitored blood glucose – SMBG) and the control (only SMBG) group. Inclusion criteria were: 1) HbA1c level $\geq$ 8%, 2) clinical diagnosis of insulin-dependent type 1 diabetes mellitus for at least 1 year, 3) patient's age 5 to 18 years, 4) availability for all office visits and compliance with the study protocol, and 5) compliance to wear a medical device for 72 consecutive hours. All the patients were using insulin aspart as their ultra short-acting insulin and insulin detemir as long-acting insulin in their therapy. Most of the patients (70%) in both groups were on intensive insulin therapy (using multiple injections per day – MDI), while the rest of them (30%) followed convential therapy (using three insulin injections per day – TDI). Exclusion criteria included history of comorbidities, and noncompliance with the study protocol. The parents and/or patients had given a written informed consent for the inclusion in the study. All the patients were followed up in the clinic at baseline, 3 and 6 months by the same investigator.

Both demographic and clinical data were collected using a standardized data collection form. The experimental group of the patients and families were instructed by the same investigator in the use of the CGMS device and they were asked to enter at least four daily blood glucose measures obtained with a personal glucometer into the instrument for the calibration purpose. They also entered all data regarding insulin administration, meals taken, exercise and other relevant events (e.g. hypoglycemic symptoms). The CGMS (Medtronic MiniMed, Northridge, CA) was applied for 72 hours including three overnight profiles and was well tolerated by patients in the experimental group. There was no evidence of infection or inflammation at the insertion site of the sensor. The CMGS data were analyzed using the Medtronic-MiniMed Solution Software version 3.0B (Medtronic). In both groups, the patients underwent 3 days of nine-point self-monitoring of blood glucose using an Accu-check glucometer (Roche), before and after each main meal, at bedtime, and during the night at 2 a.m. and 5 a.m. The postmeal measurements were taken within 2 hours after the preprandial measurements. Four of the SMBG tests were entered into the CGMS monitor for calibration. Sensor insertion and CGMS training as well as SMBG training were done in hospital, after which the patients returned home to their usual insulin therapy, diet and activity. Hemoglobin A1C (HbA1c) level was measured using the DCA 2000 (Bayer, Tarrytown, NY; non-diabetic range 4.3%–6.3%) at the beginning of the study and after 3 and 6 months. In the experimental group, CGMS data were collected and reviewed and therapeutic decisions during the first three months were made based on both CGMS and SMBG data, while in the control group, therapeutic decisions were made based solely on SMBG data. Different therapy recommendations were given based on the obtained data: e. g. change in dosage of rapid-acting insulin, change in dosage of long-acting insulin, carbohydrate intake changes, or increase in physical activity. Main outcome measures considered were HbA1c, average SMBG values and numbers of hypo- and hyperglycemic events. Hypoglycemia was defined as capillary blood glucose (CBG) level lower than 3.5 mmol/L, whereas hyperglycemia was defined as CBG level higher than 10.0 mmol/L.

Raw data were summarized and expressed as counts (categorical data) or means ± standard deviations (numeric data). Bivariate relationships were estimated with Pearson's linear correlation coefficients. Group differences were assessed with the Fisher exact tests, Wilcoxon-Mann-Whitney tests (exact probabilities), Wilcoxon signed-ranks tests (exact probabilities), and Student's *t*-tests (for independent and paired samples) corrected for unequal variances, where needed. The level of statistical significance was set at $p < 0.05$ (all tests two-tailed). The Statistical Package for Social Sciences (SPSS, Chicago) was used for all statistical analyses.

Results

As can be seen from Tables 1 and 2, the experimental and control groups were similar with regard to their average body mass indexes, sex distribution, and baseline measures of HbA1c. Yet, the experimental group included on average

older children with longer diabetes duration. Furthermore, both the baseline average insulin dose, as well as the initial average glucose levels, were significantly higher in the experimental group (Tables 1 and 2). Interestingly, in neither group did the average insulin dose change noticeably during the course of the study (Table 1), despite the modifications of therapy in individual cases (Table 3 presents the most relevant modifications).

Median duration of sensor wear was 72 h in the experimental group with the average number of CGMS readings per subject equaling 864. The mean plasma glucose level measured by SMBG (10.6 ± 1.9 mmol/L) did not differ significantly from that measured by CGMS (10.6 ± 2.3 mmol/L) (p = 0.765). These two measures correlated highly and statistically significantly (r = 0.86, p < 0.001). In contrast, linear correlation between HbA1c and mean glycemia

Table 1

Demographic and clinical characteristics of patients (n = 80)

Characteristics	Baseline	p*	3 months	p*	6 months	p*
Number of patients						
experimental group	40					
control group	40					
Female (n, %)						
experimental group	22 (55.0)	0.655				
control group	19 (47.5)					
Age (M ± SD) (yrs)						
experimental group	13.7 ± 3.3	0.016				
control group	11.8 ± 3.8					
Diabetes duration (yrs)						
experimental group	6.3 ± 4.0	0.013				
control group	4.4 ± 2.7					
Insulin (dose/kg) (M ± SD)						
experimental group	0.85 ± 0.23	0.005	0.86 ± 0.23	0.163	0.85 ± 0.22	0.150
control group	0.72 ± 0.18		0.76 ± 0.22		0.76 ± 0.23	
BMI (kg/m^2) (M ± SD)						
experimental group	19.1 ± 2.7	0.303	19.0 ± 2.6	0.438	19.6 ± 2.6	0.228
control group	18.5 ± 2.6		18.6 ± 2.5		18.9 ± 2.9	

BMI – body mass index; M – mean; SD – standard deviation;
***Statistical tests used were Fisher exact tests (sex) and *t*-tests for independent samples (age, diabetes duration, insulin/kg, BMI) – corrected for baseline levels (differences at 3 and 6 months) and unequal variances where needed**

Table 2

Changes in average hemoglobin A1C (HbA1c) and mean glycemia over 6-month period*

Period of the study	HbA1c			Mean glycemia		
	experimental group	control group	$p^{\dagger}$	experimental group	control group	$p^{\dagger}$
baseline	10.0 ± 1.6	10.2 ± 2.0	0.657	10.6 ± 1.9	9.5 ± 2.4	0.031
3 months	9.1 ± 1.5	9.4 ± 1.6	0.604	9.4 ± 2.1	9.0 ± 1.9	0.256
6 months	8.6 ± 1.2	8.9 ± 1.3	0.705	8.8 ± 1.4	9.5 ± 2.4	0.002
p Δ(1-2)‡	0.009	0.001		0.004	0.117	
p Δ(1-3)‡	< 0.001	< 0.001		< 0.001	0.813	

***The results are presented as mean ± standard deviation; †*t*-test for independent samples (between-subjects effects) controlled for baseline differences; ‡*t*-test for paired samples (within-subjects effects) examining changes from the baseline levels.**
Δ(1-2) – difference in average levels between baseline and 3-month measurement;
Δ(1-3) – difference in average levels between baseline and 6-month measurement.

Table 3

Theraupetic interventions in the course of the study (n = 80)

Interventions	Experimental group		Control group	
Long-acting insuline change	25	(62.5)	20	(50.0)
increase	22	(55.0)	17	(42.5)
decrease	3	(7.5)	3	(7.5)
Rapid/short-acting insuline change	17	(42.5)	12	(30.0)
increase	15	(37.5)	9	(22.5)
decrease	2	(5.0)	3	(7.5)
Carbohydrate intake changes	15	(37.5)	9	(22.5)
Increase of physical activity	8	(20.0)	9	(22.5)

***The results are presented as frequencies and percentages (given in parentheses)**

measured by SMBG was small in magnitude, but significant ($r = 0.14$, $p = 0.032$).

At the baseline, the mean hemoglobin A1c value in the experimental group was 10.0 ± 1.6%, whereas it was insignificantly higher in the control group 10.2 ± 2.0%. A significant improvement in HbA1c was observed in both groups after 3 and 6 months ($p < 0.001$) (Table 2). However, the degree of improvement in HbA1c was not statistically significantly different between the two groups, neither at 3 months ($p = 0.604$), nor after 6 months ($p = 0.705$).

Mean glycemia (self-monitored, both groups) were significantly improved in the experimental group after 3 and 6 months (both $p = 0.01$) (Table 2). In the control group, there was an initial decrease in mean glycemia after 3 months, which was not significant ($p = 0.117$), but after 6 months mean glycemia reversed back almost to the baseline level ($p = 0.813$, compared to the baseline). No significant difference in mean glycemia between two groups was found after 3 months ($p = 0.256$); nonetheless, mean glycemia showed to be significantly better controlled in the experimental group than in the control group after 6 months ($p = 0.002$). Table 2 shows a continuous trend of improvement of mean glycemia in the experimental group, contrasted to the control group's only temporary improvement.

A statistically significant decrease in the number of hyperglycemic events per patient per day was observed in the experimental group after 6 months ($p = 0.022$) (Table 4). Also, there was a decrease in the number of hypoglycemic events after 6 months, although it did not reach a statistically significant level ($p = 0.223$) (Table 4). In the control group there was an improvement in the average number of hypoglycemia per patient both after 3 and 6 months compared to the baseline, albeit it was not statistically significant (Table 4). A significant decrease in the average number of hyperglycemia per patient was found in the control group after 3 months ($p = 0.017$), but not after 6 months ($p = 0.828$) (Table 4).

still, the advantage over the intensive classic method (intensive self-monitoring) is not straightforward. Specifically, the improvement in average HbA1c levels was observed in both groups and the difference between them was neither clinically nor statistically significant (1.4% drop in the experimental and 1.3% in the control group). Yet, other clinically important outcomes measured (namely mean glucose levels, number of hypoglycemic and hyperglycemic events) showed higher treatment effects in the experimental group in the long run. We propose some explanations for such ambiguous results.

Firstly, all our participants had relatively high average HbA1c levels (≥ 8%), with one-third of them having values over 10% at some point of the study. The earlier-mentioned low correlation between mean glycemia and HbA1c can be thus ascribed to the restricted range of the scores; the effect of which is a reduced correlation when compared to its magnitude observed in whole population. Therefore, these two measures cannot be seen as interchangeable in assessing glycemic states in our sample. Furthermore, due to the relative protractedness of HbA1c (HbA1c known to be influenced by glycemic levels of up to four months before the measurement [12, 13]) we are inclined to believe that mean glucose levels are more appropriate measures in detecting fine and more recent changes in glycemic levels in a highly event-dynamic sample as was ours. In other words, it is possible that HbA1c was not sensitive enough to detect the changes in the control group in the last three months of the trial, and, as a result, we obtained no statistically significant differences with regard to HbA1c, which was in contrast to other relevant findings.

We also suspect that short-term improvements in the control group in the first three months could be caused by the so-called "experimental effect" which the study had on the participants. In particular, it seems reasonable that "reactivity of measurement" [14] phenomenon had taken place; namely, thanks to the frequent intensive SMBG measurements at the

Table 4

Average number of hypo- and hyperglycemic events per day*

Period of the study	Hypoglycemia			Hyperglycemia		
	experimental group	control group	$p^{\dagger}$	experimental group	control group	$p^{\dagger}$
Baseline	0.8 ± 0.7	0.7 ± 1.4	0.015	4.3 ± 1.7	3.6 ± 2.1	0.266
3 months	0.6 ± 0.7	0.5 ± 0.6	0.429	3.6 ± 2.1	3.0 ± 1.9	0.910
6 months	0.6 ± 0.6	0.6 ± 1.4	0.468	3.2 ± 1.6	3.6 ± 2.3	0.115
$p\ \Delta(1\text{-}2)^{\ddagger}$	0.180	0.715		0.089	0.017	
$p\ \Delta(1\text{-}3)^{\ddagger}$	0.223	0.175		0.022	0.828	

***The results are presented as mean ± standard deviation obtained from 27 measures per visit (9 capillary glycemic tests × 3 days) per individual. Hypoglycemia defined as blood glucose < 3.5 mmol/L; hyperglycemia as blood glucose > 10.0 mmol/L. †Wilcoxon-Mann-Whitney test (between-subjects effects) controlled for baseline differences. ‡Wilcoxon signed-ranks test (within-subjects effects) examining changes from the baseline levels.**

Δ (1-2) – difference in average number of events per day between baseline and 3-month measurement; Δ (1-3) – difference in average number of events per day between baseline and 6-month measurement.

Discussion

The results of this six-month long study suggest that even a short-term use of the CGMS method influences therapy decisions and subsequently leads to the improvement of metabolic control in young T1DM patients in the long run; baseline the participants might have changed their health-related behaviors, but the benefits of it faded out in the course of time. On the contrary, the group receiving treatment and accompanying advice on the basis of their CGMS measures persisted in showing improvements in all outcomes (quite the opposite to what Yates et al. [15] found) throughout

the study, which might indicate profound changes in treatment adherence.

Indeed, further empirical investigation is required to prove the aforementioned hypothesis, even though psychological effects on diabetes treatment adherence when monitoring blood glucose levels with memory-equipped devices have already been discussed [16, 17]. Although the experts emphasize the potential benefits of those devices, some concern was over the possible information overload for the patients [17]. With regard to this, we believe the overload would be less of an issue when the CGMS is used for a short-term only and under medical supervision, as was the case in our study. It must be stated, however, that our intention was not to imply that three-day use would be its suggested use; rather we merely tested whether short-term application of the CGMS could be of any value for clinical practice.

Conclusion

A significant reduction of mean glycemia and hyperglycemic events compared to the control group, accompanied with the observed reduction of HbA1c levels and the number of hypoglycemic events does lead us to a conclusion that the CGMS is a valuable add-on in the control of type 1 diabetes in young patients, even when used for such a brief period. It represents a newer methodology that seems to be better accepted by both younger patients and their parents since it produces comprehensible graphic-imaging of glycemia dynamics, which at least in theory, should lead to better patient's and parents' compliance with the therapy. From the practitioner's perspective, we found that the CGMS offers more information and allow for better decisions in the course of therapy, especially with regard to the insulin administration (dosage and timing) which plays a crucial role in glycemic control. Still, more research is needed in order to determine optimal parameters of CGMS use to exploit it in the most effective way. Lastly, it seems that intensified (e.g. 9-point) self-monitoring of blood glucose has its benefits as well and that at this stage of research it can be perceived as a second-choice alternative to the CGMS, especially in economically challenged countries, such as Bosnia & Herzegovina, where affording continuous glucose measuring devices could still represent an issue.

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Neurons of human nucleus accumbens

Neuroni humanog nukleusa akumbensa

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Abstract

Background/Aim. Nucleus accumbens is a part of the ventral striatum also known as a drug active brain region, especially related with drug addiction. The aim of the study was to investigate the Golgi morphology of the nucleus accumbens neurons. **Methods**. The study was performed on the frontal and sagittal sections of 15 human brains by the Golgi Kopsch method. We classified neurons in the human nucleus accumbens according to their morphology and size into four types: type I – fusiform neurons; type II – fusiform neurons with lateral dendrite, arising from a part of the cell body; type III – pyramidal-like neuron; type IV – multipolar neuron. The medium spiny neurons, which are mostly noted regarding to the drug addictive conditions of the brain, correspond to the type IV – multipolar neurons. **Results.** Two regions of human nucleus accumbens could be clearly recognized on Nissl and Golgi preparations each containing different predominant neuronal types. Central part of nucleus accumbens, core region, has a low density of impregnated neurons with predominant type III, pyramidal-like neurons, with spines on secondary branches and rare type IV, multipolar neurons. Contrary to the core, peripheral region, shell of nucleus, has a high density of impregnated neurons predominantly contained of type I and type IV – multipolar neurons, which all are rich in spines on secondary and tertiary dendritic branches. **Conclusion.** Our results indicate great morphological variability of human nucleus accumbens neurons. This requires further investigations and clarifying clinical significance of this important brain region.

Key words:
nucleus accumbens; neurons; dendrites; anatomy; histology.

Apstrakt

Uvod/Cilj. Nukleus akumbens je deo ventralnog strijatuma, poznatog kao region mozga koji je osetljiv na dejstvo opijata, pogotovo na bolesti zavisnosti. Cilj ove studije bio je istraživanje Goldži morfologije neurona humanog nukleusa akumbensa. **Metoda.** Studija je izvedena na frontalnim i sagitalnim presecima na 15 humanih mozgova prema Goldži Kopš metodi. Klasifikovali smo neurone humanog nukleusa akumbensa prema morfologiji i veličini na četiri tipa: tip I – fuziformni neuron; tip II – fuziformni neuron sa bočnim dendritom koji izlaze sa bočne strane tela neurona; tip III – telo neurona slično piramidi; tip IV – multipolarni neuron. **Rezultati.** Neuroni srednje gustine spina, koji su najviše vezani za osobine adikcije ovog regiona, bili su vezani za tip IV – multipolarni neuron. Dva regiona nukleusa akumbensa mogla su biti jasno razgraničena na Nisl i Goldži preparatima, a svaki je sadržavao dominantne neuralne tipove. Centralni deo nukleusa akumbensa, srž nukleusa akumbensa, imao je malu gustinu impregnisanih neurona sa predominantnim tipom III, piramidi sličnim telom neurona sa spinama na sekundarnim granama i retkim tip IV, multipolarnim neuronima. Suprotno od srži, periferni region, kora nukleusa akumbensa, imao je visoku gustinu impregnisanih neurona dominantnih po tipu I, fuziformnih, i tipu IV, multipolarnih neurona, sa velikom gustinom spina na sekundarnim i tercijarnim dendritima. **Zaključak.** Naši rezultati pokazali su veliku morfološku varijabilnost humanog nukleusa akumbensa. Ovo zahteva dalja istraživanja i pojašnjavanje kliničkih značajnosti ovog važnog moždanog regiona.

Ključne reči:
nukleus akumbens; neuroni; dendriti; anatomija; histologija.

Introduction

Nucleus accumbens occupies ventral part of the striatum, laying laterally on the septum. This nucleus is also known as nucleus accumbens septi. Nucleus accumbens is known to play an important role in pleasure, reward, and addiction.

Subdivisions of nucleus accumbens were described, consisting of the core and shell region. The core region is in-

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volved in motor functions and the shell region is involved in emotional and motivational processes [1]. Two neurochemical subdivisions described in the human nucleus accumbens, could be related to the core and shell regions [2].

In the beginning of the last century, Ramón and Cajal [3] investigated the morphological changes of the brain, due to neuron structure alterations. Behavioral changes initiated by drugs, altered morphological and biochemical structures of brain plasticity and synaptic connectivity [4, 5]. Recent tracing and histochemical studies provide enough evidence to consider that the medium spiny neurons of the nucleus accumbens have the specific role of striatum relation with its connectivity pattern [6]. Nucleus accumbens has the key role in reward and enforcement neuronal processes *via* glutaminergic afferent pathways originated from the basolateral amygdala, ventral subiculum and medial prefrontal cortex [7, 8]. Stimulated dopamine transmission in human nucleus accumbens is related to the addictive properties and positive reinforcement by many drugs [8].

Many cortical and subcortical parts of the brain, especially in the limbic regions are related to drug-induced neurobehavioral adaptations as well as experience. However, it is confirmed that **t**he most important role in this processes belong to the nucleus accumbens [9].

Golgi morphology of neurons in human nucleus accumbens was poorly described in the available literature, which was the reason to undertake this investigation. The aim of this study was to classify types of human nucleus accumbens. We defined several neuronal types considering their soma size and morphology, dendrite patterns and spine density.

Methods

The present study included 15 adult human brains of both genders, aged 30–65 years. All the brains were taken within 12–18 hours after death. Only normal brains with no visible malformations and without any neuropathological changes or neuropsychiatric history were used. The brains were fixed in phosphate buffered neutral solution of 10% formalin (3.7% formaldehyde) over a period of at least 3 months.

The 20 blocks, originating from 15 brains (30 hemispheres), comprising the septal region were stained according to the Golgi-Kopsch method. The other of the remaining 10 blocks, were cut along the coronal plane and divided into 4 thinner slices. The slices were stained alternately by Nissl and Kluver-Barrera methods in order to enable confirmation of the exact topographical relationships. Application of the Nissl and Kluver-Barrera method was necessary for further delineation of other septal structures and was performed on 10 μm thick coronal sections. The transparency of the Golgi-Kopsch, silver impregnation was the most favorable in 100 μm thick coronal sections without stain precipitations, blood vessels and glia.

We investigated all parts of nucleus accumbens following it rostrocaudally, which merges without a clear border with the medial septal nucleus dorsomedially, and with the basal nucleus and substantia innominata ventrolaterally.

Classification of neurons was performed according to the following criteria: a) shape and size of the cell bodies; b) dendrite organization - the position, number, length and its branching patterns; c) density of the spines covering dendrites; and d) axonal branching patterns. Neurons were drawn using a Camera Lucida Leica DMLB 2 under the magnification of 200× and were photographed under different magnifications.

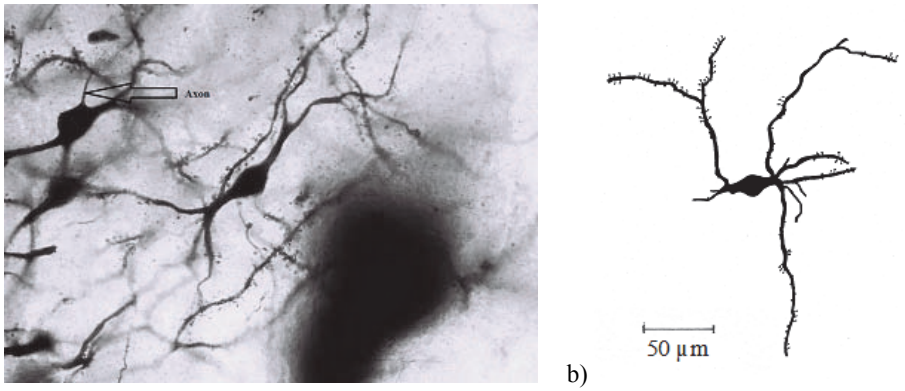
The neuronal soma investigation: maximal length (D max) and maximal width (D min) of perikarya were performed on all of the cells using the Zeiss Axiovision 3.0.6. Aditionally, total dendritic length was controlled by the Sholl analysis [10].

Results

We defined four different types of neurons in human nucleus accumbens, according to their morphology: type I – fusiform neurons; type II – fusiform neurons with lateral dendrite; type III – pyramidal-like neurons, and type IV – multipolar neurons.

Type I – fusiform neuron displayed a fusiform and elongated soma with mostly two long primary dendrites originated from both poles (Figure 1a). The average maximal length (D max) of their elongated perikaryon was 27.99 ± 3.44 μm and the width (D min) was 9.57 ± 0.8μm. The mean total dendrite length (TDL) was 283.34 ± 12.5 μm. Their secondary dendrites were longer than primary ones, but thickness was almost equal (Figure 1b). This frequent neuronal type also predominated in the peripheral region of human nucleus accumbens, the shell, and it was covered by numerous stalked spines, alone its distal dendrites.

Type II – fusiform neuron with lateral dendrite, was found mostly in shell division. In fact, the majority of human nucleus accumbens neurons in the shell division were of this type which exhibited conspicuously featuring on our sections. The specific feature of this type was the constant finding



a) b)

Fig. 1 – Type I (fusiform neuron)

of lateral dendrite arising from the lateral part of the perikaryon (Figures 2, a and b). This thick lateral dendrite was leaving soma mostly from the middle part, under different angles, so we defined as the special type fusiform neurons with lateral dendrite. The perikaryon D max was 26.43 ± 1.22 µm in its long axis, D min was 12.52 ± 0.76 µm wide with 2–3 primary dendrites. Its TDL was 290.98 ± 23.45 µm.

Type III – pyramidal-like neuron, displayed mostly pyramidal or triangular soma, with three major primary dendrite branches. Axons left soma from the part opposite to the strong apical dendrite. Primary dendrites were sparsely covered by the stalked spines, despite the secondary ones with more densely spines (Figures 3, a–f).

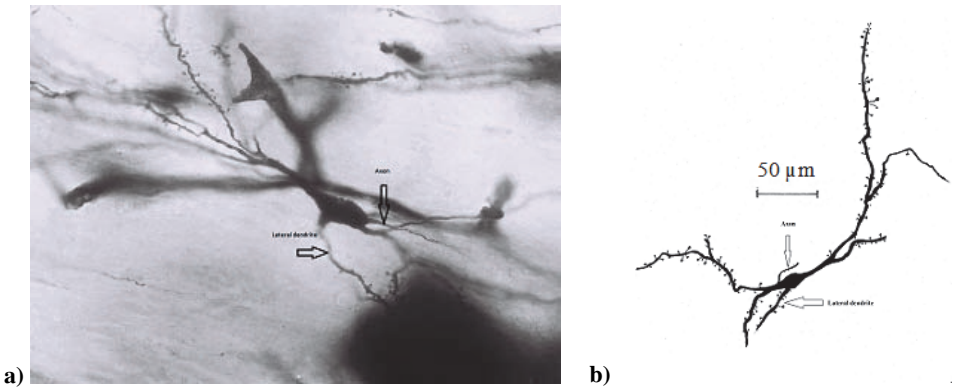


Fig. 2 – Type II (fusiform neuron with lateral dendrite)

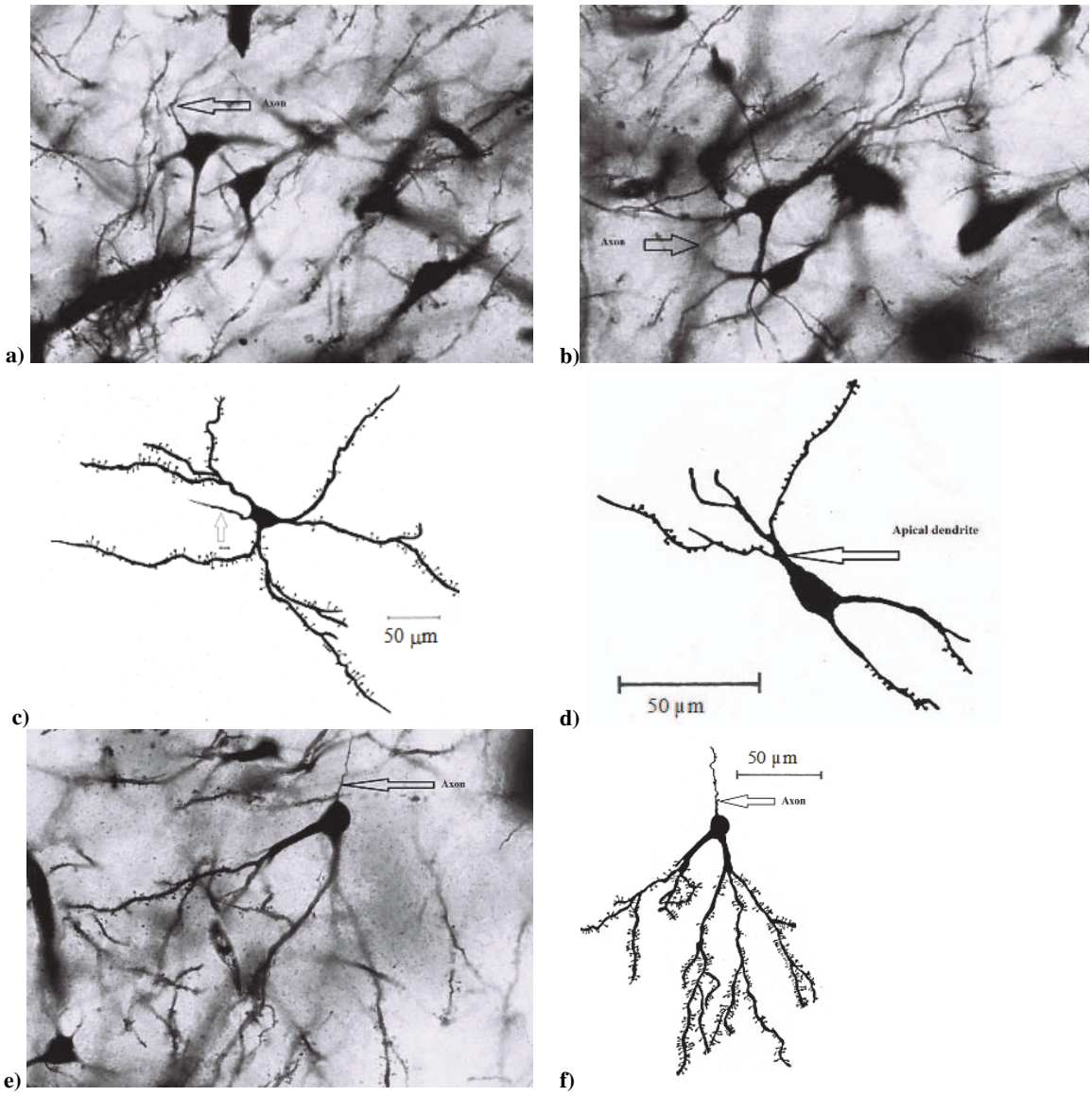


Fig. 3 – Type III (pyramidal-like neuron)

Pyramidal–like neurons had perikaryon D max – 28.3 ± 1.45 μm long, D min was 9.06 ± 0.76 μm wide, and their TDL was 366.02 ± 23.5 μm.

One of the most significant features of these neurons was the presence of the thicker dendrite which raises from the wide base of neuronal soma. Neuronal soma varies from clear pyramidal to elongated pyramidal and piriform. This type was predominant in the core of the nucleus accumbens. Notable was the dominancy of primarily dendrite branch on the "apical" end of neuronal body (Figures 3, e and f). Neurons with piriform soma were sparsely found in shell of the nucleus accumbens with the dense spines on dendrites and occasionally spine like formations on some axons. At the dendrite's end there are many protrusions with the spine clusters at their apex.

Type IV – multipolar neuron, showed different shape of their soma with average six (ranged from 4 to 9) primary dendrites (Figures 4, a–d). The D max of perikaryon was 26.3 ± 1.34 μm and D min was 14.93 ± 2.56 μm. Their numerous dendrites often had varicosities and the primary dendrites were much shorter than the few very long secondary branches which contribute considerably to their mean projected TDL of 354.89 ± 45.3 μm. This neuronal type was found in the core region of the human nucleus accumbens.

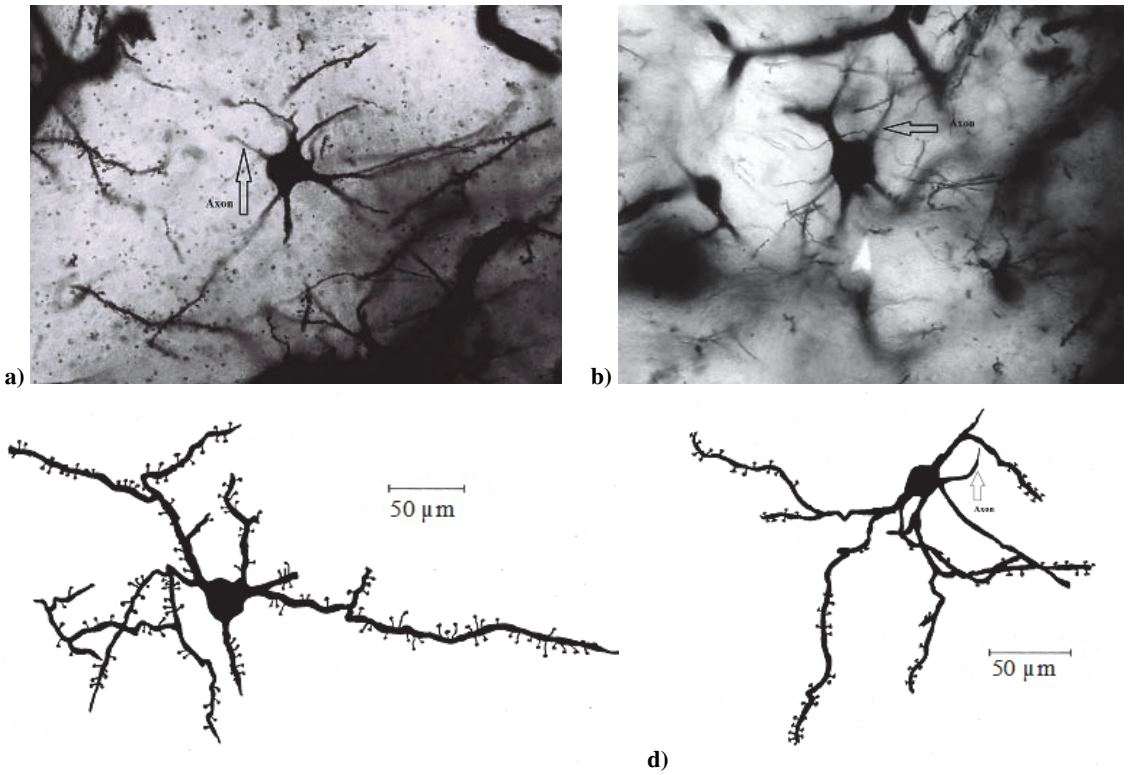


Fig. 4 – Type IV (multipolar neuron)

According to topographical neural distribution we found some difference between the core and the shell. In the shell region of human nucleus accumbens mostly fusiform (type I) and fusiform with lateral dendrite neurons (type II) were predominant, often in groups. Also, their well-arborized dendrite tree was covered by numerous spines. In the core region of human nucleus accumbens, pyramidal-like (type III) neurons were predominant types, but they were usually solitary placed, showing varicose dendrites with stalked spines, and presenting it as medium spiny neurons. Some types of neurons (fusiform, multipolar and piriform) could be classified into larger and smaller subtypes.

Discussion

According to our findings, the border of the human nucleus accumbens to the septum, the shell region, can be recognized by the accumbal fusiform neurons with their longitudinal axis parallel to the convex border to other septal nuclei. The border of human nucleus accumbens to the striatum on Golgi preparations was clearly recognizable by greater cell density and consequent darker appearance of nucleus accumbens. The presented details in the staining sensitivity or selectivity, together with the obvious cytoarchitectural differences and lower impregnation quality of some parts of septal region, may be Golgi-dependent characteristics observed in these regions. It is well-known that the Golgi method is neural and highly selective. On the other hand, this technique provides useful information on the neuronal processes and their branching patterns.

Nucleus accumbens, the shell region, is a part of the limbic region involved in brain functions from motivation and reward to feeding and drug addiction. In the available literature there were few data about Golgi morphology of neurons in human nucleus accumbens. In the cytoarchitectonic study of human nucleus accumbens on Nissl stained sections, Lauer and Hensen [11] mentioned large fusiform, pyramidal- like and granular neurons without detailed descriptions.

We found four major types of neurons in human nucleus accumbens. Fusiform and multipolar types of neurons which we found in human nucleus accumbens correspond to the spiny I type of neurons in monkey striatum described by Di Figlia et al. [12]. Our fusiform neuron (type I) could correspond to the spiny I neurons with flattened soma described by some authors [12]. However, human striate spiny neurons with six primary dendrites described by others [13] could correspond to our multipolar neurons. Our pyramidal-like neurons correspond to spiny type II of Di Figlia et al. [12] with about smaller spine density than spiny I type. Both spiny types (I and II), were considered as the efferent elements, but we did not find aspiny types also described in monkey striatum in human nucleus accumbens [12, 13]. Our finding of type IV, multipolar neuron (Figures 11 and 12), corresponds to medium spiny neurons described by other authors. Medium spiny neurons consist of 2–6 primary dendrites, different thickness, with dense spines on secondary and third dendrite branches [14–17].

We compared the morphology of rat nucleus accumbens neurons and its projection to the substantia nigra, examined by Meredith et al. [6], with our results. Their findings were different in significantly larger number of the spines in the core than in the shell region, similar morphology of perikaryons in both regions of nucleus accumbens (round to oval perikaryons), and the smaller size of perikaryon in the rat (equivalent diameter 9–15 μm). On the other hand, their findings of densely spine dendrites, less primary dendrites than secondary and core dendrites branching under the sharp angle were similar to ours. Possible differences can be attributed to the smaller, different and very specific sample of their neuronal population in rat nucleus accumbens [18, 19].

In mammalian brain psychostimulant drugs induce changes of brain plasticity such as altering dendrite branching, spine density and/or density and synaptic organisations in medium spiny neurons in the nucleus accumbens. Repeated exposure to some psychostimulant drugs is related to behavioural and long lasting changes in the brain. Some of them are transitory and others are persistent, resulting in irreversible changes in the neural structure and behaviour. Thus, well established knowledge of neural morphology is essential for understanding the drug related changes [20–24].

Despite many investigations of medium spiny neurons that exhibit morphological alteration in usage of psychoactive drugs [25–28], their neuroanatomical characteristics remain unexamined, what requires more detailed analysis.

Lower density of impregnated neurons in the core region of the human nucleus accumbens and their higher density in the shell region, with the fusiform and pyramidal-like type neurons (neuronal types most rich in the spines) which we found as the predominant types in shell region, suggest probably nucleus expansion, during the human phylogenesis, favoring the limbic functions.

Conclusion

Because of the great motivational, emotional and other limbic demands, as like as the limbic expression in humans, our results indicate a great morphological complexity of human nucleus accumbens neurons, as it was expected. In general, our findings point to the functional importance of human nucleus accumbens, as an important region for emotional and motivation processes in basal forebrain [28–30].

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Epidemiology of nosocomial colonization/infection caused by *Acinetobacter* spp. in patients of six surgical clinics in war and peacetime

Epidemiologija bolničkih infekcija/kolonizacija uzrokovanih *Acinetobacter* spp. u ratu i miru kod bolesnika iz šest hirurških klinika

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Abstract

Background/Aim. *Acinetobacter* spp. has emerged as nosocomial pathogen during the past few decades in hospitals all over the world, but it has increasingly been implicated as a serious nosocomial pathogen in military hospitals. The aim of this study was to analyse and compare the surveillance data on *Acinetobacter* nosocomial colonization/infection (NCI) collected during the wartime with the data collected in peacetime. **Methods.** We conducted a prospective study of incidence of *Acinetobacter* spp. colonization/infection. Also, the two nested case-control studies were conducted. The patients with nosocomial infection (cases) were compared with those with nosocomial colonization (controls) during the two different periods, wartime and peacetime. The patients with NCI by *Acinetobacter* spp. were identified by the case-based surveillance. The surveillance covered all the patients in 6 surgical clinics. **Results.** During the study periods a total of 166 patients had cultures that grew *Acinetobacter* spp. and the pooled rates of *Acinetobacter* spp. colonization and infection were significantly higher in wartime. When patients with NCI in wartime were compared with those with NCI in peacetime significant differences were observed. In the war year, the patients were more significantly males ($p < 0.000$). In a period of peace, most of the colonization/infections were reported from patients with certain chronic diseases ($p = 0.020$) and the survival of patients was more significant ($p = 0.049$). During the peacetime, proportions of *Acinetobacter* isolates resistent to ciprofloksacin, imipenem and meropenem were significantly higher ($p < 0.001$). **Conclusion.** This study provides additional important information about the risk factors of nosocomial *Acinetobacter* spp. infections in a large cohort of surgical patients. This is also the first study that directly examines epidemiological differences between NCI caused by *Acinetobacter* spp. during the war and peace period.

Key words:
acinetobacter; cross infection; war risk assessment.

Apstrakt

Uvod/Cilj. U poslednjih par decenija *Acinetobacter* spp. postaje značajan uzročnik kolonizacija/infekcija kod hospitalizovanih bolesnika. Njegovo širenje dobilo je poseban značaj u vojnim bolnicama. Cilj ovog istraživanja bio je da se uporede rezultati epidemiološkog nadzora nad bolničkim kolonizacijama/infekcijama uzrokovanim ovom bakterijom dobijeni u periodu rata sa rezultatima dobijenim u periodu mira. **Metode.** Istraživanje je rađeno kao prospektivna studija incidencije kolonizovanih/inficiranih bolesnika *Acinetobacter* spp. Takođe, izvedene su dve „ugnežđene" anamnestičke studije. Bolesnici sa infekcijom uzrokovanom *Acinetobacter* spp. (slučajevi) upoređeni su sa kolonizovanim bolesnicima (kontrole) istim uzročnikom u periodu rata i u periodu mira. Sveobuhvatni epidemiološki nadzor u cilju identifikacije ispitanika sproveden je u šest hirurških klinika Vojnomedicinske akademije. **Rezultati.** U periodu istraživanja registrovano je 166 bolesnika kod kojih je izolovan *Acinetobacter* spp. kao uzročnik koloniza-

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cije ili infekcije. Uočeno je da su stopa kolonizacije/infekcije na svim posmatranim klinikama bile značajno više u periodu rata. Takođe, postojale su značajne razlike između ispitanika u periodu rata u odnosu na period mira. U ratnoj godini, ispitanici su češće bili muškog pola ($p < 0,000$), dok su u godinama mira ispitanici češće bolovali od neke hronične bolesti ($p = 0,020$), a i preživljavanje ispitanika bilo je značajno učestalije ($p = 0,049$). Rezistencija izolata *Acinetobacter* spp. na ciprofloksacin, imipenem i meropenem bila je značajno učestalija u periodu mira ($p < 0,001$). **Zaključak.** Ovo istraživanje obezbeđuje dodatne informacije o faktorima rizika od nastanka infekcija izazvanih *Acinetobacter* u kohorti hirurških bolesnika. Takođe, ovo istraživanje po prvi put ukazuje na značajne epidemiološke razlike između kolonizovanih/inficiranih bolesnika u periodu mira u odnosu na ratni period.

Ključne reči:
acinetobacter; infekcija, intrahospitalna; rat; rizik, procena.

Introduction

Acinetobacter spp. has emerged as nosocomial pathogen during the past few decades in hospitals all over the world [1–5], but it has increasingly been implicated as serious nosocomial pathogen in military medical facilities [6, 7]. This Gram-negative coccobacillus is fairly stable and widely distributed both in nature and in hospital environment [8]. *Acinetobacter* may also colonize the skin of healthy humans [9] or patients without causing infection [10, 11]. Its capacity to survive on most surfaces, animate and inanimate, suggests that hospital environment and colonized patients or healthcare personnel are the major reservoirs of this microorganisms. Multidrug resistance is common among these organisms and may complicate the treatment of serious infections. While resistance to antibiotics is not a new phenomenon for *Acinetobacter*, what is new is that in the last few years some strains have begun to develop resistance to the last effective group of active drugs called carbapenems, powerfull tools for management of nosocomial infections (NI) [12, 13]. Types of infections due to *Acinetobacter* varied and included pneumonia, particularly ventilator-associated pneumonia (VAP) in intensive care units (ICU), bloodstream infections (BSI), surgical site infections (SSI), urinary tract infections (UTI), etc. [14]. Today, nosocomial colonization/infection (NCI) by *Acinetobacter* spp. are a real challenge to infectious diseases physicians, surgones and hospital epidemiologists, especially in wartime.

The aim of this study was to analyse and compare some surveillance *Acinetobacter* colonization/infection data collected during wartime with the data collected during peacetime.

Methods

The Military Medical Academy (MMA), Belgrade, Serbia, is a 1,200-bed tertiary center divided in 27 departments according to medical specialty. The surveillance of NCI by *Acinetobacter* spp. covered all patients in 6 surgical clinics of MMA: Plastic Surgery and Burns, Neurosurgery, Urology, Traumatology/Orthopedics, Cardio/Thoracic Surgery, General/Vascular Surgery.

We conducted a prospective analytic study. The patients with NCI by *Acinetobacter* spp. were identified by a case-based surveillance during the two different periods: wartime, June-September, 1999 (during NATO bombing of Serbia and 3 months after) and peacetime, June-September, 2000–2004. We gathered data on the following variables for patients with NCI: intrinsic factors, including gender, age, diabetes, neoplasm, chronic diseases (cardiac disease, pulmonary disease, hepatic disease, renal disease, malignacy, cerebrovascular accident) and survival; and factors related to health care: ICU admission, indwelling urinary catheter, central venous catheter, mechanical ventilation, nasogastric tube, previous surgery, transfusion of blood and blood products, previous antimicrobial treatment.

Clinical significance (colonization or infection) of *Acinetobacter* spp. isolation and the type of NCI in each surgical patient were assessed according to the Center for Disease Control (CDC) criteria [15].

Clinical and microbiological data also recorded were: type of NCI, as well as antimicrobial sensitivity tests. The patients with isolation of *Acinetobacter* spp. recovered within 48 h of admission were excluded. Also, duplicate isolates from the same patient were excluded; these were defined as isolates of the *Acinetobacter* spp. with the same antimicrobial resistance pattern recovered from the same patient, regardless of the site of isolation. All the patients' characteristics with NCI in wartime were compared with those in peacetime. Also, the 2 nested case-control studies were conducted, with all the patients with NCI during the 2 different periods, wartime and peacetime. Those with NI (cases) were compared with those with NC (controls).

Microbiological testing for the hospital was performed at the Institite for Medical Microbiology in MMA. Identification of isolates was done using the routine methods [16]. Susceptibility testing was done according to the national standards in selection of antibiotics for antibiogram typing [17].

Rates were estimated by dividing the number of new patients with NCI by the number of patient days or by the number of patient admissions in the study period. Statistical analysis for the data was done with SPSS software (SPSS, Chicago, version 10.00). The results were expressed as the mean $\pm$ SD or as a proportion of the total number of patients. Testing for significant differences was conducted by the χ^2 test for categorical variables and by the Student's *t*-test for continuous variables in all studies. Factors were considered significant for a *p*-value of ≤ 0.05. All *p*-values are two tailed. Risk factors (RF) independently associated with infections in a period of peace and a period of war were identified by stepwise logistic regression analysis of variables selected by univariate analysis, with a limit for entering and removing variables at 0.05.

Results

During the study periods, 166 patients had cultures growing *Acinetobacter* spp. (96% were *Acinetobacter baumannii* [*A. baumannii*]), out of which 133 patients met NI criteria. *Acinetobacter* spp. pooled rates are shown in Table 1. The mean age of the study population was 44.33 years (range 10–86 years, median 41.50 years). The patient's characteristics and characteristics related to health care are shown in Table 2. There were no significant differences between the infected and colonized patients, or patients treated in wartime and peacetime, regarding the prior use of antibiotics (Table 3). Univariate analysis of potential RFs for infection by *Acinetobacter* spp. in the wartime in MMA, showed that the patients with NI were more significantly hospitalized in the ICU, and significantly more of then had a urinary catheter and transfusion of blood and blood products than patients with NC (Table 4). But, transfusion of blood and blood

Table 1

Rates of *Acinetobacter* spp. colonization or infection in 6 surgical clinics in the Military Medical Academy, Belgrade during the study periods

Clinics	Wartime		Peacetime	
	Case per 100 admissions	Case per 1,000 patient-days	Case per 100 admissions	Case per 1,000 patient-days
Plastic Surgery and Burns	5.2	3.9	1.3	1.3
Neurosurgery	2.0	1.7	0.8	0.6
Urology	1.2	0.9	0.3	0.3
Traumatology/Orthopedics	4.6	2.6	1.0	0.7
Cardio/Thoracal Surgery	3.2	2.0	1.5	0.6
General/Vascular Surgery	1.6	1.4	0.3	0.4
Average	3.0	2.1	0.9	0.7

Table 2

Characteristics of the patients and characteristics related to helath care of patients colonized or infected with *Acinetobacter* spp. in wartime (1999) and peacetime (2000–2004) in the Military Medical Academy, Belgrade

Patient characteristics	Colonization (n = 33) n (%)	Infection (n = 133) n (%)	*p*	Wartime (n = 73) n (%)	Peacetime (n = 93) n (%)	*p*
Males	27 (81.8)	107 (80.5)	0.859	69 (94.5)	65 (69.9)	0.000
Thermal injury	4 (12.1)	16 (12.0)	0.989	11 (15.1)	9 (9.7)	0.290
Diabetes mellitus	1 (3.0)	6 (4.5)	0.705	1 (1.4)	6 (6.5)	0.106
Neoplasm	4 (12.1)	14 (10.5)	0.792	5 (6.8)	13 (14.0)	0.143
Chronic diseases	7 (21.2)	35 (26.3)	0.546	12 (16.4)	30 (32.3)	0.020
Survivors	31 (93.9)	117 (88.0)	0.324	69 (94.5)	79 (84.9)	0.049
Related to helath care						
clinics*			0.206			0.796
ICU admission	4 (12.1)	62 (46.6)	0.000	27 (37.0)	39 (41.9)	0.518
urinary catheter	11 (33.3)	87 (65.4)	0.001	39 (53.4)	59 (63.4)	0.193
central venous catheter	6 (18.2)	36 (27.1)	0.293	14 (19.2)	28 (30.1)	0.108
mechanical ventilation	4 (12.1)	26 (19.5)	0.321	10 (13.7)	20 (21.5)	0.194
nasogastric tube	5 (15.2)	31 (23.3)	0.309	11 (26.9)	11 (15.1)	0.067
surgery	28 (84.8)	116 (87.2)	0.719	62 (84.9)	82 (88.2)	0.541
transfusion	8 (24.2)	77 (57.9)	0.001	35 (47.9)	50 (53.8)	0.457

***Clinics for: PlastigcSurgery and Burns, Neurosurgery, Urology, Traumatology/Orthopedics, Cardio/Thoracal Surgery, General/Vascular Surgery**

Table 3

Prior use of antibiotics

Received antibiotics	Colonization (n = 33) (%)	*p*	Infection (n = 133) (%)	Wartime (n = 73) (%)	*p*	Peacetime (n = 93) (%)
Without antibiotics	10 (30.3)		21 (15.8)	17 (23.3)		14 (15.1)
Cephalosporins	14 (42.4)		75 (56.4)	34 (46.6)		55 (59.1)
Aminopenicillins	1 (3.0)		4 (3.0)	3 (4.1)		2 (2.1)
Aminoglycosides	3 (9.1)		17 (12.8)	13 (17.8)		7 (17.8)
Fluoroquinolones	0		9 (6.8)	4 (5.5)		5 (5.4)
Carbapenems	3 (9.1)		4 (3.0)	1 (1.4)		6 (6.5)
p		0.122			0.106	

***Clinics for: PlastigcSurgery and Burns, Neurosurgery, Urology, Traumatology/Orthopedics, Cardio/Thoracal Surgery, General/Vascular Surgery**
OR – odds ratio; CI – confidence interval

Table 4

The results of univariate analysis of potential risk factors for infection by *Acinetobacter* spp. in the Military Medical Academy, Belgrade in wartime (1999)

Patient characteristics	Infection (Case) (n = 56) n (%)	Colonization (Control) (n = 17) n (%)	*p* value	OR (95% CI)
Males	53 (94.6)	16 (94.1)	0.934	1.1 (0.1–11.4)
Thermal injury	10 (17.9)	1 (5.9)	0.252	3.5 (0.4–29.3)
Diabetes mellitus	1 (1.8)	0		1.2 (0.1–11.8)
Neoplasm	4 (7.1)	1 (5.9)	0.857	1.2 (0.1–11.8)
Chronic diseases	10 (17.9)	2 (11.8)	0.556	1.6 (0.3–8.3)
Related to helath care clinics*			0.155	1.2 (0.9–1.7)
ICU admission	25 (44.6)	2 (11.8)	0.024	6.0 (1.3–29.0)
urinary catheter	34 (60.7)	5 (29.4)	0.029	3.7 (1.1–12.0)
central venous catheter	13 (23.2)	1 (5.9)	0.144	4.8 (0.6–40.0)
mechanical ventilation	9 (16.7)	1 (5.9)	0.306	3.1 (0.4–26.1)
nasogastric tube	10 (17.9)	1 (5.9)	0.252	3.5 (0.4–29.3)
surgery	47 (83.9)	15 (88.2)	0.665	0.7 (0.1–3.6)
transfusion	32 (57.1)	3 (17.6)	0.008	6.2 (1.6–24.1)

***Clinics for Plastic Surgery and Burns, Neurosurgery, Urology, Traumatology/Orthopedics, Cardio/Thoracal Surgery, General/Vascular Surgery**
OR – odds ratio; CI – confidence interval

products was the only one independent RF for the NI of the patients in a wartime [Odds ratio (OR): 6.2, 95% confidence interval (CI): 1.6–24.1; $p = 0.008$]. Univariate analysis of potential RFs for infection of our patients by *Acinetobacter* spp. in peacetime were similar to those in wartime (Table 5). Also, multivariate logistic regression analysis identified hospitalization in the ICU as the only one independent RF for the NI of patient in peacetime (OR: 6.4, 95% CI: 1.4–30.4; $p = 0.018$).

SSI, UTI and burns were more frequent among the patients in wartime, but pneumonia, BSI, and decubitus ulcer were more frequent among the patients in peactime ($p = 0.025$) (Table 6). Clinical isolates from different culture

Table 5

The results of univariate analysis of potential risk factors for nosocomial infection by *Acinetobacter* spp. in peacetime (2000–2004) in the Military Medical Academy, Belgrade

Patient characteristics	Infection (Case) (n = 77) n (%)	Colonization (Control) (n = 16) n (%)	*p* value	OR (95% CI)
Males	54 (70.1)	11 (68.8)	0.913	1.1 (0.33–3.4)
Thermal injury	6 (7.8)	3 (18.8)	0.191	0.4 (0.8–1.7)
Diabetes mellitus	5 (6.5)	1 (6.3)	0.971	1.0 (0.1–9.5)
Neoplasm	10 (13.0)	3 (18.8)	0.548	0.6 (0.2–2.7)
Chronic diseases	25 (32.5)	5 (31.3)	0.924	1.0 (0.3–3.4)
Related to helath care clinics*			0.533	1.1 (0.8–1.5)
ICU admission	37 (48.1)	2 (12.5)	0.018	6.4 (1.4–30.4)
urinary catheter	53 (68.8)	6 (37.5)	0.023	3.7 (1.2–11.3)
central venous catheter	23 (29.9)	5 (31.3)	0.913	0.9 (0.3–3.0)
mechanical ventilation	17 (22.1)	3 (18.8)	0.768	1.2 (0.3–4.8)
nasogastric tube	21 (27.2)	4 (25.0)	0.852	1.1 (0.3–3.9)
surgery	69 (89.6)	13 (81.3)	0.353	1.9 (0.5–8.5)
transfusion	45 (58.4)	5 (31.3)	0.054	3.1 (1.0–9.8)

***Clinics of Plastic Surgery and Burns, Neurosurgery, Urology, Traumatology/Orthopedics, Cardio/Thoracal Surgery, General/Vascular Surgery; ICU – intensive care units; OR – odds ratio; CI – confidence interval**

Table 6

Type of nosocomial colonization and infection in wartime (1999) and peacetime (2000–2004) in the Military Medical Academy, Belgrade

Type of NCI*	Wartime (n = 73) n (%)	Peacetime (n = 93) n (%)
Colonization	20 (27.4)	22 (23.7)
SSI	30 (41.1)	34 (36.6)
Pneumonia	2 (2.7)	13 (14.0)
UTI	9 (12.3)	4 (4.3)
BSI	4 (5.5)	9 (9.7)
Burns	6 (8.2)	4 (4.3)
Decubitius ulcer	1 (1.4)	7 (7.5)
Skin	1 (1.4)	0

NCI – nosocomial colonisation/infection; SSI – surgical site infection;
UTI – urinary tract infection; BSI – bloodstream infection

sites were equally distributed in both groups of patients (wartime and peacetime) ($p = 0.161$) (Table 7).

Antibiotic susceptibilities of all isolates in the war and peacetime were presented in Table 8. There were no significant differences in the susceptibility frequencies for amikacin, gentamicin, ceftazidim and ampicilin/sulbactam in two study periods. During peacetime, proportions of *Acinetobacter* isolates resistent to ciprofloksacin, imipenem and meropenem were significantly higher ($p < 0.001$).

Table 7

Culture sites of clinical isolates of *Acinetobacter* spp. in wartime (1999) and peacetime (2000–2004) in the Military Medical Academy, Belgrade

Culture site*	Wartime (n = 73) n (%)	Peacetime (n = 93) n (%)
Wound	57 (73.1)	68 (78.1)
Urine	9 (12.3)	5 (5.4)
Respiratory	3 (4.1)	10 (10.8)
Blood	3 (4.1)	9 (9.7)
Central venous catheter	1 (1.4)	1 (1.1)

***Respiratory cultures include bronchoalveolar lavage, sputum and tracheal aspirat**

Table 8

Antibiotic susceptibilities of the isolates of *Acinetobacter* spp. in wartime (1999) and peacetime (2001) in the Military Medical Academy, Belgrade

Antibiotic	Wartime		Peacetime		*p*
	tested (n)	susceptible (%)	tested (n)	susceptible (%)	
Amikacin	69	10.1	88	9.1	0.824
Gentamicin	70	0	83	2.4	0.191
Ceftazidim	69	6.9	87	2.9	0.261
Ciprofloksacin	67	56.7	86	26.7	0.000
Imipenem	69	100	86	81.4	0.000
Meropenem	65	95.4	40	72.9	0.001
Ampicilin/Sulbactam	26	69.2	30	70.0	0.950

Discussion

In the available literature we found a number of studies regarding epidemiology, clinical features, resistance to antimicrobial agents of *Acinetobacter* spp infection/colonization. This study provides further important information about the RF of nosocomial *Acinetobacter* spp. infections in a large cohort of patients admitted to 6 surgical clinics of military tertiary care center in Serbia. Also this is the first study that directly examines epidemiological differences between NCI caused by *Acinetobacter* spp. during war and peace period.

Worldwide, during peacetime conditions, NI constitutes one of the greatest challenges of modern medicine. War conditions imply numerous and different injuries. Generally, war wounds are grossly contaminated, and high velocity missiles can cause massive destruction of bones and soft tissue. Extensive burn injures may be isolated or associated with severe trauma. In addition to trauma, and as direct consequence of war-related circumstances, NIs become one of the greatest challenges of military medicine, too. Some new studies emphasized that war wounds, immediately after injury during the current Iraq conflict, showed the presence of a range of less pathogenic, Gram-positive, skin-commensal bacteria. At the same time Gram-negative bacteria were rarely found, and none was multidrug resistant. So, the use of broad-spectrum antibiotics is unnecessary, but effective infection-control practice to prevent colonization with possible subsequent infections is necessary in early wound management [18].

Our study showed that the pooled rate of *Acinetobacter* NCI was 0.9 cases per 100 admissions or 0.7 cases per 1,000 patient days in peacetime. During the period of war this pooled rate was higher, 3.0 cases per 100 admissions or 2.1 cases per 1,000 patient-days. A large CDC study on *Acinetobacter* epidemiology conducted from 1987 to 1996 reported the average rates of infections of 0.72 cases per 1,000 patient days in ICUs, with persistant seasonal variation in the rate of infections, which increase in late summer months for all major infection sites [19]. During Novemeber 2000, in 25 Spanish hospitals, pooled rate in general hospitals was 0.39 cases per 1,000 patient days with highest rate in ICUs [3]. In our study, the highest *Acinetobacter* NCI rates were in Plastic Surgery and Burns Clinic, both in peace and war period (1.3 cases *vs* 5.2 per 100 admissions or 1.3 *vs* 3.9 cases per 1,000 patient days, respectively). Today, it is well-known that patients with burn injures reperesent a highly susceptible population. The outbreaks of infection caused by *A. baumannii* infection have been reported in burn units. During the outbreak due to multiresistant *A. baumannii* in Ross Tilley Burn Centre, rate of colonization/infection was 12.6 per 100 admissions [20].

Our study also showed significant differences between patients with NCI in wartime and those with NCI in peacetime. In the war year, patients were more significantly males. Similar situation regarding gender was described during the period January 1, 2002 to August 31, 2004, when military health officials reported BSI in 102 patients at 2 military medical facilities, Landstuhl Regional Medical Center (LRMC) in Germany and Walter Reed Army Medical Center (WRAMC), USA, in which service members injured in the

Iraq/Kuwait region and in Afghanistan were treated. Of the 33 patients with *A. baumannii* BSI at LRMC and of the 45 patients with *A. baumannii* BSI at WRAMC, 32 (97%) and 39 (87%) were males, respectively [7]. In the study of Lee et al. [21], conducted in patients admitted during 2000 at the Asan Medical Center in Seoul, Korea, male gender was identified as independent RF for nosocomial occurrence of imipenem-susceptible, but not for nosocomial occurrence of imipenem-resistant *A. baumannii*. In the wartime the 100% of our isolates were susceptible to imipenem.

Furthermore, as patients in hospitals all over the world [22], our patients with some chronic diseases were at the greater risk for NCI in the period of peacetime.

Sunshine et al. [23] performed retrospective, matched cohort investigation at two Baltimore hospitals, to examine outcomes of patients with multidrug (MDR) *Acinetobacter* infection compared with patients with susceptible *Acinetobacter* infections and patients without *Acinetobacter* infections. They showed that 92% of their MDR isolates were not susceptible to carbapenems. Also mortality hospital rate for patients with MDR *Acinetobacter* infections (26%) were higher than for susceptible references (18%) and uninfected references (11%), but only the difference between MDR *Acinetobacter*-infected patients and uninfected patients was statistically significant. Some study conducted in intubated patients, showed that pneumonia caused by *Acinetobacter* spp. is not significantly associated with attributable mortality rate [24]. Proportions of *Acinetobacter* isolates resistent to imipenem and meropenem, were significantly increased during the peacetime, in our study, but the patients survival was significantly higher in this period than in wartime.

Acinetobacter spp. was the most frequently recovered Gram-negative isolate from war wounds and the second most frequent bacterium causing BSI in US Marines with extremity wounds during the Vietnam War [25]. Injured soldiers from Iraq and Afghanistan have also brought an epidemic of MDR *A. baumannii* infection to military hospitals [7]. First, it was not known how this has occurred and the reservoir for infection was unclear, but recent investigation by the military medical and research community suggests that these are NI [7, 18, 26, 27]. During wartime, this bacteria was prevalent isolate in our hospital, too [6].

Univariate analysis of potential RFs for infection by *Acinetobacter spp*. in wartime in MMA, showed that patients with NI were more significantly hospitalized in the ICU and significantly more often had a urinary catheter and transfusion of blood and blood products than patients with NC. But, transfusion of blood and blood products was the only one independent RF for NI of patients in wartime. Also, a study which analyzed data on 5,366 patients over a 2-year period, showed the receipt of blood products as an independent risk factor for infection in hospitalized trauma patients. There has been controversy as to wether such an association merely represents severity of underlying illness or wether receipt of blood products may induce immunosuppressive effects [28]. Some early works of Blumberg and Heal [29], showed that infections associated with transfusions were the major factor in altering host immune defenses [29]. A Canadian national universal leukoreduction program was assocated with unadjusted in-hospital mortality rates, decrease of fever episode, and antibiotic use, but number of serious NI did not decrease [30].

Potential RFs for infection in our patients caused by *Acinetobacter* spp. in peacetime were similar to those in wartime. Our patients with *Acinetobacter* NI were more significantly hospitalized in the ICU, and significantly more often had a urinary catheter and received transfusion of blood and blood products than patients with NC. Multivariate logistic regression analysis identified hospitalization in the ICU as the only one independent RF for NI of patient in peacetime.

Similar multiple factors have also been found in previous studies conducted during peace period all over the world [20, 22, 31, 32].

In the past decades in the USA hospitals, Gram-negative bacilli have been the most frequent pathogens associated with pneumonia and UTIs. However, Gram-positive bacterial pathogens were most frequently associated with BSIs and SSIs. The proportion of ICU pneumonia episodes associated with *Acinetobacter* spp. significantly increased from 4% in 1986 to 7% in 2003 [33]. SSI, UTI and burns associated with *Acinetobacter* spp. were more frequent among the patients treated in MMA in war time, but pneumonia, BSI, and decubitus ulcer associated with *Acinetobacter* spp. were more frequent among the patients treated in peace time ($p = 0.025$).

Acinetobacter spp. isoaltes have inherent and/or easily acquired mechanisms of resistance aganist many of the available antimicrobial agents, which make this pathogen one of the most significant microbial challenges of the current era. Differences in antibiotic susceptibility have been observed among countries, probably as a result of different patterns of antimicrobial usage and environmental factors, especially infection control practice [14, 27]. Although in our hospital, broad spectrum antibiotics are extensively used there were no significant differeces between infected and colonized patients, or patients treated in wartime and peacetime, regarding prior use of antibiotics. Also, there were no significant differences in frequencies for amikacin, gentamicin, ceftazidim and ampicilin/sulbactam susceptibility in the two study periods. During peacetime, proportions of *Acinetobacter* isolates resistant to ciprofloksacin, imipenem and meropenem were significantly higher. Most of *Acinetobacter* isolates in France, which were originally susceptible to fluoroquinolones, became resistent within 5 years of introduction of these antibiotics [14]. In a report of a citywide clonal outbreak in New York City, 53% of *A. baumannii* were resistant to meropenem/imipenem and 12% were resistant to all standard antibiotics [13].

Acinetobacter spp. has been nicknamed the "Gram-negative MRSA", but the same measures cannot be used to control these two pathogens. Therapeutic approaches and control measures should be customized according to epidemiologic pattern of resistant organisms typical for each institution. So, a decision on how to prevent transmission and how to treat patients at risk is the most important step in struggle against this nosocomial pathogen [34].

The most important limitation of our systematic review is that we were unable to type isolates using molecular meth-

ods. The use of molecular typing methods in epidemiologic studies is crucial for investigation sucess.

Conclusion

When characteristics of surgical patients and characteristics related to the health care of surgical patients colonized or infected with *Acinetobacter* spp. in wartime and peacetime are compared, significant epidemiologic differences are observed.

Our results suggest that pooled rates of *Acinetobacter spp*. colonization and infection, especially NI as SSI, UTI's and NI of burns, were significantly higher during wartime. The only independent RF for NI in patients was blood and blood products transfusion during wartime and ICU admission during peacetime. No significant differences regarding the prior use of antibiotics were observed between infected and colonized patients or patients treated during war and peacetime, but proportions of *Acinetobacter* isolates resistant to ciprofloxacin, imipenem and meropenem were significantly higher during peacetime.

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Značaj morfometrijske analize tireocita u diferencijalnoj dijagnozi tireoidnih karcinoma

Thyrocyte morphomethric analysis significance in differential diagnosis of thyroid carcinoma

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Apstrakt

Uvod/Cilj. Konvencionalna citodijagnostika aspirata tireoidnih nodusa ima ograničen značaj u preoperativnom razlikovanju benignih folikularnih lezija i adenomatoznih hiperplastičnih nodusa od folikularnog karcinoma (FTC) i folikularne varijante papilarnog tireoidnog karcinoma (PTC). To otežava pouzdanu preoperativnu dijagnozu ovih lezija što je u nekim slučajevima vezano za nepotrebno operativno lečenje. Cilj istraživanja bio je da se proceni značaj kvantitativnog određivanja površine jedara tireocita (kariomorfometrije) u preoperativnom razlikovanju benignih od malignih tireoidnih nodusa, i njen doprinos rutinskoj citodijagnostici. **Metode.** Analizirani su preparati konvencionalne citodijagnostike i kariomorfometrijski parametri kod 48 bolesnika sa nodoznom strumom i histopatološki nalazi tireoidnih nodusa nakon operativnog lečenja. Prema usvojenim kriterijumima citološke interpretacije nalazi su klasifikovani u tri grupe: malignu (M), n = 15; benignu (B), n = 8, i suspektno malignu (S), n = 25. Korišćenjem računara koji je video-sistemom povezan sa svetlosnim mikroskopom, mereni su površina jedara tireocita i malih limfocita i izračunavani koeficijent varijacije površine jedara tireocita (KVPJ) i indeks anizokarioze. **Rezultati.** Definitivan histološki nalaz potvrdio je PTC kod svih 15 bolesnika sa citološkim M-rezultatom i benigne noduse [folikularni adenom (FA) i adenomatozne hiperplastične noduse] kod 8 citoloških B-nalaza. Kod 15 (60%) bolesnika sa citološkim S-nalazom, potvrđen je tireoidni karcinom. Najveća vrednost površine jedara tireocita bila je u grupi sa PTC (90,74 ± 26,71 μm²) i statistički se značajno razlikovala od svih ostalih grupa ($p < 0,001$). U grupi FTC prosečna vrednost površine jedara iznosila je 69,20 ± 27,31 μm² i pokazivala je značajnu razliku u odnosu na benigne adenomatozne noduse ($p < 0,01$). U odnosu na FA nije bilo značajne razlike. Utvrđena je značajna razlika u vrednostima KVPJ tireocita između ove dve grupe (FTC: 39,46% prema FA: 23,42%, $p < 0,001$). Koristeći kao graničnu vrednost KVPJ 18%, kod 27/30 (90%) bolesnika sa tireoidnim karcinomom nađene su visoke vrednosti KVPJ tireocita. **Zaključak.** Preoperativna kariomorfometrija, kao komplementarna metoda konvencionalnoj citodijagnostici, korisna je u preoperativnom razlikovanju tireoidnih karcinoma od benignih lezija. Koeficijent KVPJ se pokazao kao najosetljiviji morfometrijski parametar maligno transformisanih tireocita.

Ključne reči:
tireoidna žlezda, bolesti; dijagnoza, diferencijalna; tireoidne neoplazme; biopsija iglom; histološke tehnike.

Abstract

Backgraund/Aim. Conventional cytomorphology of thyroid cell aspirates is limited in preoperative differential diagnosis of follicular adenomas (FA) and hyperplastic adenomatoid nodular goiters from well differentiated thyroid follicular carcinoma (FTC) and follicular variant of thyroid papillary carcinoma (PTC). This is the reason of inaccurate presurgical differential diagnosis and in the same cases of inadequate operative managament. The aim of the study was to evaluate the role of quantitative estimation of thyreocite nuclear features (cariomorphometry) in thyroid aspirated smears in preoperative differential diagnosis of benign from malignant thyroid lesions. **Methods.** A total of 48 patients with thyroid nodular disease underwent fine needle aspiration biopsy for cytomorphology, cariomorphometric analysis of the aspirates, and histopathologic explorations conducted fully postoperatively. On the basis of cytomorphology classification the patients were divided into three groups: benign (B), n = 8; malignant (M), n = 15, and suspicious for malignancy (S), n = 25. Using a microscope connected to a computerized video system, mean nuclear area, the nuclear area coefficient of variation (NACV) and anisocariosis ratio were measured and calculated. **Results.** In all

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the 15 patients with cytologically malignant results the diagnosis of PTC was confirmed histopathologically. All cytologically benign lesions were confirmed histopathologically. Thyroid carcinoma was found in 15 out of 25 patients with suspicious lesions. The highest mean values of nuclear area were in the PCT (90.74 ± 26.71 μm²), and were significantly different from all other groups ($p < 0.001$). The mean nuclear area in FTC was 69.20 ± 27.31 μm² and was significantly higher than in the benign adenomatous group ($p < 0.01$). There was no significant difference in mean nuclear area between FTC and FA, but there was a significant difference in NACV between these two groups (FTC: 39.46 % *vs* FA: 23.42%, $p < 0.001$). In 27 out of 30 patients with thyroid carcinoma higher values of NACV than 18% were found. **Conclusion.** Preoperatively cariomorphometry is a useful method in differential diagnosis of thyroid carcinoma from benign lesions, as a complementary method to convencional cytodiagnostics. The NACV showed highest sensitivity as a parameter of malignant thyroid cell transformation.

Key words:
thyroid diseases; diagnosis, differential; thyroid neoplasms; biopsy needle; histological techniques.

Uvod

Citološka analiza aspirata dobijenog iglenom punkcijom nodusa štitaste žlezde danas je utvrđena dijagnostička metoda u preoperativnom ispitivanju tireoidnih nodusa [1, 2]. Pouzdana interpretacija prirode tireoidnog nodusa moguća je kod više od 70% bolesnika [3, 4]. Međutim, iako pouzdana, ova metoda ima i izvesna ograničenja [5, 6]. Jedno od njih predstavljaju nodusi koji se ne mogu citološki pouzadano klasifikovati niti kao benigni, niti kao maligni, zbog čega njihova klasifikacija glasi-suspektno maligno [7–9]. Ti nodusi obuhvataju 15%–30% svih nodusa u većini opisanih serija u literaturi [10, 11]. Najčešći uzrok nejasne diferencijacije kod ove kategorije suspektnih nalaza jeste nemogućnost pouzdanog razlikovanja folikularnog podtipa papilarnog tireoidnog karcinoma (PTC) i dobro diferentovanog folikularnog karcinoma (FTC) (do 50% suspektnih nalaza) od folikularih adenoma (FA) i hiperplastičnih adenomatoznih nodusa (približno 15% svih tireoidnih nodusa). Zbog toga je i pri minimalnoj sumnji na malignitet neophodno operativno lečenje i otklanjanje lezije u cilju patohistološkog pregleda.

Kao jedna od metoda koja bi mogla da pomogne u rešavanju navedenog problema, promovisana je metoda kvantitativne (kompjuterske) analize morfoloških osobina tireoidnih folikularnih ćelija. Inicijalni radovi pokazali su postojanje značajnih razlike u veličini i obliku tireocita između FTC i FA [12, 13]. Međutim, dalja istraživanja na ovom polju dala su kontradiktorne rezultate [14–16]. Kada su u pitanju PTC, većina autora se slaže da povećanje površine jedara tireocita ukazuje na malignu transformaciju ćelija [17, 18]. Međutim, problem folikularnih neoplazmi i dalje je nerešen [19, 20]. Pokušaji da se analizom većeg broja kvantitativnih pokazatelja karakteristika jedara tireocita kao što su obim jedara, stepen zakrivljenosti jedara, odnos najvećeg i najmanjeg prečnika jedara, jedarna ploidija i dr, poveća dijagnostička pouzdanost citološki suspektnih nalaza, nije dao očekivane rezultate. Kao jedan od parametara koji bi mogao biti u korelaciji sa malignom transformacijom tireocita izdvojen je koeficijent varijacije površine jedara tireocita (KVPJ), ali ni ovde stavovi nisu usaglašeni [15, 20].

Cilj ovog istraživanja bio je da se proceni značaj kvantitativne analize površine jedara tireocita i KVPJ u preoperativnom razlikovanju benignih od malignih tireoidnih nodusa.

Metode

Ovom studijom bilo je obuhvaćeno 48 bolesnika, 35 žena i 13 muškaraca, životne dobi 21–74 godine (prosečno 46,75 godina) kod kojih je kliničkim i/ili ultrazvučnim pregledom dijagnostikovan nodus u štitastoj žlezdi. Merenjem serumskih koncentracija ultrasenzitivnog tireostimulišućeg hormona (TSH), tiroksina (T4) i trijodotironina (T3) utvrđena je normalna opšta tireoidna funkcija svih ispitanika. Nakon prethodnog informisanog pristanka, urađena je aspiraciona punkcija nodusa korišćenjem tanke igle spoljašnjeg profila 0,6–0,7 mm (22–23 Gauga). Sadržaj za analizu dobijan je sa najmanje pet aspirata, ubodom kroz nodus u različitim pravcima. Aspirat je smatran dijagnostički validnim ukoliko je na svakom preparatu identifikovano najmanje šest grupa sa 10–15 tireocita. Punkcija je ponavljana ukoliko nalaz ne bi ispunio takve uslove.

Nakon sušenja na vazduhu, preparati su bojeni po standardnoj metodi May-Grünwald-Giemsa. Citološka analiza obavljana je svetlosnim mikroskopom Olympus, pri uveličanju 100×. Analizom je bilo obuhvaćeno najmanje 50 dobro očuvanih jedara tireoicita i do10 malih limfocita u susedstvu, po ispitivanom preparatu.

Prema već usvojenim citomorfološkim kriterijumima [8, 9, 21] interpretacija citoloških nalaza obuhvatila je:

– grupu benignih (B), negativnih nalaza (n = 8) koje su odlikovali: prisustvo tireocita u vidu manjih ostrvaca ili makrofolikularnog rasporeda, uniformnih, ovalnih ili sferičnih jedara, homogenog hromatina, oskudne citoplazme, sa bogatim sadržajem koloida i fagocitnim ćelijama (makrofazima). U odnosu na male limfocite ili eritrocite u susedstvu, projektovana površina ovih tireocita bila je do 1,5× projektivane površine malog limfocita;

– grupu malignih (M), pozitivnih nalaza (n = 15), sa karakteristikama papilarnog karcinoma, prema sledećim minimalnim kriterijumima: hipercelularnost sa ćelijskim rasporedom u vidu papilarnih fragmenata ili plaža ćelija, uvećanih jedara, izmenjenog, nepravilnog oblika i veličine (pleomorfizam i anizocitoza), neregularnog hromatina, prisutnih intranuklearnih citoplazmatskih inkluzija („pseudonukleolusi“) i mikronukleolusa;

– grupu suspektno (S) malignih nalaza (n = 25), koja je obuhvatala tireoidne noduse sa sledećim citomorfološkim karakteristikama: A – aglomerati tireocita u mikrofolikularnom

rasporedu, B – fenomen jedarnog preklapanja, C – odsustvo koloida i D – anizonukleoza i pleomorfizam tireocita.

Analiza morfometrijskih parametara pomoću računara, obavljena je u Institutu za patologiju Vojnomedicinske akademije, Beograd korišćenjem svetlosnog mikroskopa koji je video-kamerom povezan sa personalnim računarom, sa originalnim računarskim programom CAMIA (*Computer Assisted Morphometry Image Analysis*) autora PK.

Manuelno, koristeći kurzor, obeležena su jedra slučajno izabranih ćelija na vidnom polju, i prikazana na monitoru. Kompjuterski je izračunavana (u pikselima) površina jedara i, takođe, površina malih limfocita i eritrocita u susedstvu. Korišćenjem baždarene mikromrežice veličine 1 piksel = 0,16 μm, dobijeni podaci su preračunavani u μm^2 površine. Na osnovu dobijenih vrednosti površine jedara u individualnom slučaju izračunavana je prosečna vrednost površine jedara (PVPJ), KVPJ, i indeks anizokarioze (IA = prosečna vrednost površine jedara tireoicita / prosečna vrednost površine limfocita).

Dobijeni rezultati korelisani su zatim sa definitivnom patohistološkom dijagnozom.

Odluka o operativnom lečenju bolesnika bila je donošena na osnovu prihvaćenih medicinskih indikacija za operativno lečenje nodoznih struma [3]. Definitivna patohistološka dijagnoza dobijana je analizom hirurški odstranjenog nodusa koji je radio nezavisni patolog po kriterijumima WHO klasifikacije karcinoma štitaste žlezde, koji prethodno nije bio upoznat sa analizom morfometrijskih parametara.

Rezultati dobijeni merenjem zadatih morfometrijskih parametara upoređivani su sa definitivnom patohistološkom dijagnozom i na osnovu toga izračunavana je dijagnostička osetljivost i specifičnost zadatih morfometrijskih parametara. U kategoriju pozitivnog nalaza svrstani su bolesnici sa citološkom klasifikacijom malignih nalaza (M = 15) i suspektno malignih nalaza (S = 25) s obzirom na to da su ovakvi citološki nalazi upućivali na operativno lečenje bolesnika sa nodoznom strumom. Kategorija negativnih nalaza obuhvatila je bolesnike sa citološki benignim nalazom (B = 8).

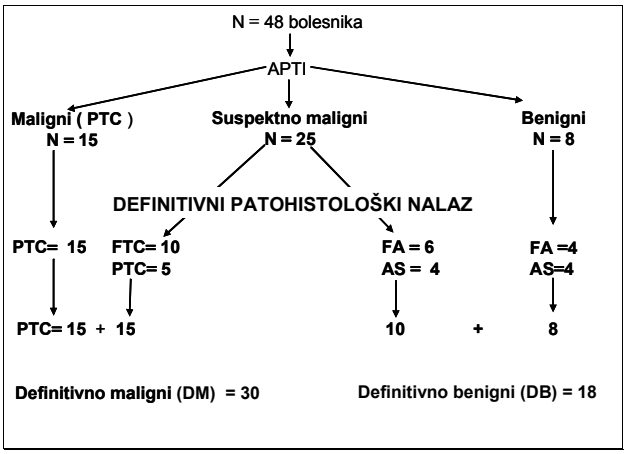
Istraživanje je sprovedeno na principima komparativne studije.

Svi rezultati u tekstu, tabelama i slikama prikazani su kao srednja vrednost ± standardna devijacija (SD). Kada je to bilo neophodno, date su i minimalne, odnosno maksimalne vrednosti pojedinih obeležja. Varijabilnost pojedinih bazičnih skupova (benigna odnosno maligna grupa) iskazivana je primenom koeficijenta varijacije. Statistička značajnost između grupa određivana je, u zavisnosti od tipa i raspodele podataka, primenom Studentovog *t*-testa, χ^2 testa i Fisherovog testa tačne verovatnoće.

U cilju lakšeg praćenja i analize karakteristika ispitivanih parametara utvrđena su tri nivoa statističke značajnosti $p < 0,05$ (*); $p < 0,01$ (**) i $p < 0,001$ (***).

Rezultati

Nezavisno od citomorfološkog nalaza, tireoidni karcinom je verifikovan kod 30 bolesnika (62,5%) od ukupno 48 ispitivana bolesnika. Kod svih 15 bolesnika sa citološki pozitivnim, M-nalazom, ali i kod pet bolesnika sa citološki suspektnim nalazom (grupa S) potvrđen je PT. Svi bolesnici sa FTC pripadali su grupi sa citološki suspektnim nalazom (slika 1).



Sl. 1 – Rezultati citoloških nalaza u poređenju sa definitivnom patohistološkom dijagnozom

APTI – aspiraciona punkcija tankom iglom; PTC – papilarni tireoidni karcinom; FTC – folikularni tireoidni karcinom; FA – folikularni adenom; AS – adenomatozna struma

U odnosu na ukupan broj tireoidnih karcinoma (n = 30), bilo je 66,7% PTC i 33,3% FTC.

Na osnovu zastupljenosti tačnih i lažnih patohistološki nalaza dijagnostička osetljivost citološkog nalaza iznosila je 100%, specifičnost 44,4%, a tačnost 69,7%.

Analiza kariometrijskih parametara, PVPJ tireocita bila značajno veća u grupi tireoidnih karcinoma (81,68 ± 28,98 μm^2), u odnosu na grupu benignih entiteta (63,95 ± 16,04 μm^2), ($p < 0,001$). Najveća PVPJ tireocita nađena je u grupi bolesnika sa PTC, a najmanja u grupi sa adenomatoznom strumom (tabele 1 i 2). U grupi sa FTC, PVPJ tireocita iznosila je 69,20 ± 27,31 μm^2 i nije se razlikovala od vrednosti ovog parametra u grupi FA (tabela 1).

U cilju procene dijagnostičke tačnosti ovog parametra, analizirane su PVPJ pojedinačnih bolesnika u odnosu na graničnu, *cutt-off* vrednost. S obzirom na to da je najveća PVPJ

Tabela 1

Vrednosti kariometrijskih parametara u odnosu na entitete

Patohistološki entiteti	Površina jedara tireocita (μm^2)			KVPJ	IA
	$\bar{x}$ ± SD	min	max	(%)	
Papilarni tireoidni karcinom	90,74 ± 26,71	46,23	170,5	29,44	2,28
Folikularni tireoidni karcinom	69,20 ± 27,31	41,14	133,75	39,46	1,74
Folikularni adenom	68,74 ± 16,10	39,63	107,50	23,42	1,73
Adenomatozne strume	57,30 ± 13,35	31,50	88,81	20,40	1,44

KVPJ – koeficijent varijacije površine jedara tireocita; IA – indeks anizokarioze

Tabela 2

Značajnost između pojedinih patohistoloških entiteta, u pogledu PVPJ (određivana t-testom)

Patohistološka dijagnoza	PTC	FTC	FA	Adenomatozna struma
PTC	–	< 0,001	< 0,001	< 0,001
FTC	< 0,001	–	ns	< 0,001
Folikularni adenom	< 0,001	ns	–	< 0,001
Adenomatozna struma	< 0,001	< 0,001	< 0,001	–

PTC – papilarni tireoidni karcinom; FTC – folikularni tireoidni karcinom; PVPJ – prosečna vrednost površine jedara tireocita; ns – nije značajno

u individualnom slučaju bolesnika grupe adenomatoznih struma bila 72,48 μm^2, kao granična vrednost uzeta je površina jedara 72,5 μm^2 (tabela 3). Više vrednosti od granične nađene su kod 23 bolesnika sa tiroidnim karcinomom (76,7%), ali i kod tri bolesnika sa benignim entitetima. Posmatrano u grupi folikularnih neoplazmi, više vrednosti od graničnih nađene su kod 50% bolesnika sa FTC i kod 30% bolesnika sa FA (tabela 4). Koristeći navedenu graničnu vrednost, osetljivost PVPJ u razlikovanju benignih od malignih lezija iznosi 76,7%, a specifičnost 83,3% (tabela 5).

U grupi PTC kod 18 bolesnika (90%) vrednosti PVPJ bile su više od graničnih. Kod preostala dva bolesnika (10%) ove grupe vrednosti PVPJ bile su manje od granične. Oba bolesnika su citološki pripadala grupi S, a histopatološki folikularnom podtipu PTC (tabela 6). Za razliku od visoke osetljivosti ovog parametra u grupi PTC u grupi FTC kod 5 bolesnika (50%) vrednosti PVPJ bile su niže u odnosu na graničnu (tabela 7), dok su u grupi FA kod tri bolesnika bile više od graničnih (tabela 4). Odstupanje od PVPJ bilo je značajno veće u grupi FTC (SD ± 27,31) u odnosu na grupu FA (SD ±16,10),

Tabela 3

Rezultati morfometrijskih analiza u grupi adenomatoznih struma (individualni prikaz)

Inicijali bolesnika	Površina jedara tireocita (μm^2) $\bar{x} \pm SD$	KVPJ (%)	IA
OR*	55,83 ± 8,32	14,9	1,40
IR	61,67 ± 7,15	11,6	1,55
0M	61,15 ± 10,57	17,3	1,54
BF	72,48 ± 8,86	12,2	1,82
SS	53,30 ± 6,60	12,4	1,34
KD*	55,08 ± 11,26	20,4	1,34
VJ*	37,69 ± 6,63	17,6	0,95
SI*	52,30 ± 6,95	13,2	1,31

***bolesnici sa citološki suspektno malignim nalazom; KVPJ – koeficijent varijacije površine jedara; IA – indeks anizokarioze**

Tabela 4

Vrednosti morfometrijskih parametara u grupi patohistološki dokazanog folikularnog adenoma (individualni prikaz)

Inicijali bolesnika	Površina jedara tireocita (μm^2) $\bar{x} \pm SD$	KVPJ (%)	IA
IJ*	67,03 ± 9,78	14,59	1,69
ŠT*	95,36 ± 18,27	19,2	2,40
KA*	61,39 ± 9,78	15,9	1,54
IB*	59,30 ± 8,48	14,3	1,49
PB*	53,29 ± 11,58	21,7	1,34
OE*	71,07 ± 12,12	17,1	1,79
IG	72,25 ± 8,95	12,4	1,82
RD	77,86 ± 11,62	14,9	1,96
JB	68,58 ± 12,19	17,8	1,73
VA	77,45 ± 17,81	23,0	1,95

***bolesnici sa citološki suspektno malignim nalazom; KVPJ – koeficijent varijacije površine jedara; IA – indeks anizokarioze**

Tabela 5

Poređenje grupa prema prosečnoj vrednosti površine jedara (PVPJ) tireocita (*cutt-off* 72,5 µm2)

PVPJ (μm^2)	Definitivna patohistološka dijagnoza	
	benigno	maligno
< 72,5	15	7
> 72,5	3	23

***p* < 0,001 (χ^2 test)**

Tabela 6

Vrednosti morfometrijskih analiza u histološki potvrđenom papilarnom tireoidnom karcinomu (PTC) u odnosu na citološki nalaz

Citološki nalaz	Površina jedara tireocita (μm^2) $\bar{x} \pm SD$	KVPJ (%)	IA
Suspektan (n = 5)	87,97 ± 23,57	26,8	2,21
	69,46 ± 13,21	19,0	1,75
	88,95 ± 25,40	28,6	2,24
	71,10 ± 18,69	26,3	1,79
	97,82 ± 25,25	25,8	2,46
Maligan (n = 15)	84,62 ± 17,53	20,7	2,13
	103,67 ± 27,26	26,8	2,61
	83,95 ± 15,26	18,2	2,14
	89,61 ± 17,86	19,9	2,26
	105,03 ± 24,74	23,6	2,64
	116,6 ± 24,11	20,7	2,94
	72,84 ± 2,78	31,3	1,84
	72,70 ± 21,00	28,9	1,83
	91,66 ± 21,74	23,7	2,31
	90,69 ± 18,32	20,2	2,28
	75,74 ± 11,44	17,46	1,91
	122,25 ± 22,99	18,8	3,08
	116,66 ± 24,11	27,2	2,94
	100,02 ± 17,48	17,5	2,52
	114,23 ± 30,86	27,0	2,87

KVPJ – koeficijent varijacije površine jedara tireocita; IA – indeks anizokarioze

Tabela 7

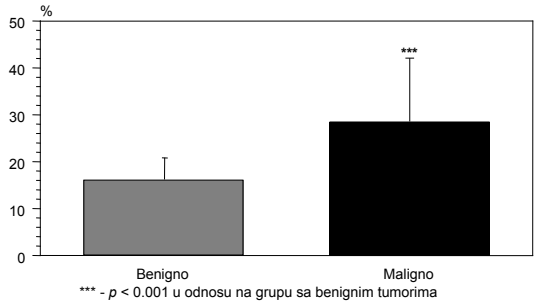
Vrednosti morfometrijskih parametara u patohistološkoj grupi FTC (individualni prikaz)

Inicijali bolesnika	Površina jedara tireocita (μm^2) $\bar{x} \pm SD$	KVPJ (%)	IA
AS*	99,52 ± 30,41	30,6	2,50
AA*	121,29 ± 25,53	21,1	3,05
ID *	49,65 ± 10,12	20,4	1,25
MLJ*	86,28 ± 29,38	34,1	2,17
SR*	78,13 ± 25,04	32,5	1,97
JR*	75,17 ± 16,78	22,3	1,89
IM*	58,26 ± 13,94	23,9	1,47
OP*	61,28 ± 15,85	25,9	1,54
SN*	48,71 ± 8,59	17,63	1,23
RD*	50,77 ± 18,70	36,8	1,28

***bolesnici sa citološki suspektno malignim nalazom; KVPJ – koeficijent varijacije površine jedara tireocita; IA – indeks anizokarioze**

što se odražavalo i značajnije većim KVPJ u grupi FTC (KVPJ = 39,46%) u odnosu na grupu FA (tabela 1).

Analizirane vrednosti KVPJ u grupi svih bolesnika sa tireoidnim karcinomima bile su značajno veće od vrednosti ovog parametra u grupi benignih entiteta ($p < 0,001$) (slika 2).



Sl. 2 – Koeficijent varijacije površine jedara tireocita u grupama sa benignim i malignim tumorima štitaste žlezde

S obzirom na to da se raspon vrednosti KVPJ u grupi tireoidnih karcinoma kretao od 17,46%–36,8% kao granična (*cut-off*) vrednost, uzeta je vrednost KVPJ od 18% (tabela 8). Koristeći ovu vrednost kao graničnu, 90% bolesnika sa tireoidnim karcinomom imalo je veće vrednosti KVPJ od graničnih ($p < 0, 001$, tabela 9).

Poređenje grupa bolesnika sa malignim i benignim lezijama prema parametru KVPJ prikazano je u tabeli 8, a dijagnostička osetljivost, specifičnost i tačnost analiziranih morfometrijskih parametara (PVPJ, KVPJ i indeks anizokarioze) u tabeli 10.

Indeks anizokarioze u grupi bolesnika sa patohistološkom dijagnozom tireoidnog karcinoma pokazuje više vrednosti u odnosu na bolesnike sa patohistološki benignim entitetima, i dobar je pokazatelj veličine maligne ćelije u odnosu na veličinu normalnog tireocita (tabela 1).

Tabela 8
Poređenje grupa prema koeficijentu varijacije površine jedara (KVPJ) tireocita (*cut-off* vrednost 18%)

KVPJ	Definitivna patohistološka dijagnoza	
	benigno	maligno
< 18%	14	3
> 18%	4	27

$p < 0{,}001$ (χ^2 test)

Tabela 9
Značajnost razlike između grupa za koeficijent varijacije površine jedara (KVPJ) tireocita

Patohistološka dijagnoza	Papilarni tireoidni karcinom	Folikularni tireoidni karcinom	Folikularni adenom	Adenomatozna struma
Papilarni tireoidni karcinom	–	ns	< 0,01	< 0,001
Folikularni tireoidni karcinom	ns	–	< 0,05	< 0,01
Folikularni adenom	< 0,01	< 0,05	–	ns
Adenomatozna struma	< 0,001	< 0,01	ns	–

ns – nije značajno (Fišer-ov test tačne verovatnoće)

Tabela 10
Dijagnostička osetljivost, specifičnost i tačnost pojedinih morfometrijskih parametara

Morfometrijski parametri	Osetljivost (%)	Specifičnost (%)	Tačnost (%)
PVPJ	76,7	83,3	79,2
KVPJ	90	77,8	85,4
IA	76,7	72,2	75,0

PVPJ – prosečna vrednost jedara tireocita; KVPJ – koeficijent varijacije površine jedara tireocita; IA – indeks aminokarioze

Diskusija

Doprinos kvantitativnih tehnika (morfometrije) jasnijem razlikovanju benignih od malignih proliferacija štitaste žlezde i dalje je kontroverzan. Za razliku od optimizma koji su donela početna istraživanja, kasniji radovi nisu u potpunosti potvrdila ovaj značaj.

Iako rezultati našeg istraživanja pokazuju značajno veću PVPJ tireocita kod bolesnika sa tireoidnim karcinomima, ovo je rezultat predominacije PTC koji je bio prisutan kod 60% ispitivanih bolesnika. Luck i sar. [15] istražujući morfometrijske karakteristike tireocita upravo najčešćih patohistoloških entiteta, koji predstavljaju citomorfološku diferencijalnodijagnostičku poteškoću, nalazi značajno veću vrednost jedarne površine samo kod PTC, dok kod drugih entiteta, FTC, adenoma i adenomatoznih nodusa nije bilo razlike ni u ovoj, niti u drugim morfološkim karakteristikama jedara tireocita. Wright i sar. [20], ukazuju na široki raspon vrednosti površine jadara tireocita u individualnim slučajevima, sa preklapanjem između benignih i malignih folikularnih lezija, što otežava interpretaciju dobijenih rezultata. Naši rezultati, takođe, ukazuju na to da nema značajnije razlike u jedarnoj površini tireocita između folikularnih karcinoma i adenoma, što odgovara opisanim morfološkim karakteristikama folikularnih neoplazmi. Preklapanje vrednosti površine jedara između benignih i malignih folikularnih lezija uočili su i drugi autori [18, 22–24]. Ravinsky i Safneck[12] ističu da su morfološke karakterstike folikularnih neoplazmi, pa čak i adenomatoznih struma i folikularne varijante PTC slične, te da su ćelije retko kada tako izmenjene morfologije da bi se odmah mogle prepoznati kao maligne. Razlog za postojanje ili nepostojanje fenotipskih ćelijskih razlika nije u potpunosti jasan. Proliferacija i diferencijacija tireoidnih epitelijalnih ćelija pod uticajem je brojnih faktora rasta, onkogena, citogena, hormona i drugih mitogena. Odgovor tireocita na ove faktore uslovljen je postojanjem različitih, tačno određenih, puteva transmisije signala čiji je osnovni cilj aktivacija jedarne transkripcije i formiranje ćelijskog fenotipa. Ovo može delimično objasniti razlike, ali i sličnosti u transformisanom ćelijskom fenotipu pojedinih oblika tireoidnih karcinoma, njihovom karakterističnom morfološkom i biološkom ponašanju. Izražen polimorfizmam ćelija sa krupnim nepravilnim jedrima, neregularnog, svetlog hromatina i karakterističnim prisustvom citoplazmatskih invaginacija, „pseudonukleolusa“, tipične su citomorfološke karakteristike PTC [16]. Kariomorfometrijski, ovakva jedra su značajno veće projektovane površine od regularnih. Ovo, međutim, nije slučaj sa folikularnom varijantom PTC koji je po svojim morfološkim karakteristikama sličniji folikularnim lezijama [9–11, 21]. Kod 10% naših bolesnika sa PTC koji su histološki potvrđeni kao folikularni podtip, vrednosti PVPJ bile su niže od graničnih. Isto tako, kod 50% bolesnika sa FTC vrednosti istog parametra bile su značajno manje i bliže vrednostima FA, što sve ukazuje na to da jedarna površina kao izolovan, samostalni parametar ne može biti pouzdan pokazatelj maligne transformacije tireocita. U analizi bolesnika sa citološkom interpretacijom suspektno maligno, Nagashima i sar. [17, 18] ne nalaze povezanost analizirane površine jedara i perimetra jedara sa određenim histopatološkim podtipovima folikularnih lezija. Izražena varijacija u površini jedara tireocita ogleda se u značajnim razlikama KVPJ tireocita između FTC i benignih lezija. Kod 27/30 (90%) naših bolesnika sa tireoidnim karcinomom, vrednosti KVPJ pokazuju značajno više vrednosti. Ukoliko se vrednosti ovog parametra analiziraju u grupi FTC, uzimajući kao graničnu vrednost 18%, samo kod jednog od 10 (90%) bolesnika nalazimo nižu vrednost od granične, što ukazuje na visoku osetljivost navedenog parametra u dijagnostici FTC. Ukoliko se slična analiza primeni na

grupu benignih entiteta, kod 14 (85,5%) nalazimo niže vrednosti KVPJ, što ovom parametru obezbeđuje specifičnost od 77,8% i tačnost od 85,4%.

Frasoldati i sar. [25] kao meru brze procene veličine jedra analiziranog tireocita uvode i odnos jedarne površine prema konstanti, a to je mali limfocit. Analizirajući sva tri parametra ovi autori, zaključuju, takođe, da iako vrednost površine jadara kao samostalni parametar ne može doprineti pouzdanijem razlikovanju benignih od malignih promena, određivanje koeficijenta varijacije i indeksa anizokarioze povećava osetljvost metode do 82,5% u slučajevima suspektno malignih nalaza.

Kod naših bolesnika indeks anizokarioze pokazao se kao parametar za jednostavniju procenu veličine jedra maligne ćelije u odnosu na normalan tireocit. Naime, fiziološke varijacije površine normalnog tireocita odgovaraju 1–1,5 × površina malog limfocita. S obzirom na to da je izveden iz PVPJ, IA pokazuje sličnu dijagnostičku osetljvost i specifičnost kao i PVPJ.

Zaključak

Analizirajući rezultate našeg istraživanja možemo zaključiti da kvantitativna procena morfometrijskih parametara jedara tireocita, kao dopunska metoda citološkoj analizi, omogućava pouzdanije razlikovanje benigne od maligne tireoidne promene, a kao najosetljiviji parametar izdvaja se KVPJ tireocita. Može se izvoditi istovremeno sa citološkom analizom i, osim video-kamere i računara, ne zahteva dodatnu opremu, a analizirani podaci se mogu memorisati i po potrebi analizirati i kasnije. Međutim, prema našem iskustvu, kao samostalna analiza ne može zameniti citomorfološku dijagnostiku, tako da bi dalja ispitivanja trebalo da budu usmerena ka otkrivanju osetljivijih strukturnih parametara kao što su onkogeni i njihovi produkti, molekularni markeri i dr, koji mogu pomoći u rešavanju problema diferencijalne dijagnoze određenih oblika benignih od malignih tireoidnih lezija.

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ORIGINAL ARTICLE

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The effects of education with printed material on glycemic control in patients with diabetes type 2 treated with different therapeutic regimens

Efekti informisanja štampanim materijalom o kontroli glikemije kod bolesnika sa dijabetesom tip 2 lečenih različitim terapijskim režimom

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Abstract

Background/Aim. Diabetes mellitus (DM) is considered to be an epidemical, chronic and progressive disease. The treatment of DM reqiures substantial effort from both the diabetes treatment team and a patient. Patient education is one of the treatment elements. The most efficacious form and content of education has not yet been established. However, every DM education must include introduction to a substantial number of facts about diabetes. The aim of our study was to estimate the levels of DM knowledge and glycemic control in Serbian patients with DM type 2 as well as to estimate the effects of education using printed material on the levels of glycemic control and knowledge about DM. Also, the effects of education on glycemic control and the level of knowledge in differently treated patients were estimated. **Methods.** The patients with DM type 2 (n = 364), age 40 to 65 years, from three regional health centers, were randomized for the study. After informed consent, patients fell out the questionnaire, and were checked for HbA1c and fasting blood glucose. Finally, booklet „Healthy lifestyle with diabetes mellitus type 2“ was given to them. The same procedure was repeated after 3, 6 and 18 months. **Results.** There was a significant improvement in HbA1c levels after 3 months (8.00 ± 1.66% *vs* 9.06 ± 2.23%, $p < 0.01$) and after 6 months (7.67 ± 1.75% *vs* 9.06 ± 2.23%, $p < 0.01$). There was no further improvement in HbA1c after 18 months (7.88 ± 1.46% *vs* 7.67 ± 1.75%, $p > 0.05$). There was a significant improvement in the average test score (percent of correct answers per test sheet) after three monts (64.6% *vs* 55.6%, $p < 0.01$). There were no further statistically significant changes in the general level of DM knowledge after 6 months (65.0 ± 32.5% *vs* 64.5 ± 33.7%, $p > 0.05$) and after 18 months (64.8 ± 32.7 *vs* 64.5 ± 33.7%, $p > 0.05$). There was a significant diffrence in educational intervention response in DM type 2 patients on different therapeutic regimens. **Conclusion.** Education with printed material led to improvement in glycemic control and level of DM knowledge in our patients. Education with printed material may be a useful adjunct to DM treatment and should be structured according to the treatment modality.

Key words:
diabetes mellitus, type 2; patient education as topic; questionnaires; blood glucose.

Apstrakt

Uvod/Cilj. Dijabetes melitus DM tip 2 je hronično progresivno oboljenje epidemijskog karaktera. Lečenje DM zahteva potpunu posvećenost medicinskog tima, ali i bolesnika. Informisanje bolesnika jedan je od faktora u lečenju ove bolesti, a efikasna forma i sadržaj informisanja ni danas nisu jasno određeni. Poznavanje činjenica o DM je neophodni sadržaj svake edukacije. Cilj našeg rada bio je da procenimo nivo glikemijske kontrole i nivo poznavanja činjenica DM o kod naših bolesnika sa DM tip 2, kao i efekat informisanja štampanim materijalom na nivo glikemijske kontrole kod bolesnika lečenih različitim terapijskim modalitetima. **Metode.** Bolesnici sa DM tip 2 (n = 364), starosti od 40 do 65 godina, na redovnim kontrolama u regionalnim centrima za lečenje DM potpisali su posle informisanja pristanak i ispunili upitnik. Mereni su im HbA1c i glikemija našte, nakon čega su dobili štampani materijal - brošuru „Zdrav život sa dijabetesom”. Procedura je ponovljena posle 3, 6 i 18 meseci. **Rezultati.** Došlo je do statistički zna-

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čajnog poboljšanja nivoa HbA1c nakon 3 meseca (8,00 ± 1,66 *vs* 9,06 ± 2,23%, $p < 0,01$) i nakon 6 meseci (7,67 ± 1,75 *vs* 9,06 ± 2,23%, $p < 0,01$). Nakon 18 meseci nije došlo do daljeg sniženja nivoa HbA1c (7,88 ± 1,46% *vs* 7,67 ± 1,75%, $p > 0,05$). Test skor (prosečan broj traženih tačnih odgovora) značajno je poboljšan nakon 3 meseca (64,6% *vs* 55,6%, $p < 0,01$). Posle 6 i 18 meseci nije bilo značajnih promena u broju tačnih odgovora (6 meseci: 65,0 ± 32,5% *vs* 64,5 ± 33,7%, $p > 0,05$; 18 meseci: 64,8 ± 32,7% *vs* 64,5 ± 33,7%, $p > 0,05$). Ustanovljena je značajna razlika u odgovoru na pitanja o DM kod bolesnika sa različitim terapijskim režimima. **Zaključak.** Informisanje štampanim materijalom bez uticaja instruktora dovelo je do poboljšanja glikemijske kontrole kod naših ispitanika. Nivo poznavanja pojedinih činjenica o DM značajno je poboljšan. Ovaj tip informisanja korisna je dopunska intervencija u lečenju DM. Sadržaj informisanja treba prilagoditi terapijskom režimu.

Ključne reči:
dijabetes melitus, insulin-nezavisni; obrazovanje bolesnika; upitnici; glikemija.

Introduction

Diabetes mellitus (DM) is a chronic and progressive disease. Treatment of DM is nowdays in focus due to an unexpectedly large number of people affected with the disease [1, 2], various treatment options [3–9], the possibilities for DM prevention [10–13] and fact that the patient plays an active part in the treatment [14, 15]. Patient education is an integral part of the treatment [16–19]. The best way and the best content of DM patient education has not yet been established [20]. Patient education in DM that only increases patient knowledge on diabetes facts does not neccessarily lead to better glycemic control [21, 22]. Besides, good glycemic control is not the only positive DM treatment outcome [23–25]. It is postulated that patient education should be structured and enable the patient to make decisions that lead to good DM control, as well as to make him/her as independent from the medical team as much as possible. Diabetes treatment guidelines recommend diabetes patient education as an integral part of treatment [26–28]. However, inspite of treatment guidelines (for DM treatment, patient education and self-management of the disease) and variety of treatment options, the level of glycemic control is generally unsatisfied. It is estimated that less than one third of patients achieve good glycemic control. Diabetes education was mentioned as one of the elements of treatment that leads to good glycemic control [29]. However, there is neither a standardized educational program nor the recommended amount of facts that should be presented to DM patients in Serbia.

In 2004 UK Department of International Development provided the printed material „Healthy lifestyle with diabetes type 2“ and the HbA1c assays, as a donation to help DM care in Serbia. The printed material included relevant facts about t DM type 2. The aim of our study was to estimate the impact of education with this printed material on glycemic control in DM type 2 patients treated with different therapeutic modalities.

Methods

Diabetes mellitus type 2 patients (age 40 – 65 years), having the disease for more than 6 months, were informed about the study during their regular checkup. The study was performed at two Clinical Centers and one General Hospital in Serbia. After signed informed consent, the patients were randomized for the study. In all the patients fasting plasma glucose (FPG) and HbA1c were measured and subsequently the patients fullfiled the Questionnaire. They answered the questionnaire without interference of medical stuff. At the end of visit the patients were given the printed material „Healthy lifestyle with diabetes type 2“. The same procedure was repeated after 3, 6 and 18 months. The printed material was given to the patient only at the first visit. According to the Study Protocol DM therapy should not be changed during the first 6 months of the trial. The printed material was made in accordance with the educational programs of British Association for Diabetes and University of Michigan Diabetes Research and Training Center (UMDRTC). A two-way translation of the material was done prior to publishing. The material included Chapters (Table 1).

Table 1
Content of the printed educational material “Healty lifestyle with diabetes type 2“

Chapter	Title
1	**Introduction**
	What is diabetes type 2
2	**Be active**
3	**What is a hypoglycemia**
	How to manage hypoglycemia
4	**Food groups that we sholud be aware of**
	Body mass index
	How to calculate your body mass index
	Fiber
	Fat
	Salt
5	**Healthy life**
	Alchohol
	Smoking
6	**Diabetes complications/ prevention**
	Foot care
	Neuropathy
	Stress and relaxation
	Exercise and hypoglycemia
	Erectile dysfunction
	Prevention of diabetic retinopathy

The content was mainly quantitative except for the part referring to body mass index (BMI). The Questionnaire was created at the Clinic for Endocrinology, Diabetes and Metabolic Disease, Clinical Center of Serbia, according to the standard Diabetes Knowledge Test developed at UMDRTC and consulting the Diabetes Empowermet Scale and contained 40

questions. The questions were constructed following the text in the printed material. Simple fact reproduction was required. There were 8 questions that were connected to the patients attitude towards diabetes. The Questionnaire was identical in all examining series. The Questionnaire contained multiple choice, open type questions. Fasting blood glucose was determined using glucose-oxidase procedure, using a polarographic oxygen analyzer (Bekman Glucose Analyzer). HbA1c was determined using Bayer DCA 2000 Reagent Kit, which is based on a latex immunoagglutination inhibition methodology.

beginning of the study. From 6th to 18th month of the study, glycemic control, as seen through HbA1c, was not significantly improved (HbA1c 7.88 ± 1.46% *vs* 7.67 ± 1.75%, $p > 0.05$) (Figure 1). As expected, the patients treated with insulin had the highest level of HbA1c at the beginning of the study (9.99 ± 2.25%), followed by the patients treated with oral hypoglycemic drugs (8.79 ± 1.64%), and finally those controlled with lifestyle intervention (7.63 ± 1.94 %). Only the patients treated with lifestyle intervention did not show significant changes in the level of HbA1c during the study (Table 2).

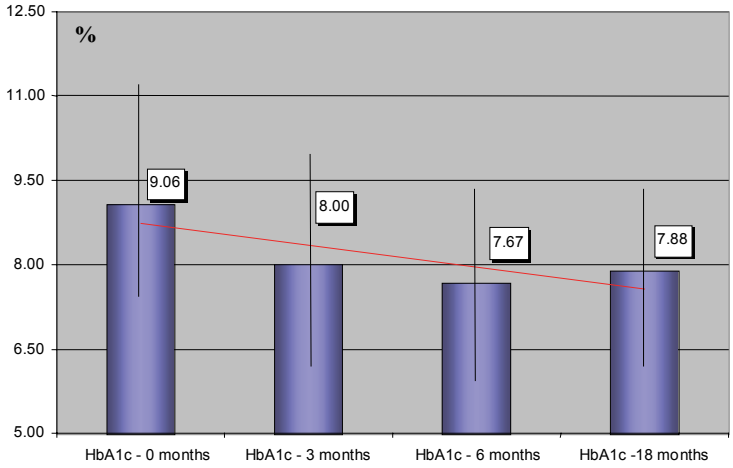

Fig. 1 – Change in HbA1c after educational intervention

Table 2

Influence of the educational intervention on changes in HbA1c level with recpect to therapeutic regimens during the study

Duration of the study (months)	Average HbA1c level (%)		
	insulin	oral hypoglycemic drugs	lifestyle change
0	9.9	8.79	7.63
3	8.38*	7.89*	7.31
6	7.84*	7.64*	6.68†
18	8.03*	7.85*	6.96

*** $p < 0.01$ *vs* 0 months ; † $p < 0.05$ *vs* 0 months**

Statistical analysis was done using methods of descriptive and analytical statistics (Student`s *t*–test, $x^2$ test, Friedman`s test). Statistical package SPSS 10 was used for analysis.

Results

The study included 364 DM type 2 patients. Of them, 276 finished the study (2 patients died, 86 did not give sufficient data). There was no statistical significance between the number of males and females included in the study. Regarding age of the participants 19% were 41-50 yeras old, 42.3% were 51-60 years old and 38.2% were over 65 years old. The majority of patients (44.8%) had high school education (44.8%), 2.5% of the patients did not have elementary school education and 16.4% of patients had college/BA education. Average body mass index (BMI) was 27.6 ± 0.3 kg/m^2, which is in the overweight range.

A significantly better glycemic control was achieved at 3 months (HbA1c: 8.00 ± 1.66% *vs* 9.06 ± 2.23%, $p < 0.01$) and 6 months (7.67 ± 1.75% *vs* 9.06 ± 2.23%, $p < 0.01$) from

Test score (seen as the percent of the required correct answers (RCA) per test sheet improved significantly after three months of the study (64.5 ± 33.7% *vs* 55.6 ± 33.2%, $p < 0.01$). No further significant change in the percent of RCA was observed after 6 and 18 months of the study in comparison with the 3rd month (65.0 ± 32.5% *vs* 64.5 ± 33.7%, $p > 0.05$; 64.8 ± 32.7% vs. 64.5 ± 33.7%, $p > 0.05$). However, the test score remained significantly higher at 18 months in comparison with the introductory test score (64.8 ± 32.7% *vs* 55.6 ± 33.2%, $p < 0.01$) (Figure 2).

The questionnaire analysis enabled us to differentiate three groups of questions:

Type A – questions about general health issues (smoking, salt in everyday diet, hypertension, etc). The patients gave high percent of correct answers at the introductory test (95.5%), at 3 months (98.55%), 6 months (98.50%) and 18 months (99%). A change between the three series of answers was not statistically significant. This type of question was not further examined with regard to therapeutic regimens.

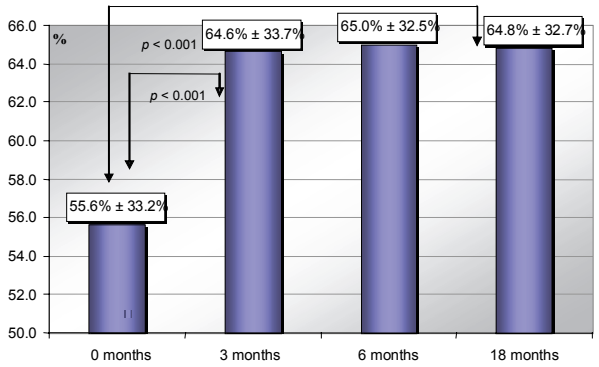

Fig. 2 – Average test score (the percent of required correct answers per test sheet) during the study

Type B – the questions that were testing knowledge of basic facts about DM (example question: What are the symptoms of diabetes?). At last one correct answer was given by the majority of examinees (Table 3). There was a very low percent of RCA in all time series (introductory 4% *vs* 3 months – 9% *vs* 6 months – 6.5% *vs* 18 months: 6.0%). A significant change was achieved at three months and 6 months form baseline ($p < 0.01$; Figure 3) There was no difference between the percent of correct answers between the patients on different therapeutic regimens at the introductory testing. A statistically significant difference was registered in the number of RCA in the patients treated with insulin, 3 months after the beginning of the study (14% *vs* 7%, $p < 0.05$) and in patients treated exclusively with lifestyle change, in whom the difference was statistically highly significant after 3 months and was sustained at 18 months at the beginning of the study: 5.9%, 3 months 23.5%, 18 months 17.6%, $p < 0.05$) (Table 4).

Table 3

Type B questions (Example question: What are the symptoms of diabetes?) and answers at the introductory testing and 18 months beginning of the study

Symptoms	Required correct answers (%) during the study	
	at the introductory testing	at 18 months
Fatique, thirst, insomnia	4.5	5.0
Fatique, pruritus, insomnia	0.5	0.5
Thirst, insomnia	1.5	2.0
Genital pruritus, thirst, insomnia	–	0.5
Genital pruritus, insomnia	–	0.5
Genital pruritus, thirst	1.5	2.5
All named symptoms	7.5	10.0
Fatique, pruritus, thirst	4.0	6.0.
Fatique, insomnia	4.0	0.5
Fatique, thirst	18.4	33.8
Fatique, pruritus	2.0	0.5
Insomnia	5.5	2.0
Thirst	30.8	26.4
Genital pruritus	2.0	1.0
Fatique	17.9	9.0

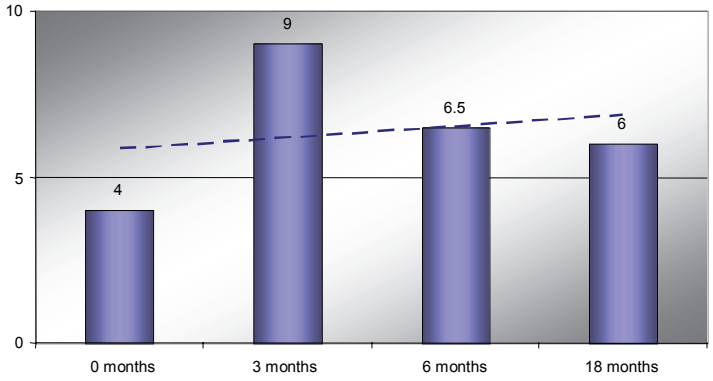

Fig. 3 – Required correct answers to type B questions (example question: What are the symptoms of diabetes?)

Table 4

Average of required correct answer to type B questions (example question: What are the symptoms of diabetes?) with respect to therapeutic regimens of diabetes mellitus * $p < 0.05$; † $p < 0.01$ ‡ $p < 0.001$ *vs* 0 months

Months after beginning of the study	Required correct answers (%)		
	insulin	oral hypoglycemic drugs	lifestyle changes
0	7.0	2.8	5.9
3	14.0*	5.7	23.5‡
6	2.3†	6.4	17.6‡
18	2.3†	5.7	17.6‡

*** $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$ *vs* 0 months**

Type C – questions that deal with acute complications of diabetes (example question: How to resolve hypoglycemia?). There was a high percent of at least one correct answer (Table 5). There was a small number of in this type of questions also (Figure 4). Patients treated with insulin had the greatest number of correct answers in all test series. The percent of RCA increased significantly during the study in the patients treated with insulin ($p < 0.01$) and in the patients treated with oral hypoglycemic drugs ($p < 0.01$). There were no RCA in the patients treated with lifestyle change after 3 and 18 months, which being a statistically significant change itself (Table 6).

Type D – questions that concern chronic complications of diabetes (example question: Diabetic foot, comprehension). There is a significant increase in the number of RCA in all test series and in patients with all therapeutic regimens, in comparison with the introductory testing (Table 7).

Table 5

Type C questions (example question: What scould you do when your blood sugar goes low?) And answers at the introductory testing and 18 months after the beginning of the study

Lifestyle changes	Required correct answers (%)	
	at the introductory testing	at 18 months
Juice, sandwich, chocolate	2.0	5.5
Juice, sandwich, rest	0.5	0.5
Juice, sandwich	0.5	0.5
30 min rest, sandwich	0.5	0.0
30 min rest, chocolate	1.5	2.0
All named	1.0	0.5
Juice, 30 min rest, chocolate	0.5	2.0
Juice, sandwich	3.0	4.0
Juice, chocolate	11.4	22.4
Juice, 30 min rest	2.0	6.5
Sandwich	8.5	2.5
Chocolate	30.8	22.4
30 min rest	6.0	1.0
Juice	30.8	28.9

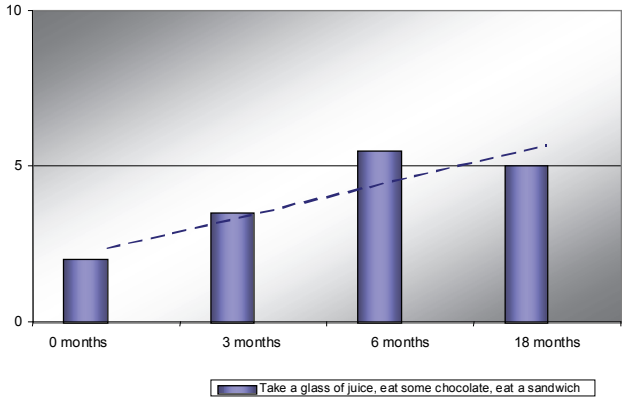

Fig. 4 – Percent of required correct answers to type C questions (example question What should you do when your blood sugar goes low?)

p < 0.01 for 0 months vs 3 months, 0 month vs 6 months and 0 months vs 18 months

Table 6

Distribution of type C required correct answers (example question: What should You do whwn your blood sugar goes low?) with respect to therapeutic regimens of diabetes mellitus

Months after beginning of the study	Average of required correct answers (%)		
	insulin	oral hypoglycemic drugs	lifestyle changes
0	2.3	1.4	5.9
3	7.0*	2.8*	0.0
6	9.3*	5.0*	0.0
18	9.3*	4.3*	0.0

***p* < 0.01 *vs* 0 months**

Table 7

Distribution of type required correct answers (example question: What would you do When you have painful feet, foot ulcers?) with respect to therapeutic regimens of diabetes mellitus

Months after beginning of the study	Average of required correct answers (%)		
	insulin	oral hypoglycemic drugs	lifestyle changes
0	20.9	8.5	11.8
3	34.9*	12.1*	17.6*
6	41.9*	19.9*	29.4†
18	32.6*	17.7*	23.5†

Discussion

The level of glycemic control seen through HbA1c is not satisfactory in our group of patients. It is, however, comparable to the other tested groups of patients in whom the education was done using printed material or where the patient was educated for self-management of DM [30–40]. The estimates of the average national HbA1c in the USA are 8.8 – 8.9% [41]. Our group of patients showed glycemic control that was similar to the described ethnically – specific or low literacy groups [42–46].

Since our examinees had a longer duration of DM and were regularly followed in the referent centers, we expected a somewhat lower level of HbA1c. Our results are comparable to the study that evaluated DM treatment at the University Center in the USA in which only 26.7% of patients reached the predefined HbA1c and only 3.2% achieved all guidelines proposed values of FPG, blood lipids and blood pressure [47].

After educational intervention with the printed material the level of HbA1c was significantly reduced in patients treated both with insulin and oral hypoglycemic drugs.

The printed educational material led to a significant increase in the knowledge of diabetes facts, independently of the treatment regimen, similar to some previously reparted studies [48–51]. The effects of educational intervention differ in patients treated with different therapeutic modalities - basic diabetes facts were best accepted by the patients that were treated with lifestyle change. Facts that reffer to self-management and that influence daily decisions are best accepted by insulin treated patients. This group of patients also showed the greatest reduction in HbA1c levels throughout the study. Insulin patients should therefore be educated more often and with structured educational content – different skills in DM self-management should be involved (self glucose blood monitoring, use and dose correction of insulin, treatment of hypoglycemia etc.). Low level of RCA suggests that patient education and evaluation of DM knowledge (test form and the structure of questions for example) should be adapted to patient`s educational and literacy level [14, 15]. We can also speculate that good effects of education with printed material in our group of patients was also due to the fact that this form of education is not a common way of health provider - patient communication in our country. It is also one of the reasons that we got a low percent of correct answers on some (predominantly multiple choice) questions. The printed material was available to the entire family [52], and it could have led to better implementation of lifestyle change or medication adherence and consequently to better glycemic control. The Questionnaire was in part insufficiently accustomed to everyday life of our patients. Therefore, it required a somewhat different approach to some of diabetes problems suggesting that educational programs should adapt to the previous patients knowledge and answer patient's needs in a culturally competent manner.

Conclusion

Education using printed material, without the intervention of a diabetes educator, led to increase in diabetes knowledge as well as to improvement of glycemic control in our group of patients with DM type 2. We believe that this form of education among DM type 2 population can be a useful adjunct to DM treatment and it should be structured according to the disease treatment modality.

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In 2002 UK Department for International Development and Access 2 Information supported the population of diabetes patients in Serbia. Their grant provided the printing material - educational booklet ″Healthy living with diabetes type 2″ and the necessary laboratory material for the measurement of FBG and HbA1c. The educational booklet was written in accordance with the educational standards of UK Diabetes Association and surveys of the University of Michigan Diabetes Research and Training Center. A two way translation of the booklet was made prior to distribution. The booklet was intended for distribution to some 10 000 diabetes patients in Serbia.

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Efikasnost lasera male snage u terapiji inflamirane gingive kod parodontopatije dijabetesnih bolesnika

Low power laser efficacy in the therapy of inflamed gingive in diabetics with parodontopathy

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Apstrakt

Uvod/Cilj. Postoje jasni dokazi o direktnoj vezi parodontopatije i dijabetesa melitusa (DM). Ističe se daleko veća prevalencija i težina parodontopatije kod dijabetesnih bolesnika u odnosu na zdravu populaciju. Poslednjih decenija u terapiji parodontopatije primenjuje se terapija laserima male snage (LLLT) koja ima biostimulativni efekat, ubrzava zarastanje rana, smanjuje bol i otok, a gotovo da nema kontraindikacija. Cilj rada bio je da se ispita efikasnost terapije laserima male snage i bazične terapije parodontopatije u eliminaciji inflamacije gingive kod dijabetesnih bolesnika sa parodontopatijom. **Metode.** Ispitivanje je obuhvatilo 150 ispitanika podeljenih u tri grupe iste veličine: grupa I (ispitanici sa DM tip 1 i parodontopatijom), grupa II (ispitanici sa DM tip 2 i parodontopatijom), grupa III (ispitanici sa parodontopatijom). Procena stanja zdravlja gingive utvrđena je uz pomoć gingivalnog indeksa Löe-Silness (Gi). Zatim su uklonjene naslage sa zuba, obrađeni parodontalni džepovi, i primenjena LLLT (5 mW) tokom pet uzastopnih dana koja je izostavljena na levoj strani vilice radi upoređivanja efekata terapije. Nakon prve, treće i pete terapije, kao i mesec dana nakon poslednje posete određen je Gi. Pre terapije i posle pete terapije uzeti su brisevi gingive i citomorfometrijskom analizom praćena površina jedra ćelija. **Rezultati.** Nakon svih ispitivanih perioda došlo je do statistički značajnog smanjenja Gi i površine jedara u odnosu na vrednost pre terapije ($p < 0{,}001$), sa značajnijim smanjenjem na strani vilice na kojoj je primenjena bazična parodontalna i LLLT. **Zaključak.** Na osnovu dobijenih rezultata terapija laserima male snage pokazala se efikasnom u eliminaciji inflamacije gingive i preporučuje se kao dodatak bazičnoj terapiji parodontopatije dijabetesnih bolesnika.

Ključne reči:
dijabetes melitus; periodontalne bolesti; lečenje laserom male snage; periodontalni indeks; biologija ćelije.

Abstract

Background/Aim. There is clear evidence on direct relationship between periodontal disease and diabetes mellitus. Many investigations point out greater prevalence and severity of periodontal disease among diabetic patients. During last decade, low level laser therapy has been used in periodontal therapy. It has biostimulative effect, accelerates wound healing, minimizes pain and swelling, and there is almost no contraindication for its usage. The aim of the paper was to investigate the efficiency of low level laser therapy as adjuvant tool in reduction of gingival inflammation in diabetic patients. **Methods.** The study incuded 150 participants divided into three groups: group I (50 participants with diabetes mellitus type 1 and periodontal disease), group II (50 participants with diabetes mellitus type 2 and periodontal disease), group III (nondiabetic participants with periodontal disease). Gingival health evaluation was done using gingival index Löe-Silness. Soft and hard deposits were removed, periodontal pockets cleaned and GaAlAs low level laser therapy (5 mW) applied five consecutive days. In each patient, low level laser therapy was not applied on the left side of the jaw in order to compare the effects of the applied therapy. After the first, third and fifth therapy and one month after the last visit gingival index was evaluated. Before the first and after the fifth therapy exfoliative cytology of gingiva was done and nuclei areal was analyzed morphometrically. **Results.** After all investigated periods, gingival index and nuclei areal were significantly decreased comparing to values before the therapy, at both jaw sides ($p < 0.001$). After the 1st, 3rd and 5th therapy, the *t*-test showed a significantly decreased gingival index at the lased side of jaw comparing to non-lased side. **Conclusion.** Low level laser therapy is efficient in gingival inflammation elimination and can be proposed as an adjuvant tool in basic periodontal therapy of diabetic patients.

Key words:
diabetes mellitus; periodontal diseases; laser therapy, low-level; periodontal index; cell biology.

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Uvod

Parodontopatija predstavlja inflamacijsko oboljenje potpornog aparata zuba izazvano dejstvom specifičnih bakterija oralnog biofilma [1]. Patološki proces počinje inflamacijom gingive, a zatim se širi u dublje delove parodoncijuma [1–3]. Međutim, ako se uspostavi ravnoteža između štetnih agenasa i odbrambenih mehanizama domaćina, ona može perzistirati duži vremenski period bez prodora inflamacije sa gingive u ostale delove parodoncijuma. Od velikog praktičnog značaja za utvrđivanje prognoze i plana terapije jeste utvrditi stepen aktivnosti parodontopatije. Smatra se da intenzitet inflamacije gingive predstavlja dobar pokazatelj aktivnosti i progresije oboljenja [4, 5]. Istraživači preporučuju upotrebu kliničkih parametara kao što je gingivalni indeks (Gi) koji određuje stepen inflamacije gingive i pokazuje mesta aktivne parodontopatije [6–10].

Jedan od veoma značajnih faktora rizika od progresije parodontopatije je dijabetes melitus (DM) [1, 3]. Ovo oboljenje predstavlja hroničnu bolest praćenu poremećajem metabolizma ugljenih hidrata, proteina i masti, udruženu sa aktivacijom kompenzatornih mehanizama većine žlezda sa unutrašnjom sekrecijom, a u osnovi je uslovljena relativnim ili apsolutnim nedostatkom dejstva insulina u većini ćelija organizma [11]. Brojna istraživanja potvrđuju daleko veću prevalenciju parodontopatije kod dijabetesnih bolesnika u odnosu na sistemski zdrave ispitanike. Ističe se da je intenzitet parodontopatije veći u slučajevima kada DM nije dobro kontrolisan i kada su prisutne dijabetesne komplikacije [12–16].

Eksfolijativna citologija je neinvazivna tehnika veoma značajna za određivanje inflamacijskih procesa u usnoj duplji, a omogućuje jednostavno i bezbolno uzorkovanje ćelija koje se zatim mikroskopski posmatraju [17, 18]. Deskvamacija ćelija višeslojnog pločastog epitela gingive zavisi od mitotičke aktivnosti ćelija bazalnog sloja, enzimskih procesa u ćelijama i delovanja mehaničkih iritacija [17]. U toku inflamacije gingive koja je česta kod dijabetesnih bolesnka dolazi do odstupanja u veličini i obliku ćelija pločastoslojevitog epitela i do uvećanja njihovih jedara nezavisno od stepena diferencijacije ćelija.

Otkriće i primena lasera predstavlja značajan doprinos savremenoj stomatologiji [19, 20]. Poslednjih decenija u terapiji parodontopatije primenjuje se dodatna terapija laserima male snage (LLLT) [21–25]. Ovaj vid terapije ima biostimulativno dejstvo, ubrzava zarastanje rana, smanjuje bol i otok, a gotovo da ne postoje kontraindikacije za njegovu primenu. Posebno je od značaja činjenica da je primena LLLT potpuno bezbolna, neinvazivna i bez štetnih efekata [19, 20, 26–29].

Cilj rada bio je da se ispita efikasnost terapije laserima male snage kao dodatak bazičnoj terapiji parodontopatije u eliminaciji inflamacije gingive u toku parodontopatije dijabetesnih bolesnika.

Metode

Ispitivanje je sprovedeno u Klinici za stomatologiju, Klinici za endokrinologiju i Institutu za patološku anatomiju Medicinskog fakulteta u Nišu. Polazeći od postavljenih ciljeva, koncipirana je metodologija za njihovu realizaciju odobrena od strane Etičkog komiteta Medicinskog fakulteta u Nišu odlukom broj 01-2800-7.

Osnovni kriterijumi za izbor ispitanika utvrđeni su pre pristupa ispitivanju, a zatim u Klinici za stomatologiju prikupljani potrebni anamnestički podaci i urađen stomatološki pregled. Izabrani bolesnici imali su parodontopatiju sa prisutnim kliničkim simptomom inflamacije gingive. U ispitivanje nisu uključeni bolesnici mlađi od 18 godina, trudnice, bolesnici sa krvnim oboljenjima, akutnim i hroničnim infekcijama, autoimunim oboljenjima, bolesnici na imunosupresivnoj terapiji ili fototerapiji, fotosenzitivne osobe i osobe koje su bile, iz bilo kog razloga, na antibiotskoj ili kortikosteroidnoj terapiji u poslednja tri meseca.

Od 150 ispitanika, 74 su bila muškog, a 76 ženskog pola, starosti od 22 do 83 godine (prosečna starost 44,6 godina), a prosečna dužina trajanja DM bila je 10,85 godina. Ispitanici su bili podeljeni u tri grupe od po 50 ispitanika: grupa I – ispitanici oboleli od DM tip 1 sa parodontopatijom, grupa II – ispitanici oboleli od DM tip 2 sa parodontopatijom, grupa III – ispitanici sa parodontopatijom koji nisu bolovali od DM.

Pre terapije procena stanja gingive izvršena je uz pomoć gingivalnog indeksa Löe-Silness (Gi) koji je meren sa svake strane prisutnih zuba regiona 11-17 i 41-47, te 21-27 i 31-37. Praćena je promena boje, površinske strukture, otok i krvarenje gingive na blag pritisak parodontalnom sondom. Indeks 0 označava zdravu gingivu bledoružičaste boje, čvrste konzistencije, sitnozrnaste površine. Indeks 1 obuhvata vrednosti 0,1–1 i označava blago izraženu inflamaciju (ivica gingive je blago crvenije boje u odnosu na zdravu gingivu, prisutan je blag edem i povećano izlučivanje gingivalnog eksudata, gingiva ne krvari na blagu provokaciju tupom sondom). Indeks 2 obuhvata vrednosti 1,1–2 i označava umereno izraženu inflamaciju (gingiva je crvene boje sa izraženim edemom i uvećanjem slobodne gingive, krvari na blag pritisak tupom sondom). Indeks 3 obuhvata vrednosti 2,1–3 i označava jako izraženu inflamaciju (gingiva je jasno crvene ili crvenoplavičaste boje, veoma uvećana, sa ulceracijama i tendencijom ka spontanom krvarenju) [3, 29]. Dobijene vrednosti podeljene su brojem strana i zuba na kojima je izvršeno merenje.

Pri prvoj seansi primenjena je bazična parodontalna terapija čiji je cilj eliminacija inflamacije. Ona obuhvata identifikaciju i uklanjanje oralnog biofilma i ostalih naslaga sa zuba, motivaciju i obučavanje bolesnika za održavanje oralne higijene, eliminaciju mogućih faktora rizika. Meke naslage uklonjene su pomoću paste (Vantal, Galenika) i rotirajućih četkica. Čvrste naslage (zubni kamenac i konkrementi) uklonjeni su ultrazvučnim aparatom Woodpecker (UDS-J) i srpastim instrumentom. Dodatno, parodontalni džepovi su obrađeni parodontalnom kiretom i ispiranjem 3% vodonik peroksidom. Zatim je primenjena terapija GaAlAs diodnim laserom male snage (Mils 94), talasne dužine od 670 nm sa prečnikom sonde od 2 mm. Izlazna snaga bila je 5 mW u kontinuiranom režimu rada, a ekspozicija 14 min po tretiranoj površini gingive oko zuba od 17 do 11,

i 47 do 41. Terapija laserom je izostavljena na levoj strani vilice svakog bolesnika, radi upoređivanja efekata terapije sa i bez lasera unutar svake grupe. U toku sledeća četiri dana pri svakoj poseti uklanjane su meke naslage i primenjena terapija laserom. Nakon prve, treće i pete terapije pri kontrolnoj poseti sutradan određen je Gi. Ispitanici su obučeni kako da pravilno održavaju oralnu higijenu, a kontrolni pregled je urađen mesec dana nakon poslednje posete kada je ponovo određena vrednost Gi.

Svim bolesnicima pre terapije i sutradan pri kontrolnom pregledu posle pete terapije uzimani su sterilnom vaticom brisevi gingive i pravljen direktan razmaz na pločici. Uzorci su, fiksirani i sušeni na vazduhu u trajanju najmanje jedan sat i bojeni Papanikolau metodom u Institutu za patološku anatomiju. Za morfometrijsku analizu korišćen je program *Image*, na mikroskopu NU2 (Carlzeiss Germany), objektiv × 63 (NA 0.8) Na citološkom materijalu morfometrijskom analizom praćena je veličina (areal) jedra ćelija.

Parametri su predstavljeni srednjim vrednostima ($\bar{x}$) i standardnim devijacijama (SD). Koeficijent varijacije određivan je kao mera homogenosti ispitivanih uzoraka u odnosu na ispitivane parametre. Studentovim *t*-testom nezavisnih uzoraka vršeno je testiranje statističke značajne razlike srednjih vrednosti dveju grupa, a ANOVA metodom vršeno je poređenje srednjih vrednosti između više od dve grupe ispitanika. Posle ispitivanja homogenosti varijansi (Levenov metod) urađena je sledstvena *post hoc* analiza. Unos i tabelarno prikazivanje rezultata obavljeno je korišćenjem MS *Office Excel* programa, a proračuni su vršeni programom SPSS, verzija 15.0.

Rezultati

Srednja vrednosti Gi na strani vilice na kojoj je primenjena bazična terapija i LLLT i strani na kojoj je primenjena samo bazična terapija, po grupama pre, tokom i posle terapije, prikazana je u tabeli 1. Kod obe strane, nakon svih perioda terapije došlo je do statistički značajnog smanjenja srednjih vrednosti Gi u odnosu na srednju vrednost pre terapije ($p < 0,001$). Poređenje vrednosti između grupa uz pomoć *t*-testa nakon prve terapije ($p < 0,001$), treće terapije (grupa I $p < 0,001$, grupa II $p < 0,01$, grupa III $p < 0,05$) i pete terapije ukazuje na statistički značajnije smanjenje srednjih vrednosti Gi na strani vilice na kojoj je primenjena bazična terapija i LLLT. Kod iste strane srednja vrednost Gi ostaje ista mesec dana nakon završene laseroterapije. Sa suprotne strane, takođe, postoji pozitivan efekat, iako se on sporije javlja.

Analizom vrednosti areala jedara u ispitivanim grupama pre terapije utvrđena je statistički značajna razlika njihovih srednjih vrednosti između ispitivanih grupa, sa maksimalnim nivoom značajnosti ($p < 0,001$). Daljom *post hoc* analizom multiplih poređenja utvrđivane su statistički značajne razlike između grupa ponaosob. Na osnovu podataka u tabeli 2 evidentno je da su srednje vrednosti areala jedra bile statistički značajno veće u grupama I i II (dijabetesni bolesnici), u odnosu na vrednosti u grupi III.

Tabela 1

Vrednosti gingivalnog indeksa (Gi) u ispitivanim grupama pre, tokom i posle terapije na strani sa primenjenom bazičnom terapijom (BT) i terapijom laserima male snage (LLLT) i na strani na kojoj je primenjena samo BT

Grupa ispitanika	Terapija (T)	Pre T $\bar{x} \pm SD$	Posle 1. T $\bar{x} \pm SD$	Posle 3. T $\bar{x} \pm SD$	Posle 5. T $\bar{x} \pm SD$	Jedan mesec posle T $\bar{x} \pm SD$
I	BT +LLLT	1,86 ± 0,35	0,78 ± 0,46*†	0,16 ± 0,37*†	0,16 ± 0,37*	0,16 ± 0,37*
	BT	1,86 ± 0,35	1,22 ± 0,55*	0,40 ± 0,49*	0,26 ± 0,44*	0,16 ± 0,37*
II	BT + LLLT	1,74 ± 0,44	0,64 ± 0,48*†	0,16 ± 0,37*‡	0,14 ± 0,35*	0,14 ± 0,35*
	BT	1,74 ± 0,44	1,00 ± 0,49*	0,54 ± 0,50*	0,20 ± 0,40*	0,16 ± 0,37*
III	BT + LLLT	1,20 ± 0,45	0,34 ± 0,48*†	0,12 ± 0,33*§	0,12 ± 0,33*	0,12 ± 0,33*
	BT	1,20 ± 0,45	0,80 ± 0,40*	0,32 ± 0,47*	0,20 ± 0,40*	0,12 ± 0,33*

***$p < 0,001$ u odnosu na vrednost gingivalnog indeksa (Gi) pre terapije; †$p < 0,001$, ‡$p < 0,01$, §$p < 0,05$ u odnosu na vrednost Gi na strani sa BT**

Tabela 2

Vrednosti veličine jedara () u ispitivanim grupama pre i posle terapije na strani sa primenjenom bazičnom terapijom (BT) parodontopatije i terapijom laserima male snage (LLLT) i na strani sa primenjenom samo bazičnom terapijom (BT) parodontopatije

Grupa ispitanika	Terapija (T)	Pre T $\bar{x} \pm SD$	Posle T $\bar{x} \pm SD$
I	BT + LLLT	107,283 ± 15,427 *	40,695 ± 4,911*‡
	BT	112,604 ± 31,458‡,§	52,035 ± 4,970*†
II	BT + LLLT	103,509 ± 14,528†	39,919 ± 3,395*‖
	BT	99,482 ± 11,695‖	54,307 ± 5,070*†
III	BT + LLLT	77,290 ± 7,870	39,404 ± 3,713*‡
	BT	76,815 ± 8,501	51,098 ± 5,996

Pre terapije: *grupa I *vs* grupa III – $p < 0,01$; †grupa II *vs* grupa III – $p < 0,001$; ‡grupa I *vs* grupa II – $p < 0,05$; §grupa I (BT) *vs* grupa III (BT) – $p < 0,01$; ‖grupa II (BT) *vs* grupa III (BT) – $p < 0,001$.
Posle terapije: *$p < 0,001$ *vs* odgovarajuće vrednosti pre terapije (Studentov *t*-test); †$p < 0,05$ *vs* grupa III (BT); ‡$p < 0,001$ (BT + LLLT *vs* BT); ‖$p < 0,01$ (BT + LLLT *vs* BT)

Studentovim *t*-testom utvrđeno je da je u sve tri ispitivane grupe na strani vilice na kojoj je primenjena bazična terapija i LLLT, kao i na strani gde je primenjena samo bazična terapija, došlo do statistički značajnog smanjenja srednjih vrednosti areala jedra sa maksimalnim nivoom statističke značajnosti u odnosu na vrednost pre početka terapije

($p < 0,001$). Koeficijenti varijacije manji od 30 ukazuju na izuzetnu homogenost vrednosti ispitivanog parametara pre, ali i posle terapije (tabela 2).

Na strani vilice na kojoj je primenjena bazična terapija i LLLT posle terapije ANOVA analizom nije utvrđena statistički značajna razlika srednjih vrednosti areala jedra između grupa. Na strani na kojoj je primenjena samo bazična terapija, posle terapije ANOVA analizom utvrđene su statistički značajne veće srednje vrednosti areala jedra u grupama I i II (dijabetesni bolesnici) u odnosu na grupu III ($p < 0,05$). Posle sprovedene terapije Studentovim *t*-testom, utvrđeno je da je unutar sve tri ispitivane grupe, na strani na kojoj je primenjena bazična terapija i LLLT, u odnosu na stranu na kojoj je primenjena samo bazična terapija, dobijena statistički značajno niža vrednost areala jedra (sa nivoima značajnosti: grupa I – $p < 0,001$, grupa II – $p < 0,01$, grupa III – $p < 0,001$) (tabela 2).

Diskusija

Masovna pojava i česti recidivi parodontopatije čine ovo oboljenje pravim socijalnim problemom koji ističe sve veći značaj profilakse. Zbog toga javlja se potreba za traženjem novih, efikasnijih sredstava i metoda lečenja. Problem je posebno istaknut kod obolelih od DM koji su zbog svoje osnovne bolesti opterećeni velikim brojem medikamenata i slabijim imunim odgovorom. U ispitivanju Yucekal-Tuncer i sar. [30] upoređivana je hronična parodontopatija kod sistemski zdravih i dijabetesnih ispitanika uz pomoć odgovarajućih indeksa. Istraživači su uočili veće vrednosti Gi indeksa u grupi dijabetesnih bolesnika i sugerisali da je parodontopatija bila težeg stepena. Smatra se da poremećaj glikemijske kontrole kod obolelih od DM doprinosi bržoj progresiji parodontopatije. Slično, u ovom istraživanju uočeno je da je Gi bio većih vrednosti na početku istraživanja u grupama dijabetesnih bolesnika u odnosu na kontrolnu grupu ($p < 0,001$).

Istraživači ističu da dodatna terapija LLLT nakon primenjene bazične parodontološke terapije redukuje gingivalnu inflamaciju i da se uspešno može koristiti u terapiji gingivitisa i parodontopatije [21, 25, 31]. Sličan nalaz uočen je i u ovom istraživanju gde je nakon primenjene bazične terapije i LLLT došlo do značajnog smanjenja stepena inflamacije koji je dokumentovan merenjem Gi. Vrednost Gi pre terapije u grupama dijabetesnih ispitanika bile su približnih vrednosti. Pri svakoj poseti, nakon primenjene bazične terapije, desne strane vilica podvrgnute su i terapiji laserom, a leve su služile kao kontrola. Na obe strane vilica nakon svih perioda terapije u odnosu na vrednost pre terapije, došlo je do statistički značajnih smanjenja srednjih vrednosti Gi ($p < 0,001$), što je u skladu sa navodima iz literature koji ističu izuzetan značaj bazične parodontalne terapije u unapređenju parodontalnog zdravlja [32–34]. Poređenjem vrednosti između grupa uočena je statistički značajno manja vrednost Gi na strani gde je primenjena dodatno i LLLT, u odnosu na stranu na kojoj nije, u periodima nakon 1. terapije ($p < 0,001$ u sve tri grupe), nakon 3. terapije ($p < 0,05$ u grupi III, $p < 0,01$ u grupi II, i $p < 0,001$ u grupi I), što je u skladu sa navodima iz literature [21, 25, 31].

Zapaža se da na strani na kojoj je primenjena dodatno i LLLT, Gi ostaje isti i mesec dana nakon terapije, što govori u prilog dugotrajnog efekta LLLT. S druge strane, na strani na kojoj nije primenjena dodatno i LLLT, takođe postoji pozitivan efekat, iako se on sporije javlja, što sugeriše da ne treba zanemariti izuzetan značaj bazične parodontalne terapije [35, 36].

Zahvaljujući činjenici da je eksfolijativna citologija moderna i neinvazivna tehnika primenjena je u ovom ispitivanju u cilju bolje interpretacije promena prisutnih u gingivi dijabetesnih bolesnika pre i posle terapije. Poznato je da tokom zapaljenskih reakcija dolazi do odstupanja u veličini i obliku ćelija pločasto-slojevitog epitela i do uvećanja njihovih jedara, nezavisno od stepena njihove diferencijacije [17]. Analizom veličine jedara pre terapije između ispitivanih grupa, uočeno je da je veličina jedara bila statistički značajno veća u grupama dijabetesnih bolesnika (grupe I i II), u odnosu na grupu ispitanika koji nisu bolovali od DM (grupa III). Ovaj nalaz je u skladu sa navodima iz literature koji sugerišu postojanje inflamacije i parodontalne bolesti težeg stepena kod dijabetesnih bolesnika u odnosu na osobe koje ne boluju od DM [37–43].

Poredeći veličinu jedara u svakoj grupi ponaosob pre i posle primenjene terapije na obe ispitivane strane vilica, uočeno je statistički značajno smanjenje veličine jedara nakon terapije u svakoj ispitivanoj grupi. Ovaj nalaz sugeriše da su primenjena samo bazična parodontološka terapija i bazična parodontološka terapija zajedno sa LLLT dovele do značajnog smanjenja inflamacije što je u skladu sa velikim brojem navoda iz literature koji ističu značaj ove dve terapeutske procedure u unapređenju i održanju parodontalnog zdravlja [44–46]. Igić [17] je pomoću eksfolijativne citologije gingive dece sprovela uporednu analizu terapije kataralnog gingivita laserima male snage, hijaluronskom kiselinom i samo bazičnom terapijom. Dobijeni rezultati ukazali su na efikasnost terapeutskih procedura u eliminaciji kataralnog gingivitisa, što je u skladu sa nalazima u ovom ispitivanju.

Na strani sa primenjenom bazičnom parodontološkom terapijom i LLLT, analizom veličine jedara posle terapije, između ispitivanih grupa nije uočena statistički značajna razlika, što sugeriše da se primenom obe terapije zajedno može postići zdravlje gingive dijabetesnih bolesnika kao kod ispitanika koji ne boluju od DM. Na strani gde nije primenjena LLLT, nakon terapije uočava se da su veličine jedara u grupama dijabetesnih bolesnika (grupe I i II) bile veće u odnosu na veličinu jedara kod ispitanika koji ne boluju od DM. Ovakav nalaz nam sugeriše da je verovatno potrebno kod dijabetesnih bolesnika upotpuniti bazičnu parodontalnu terapiju drugim terapeutskim procedurama koje imaju izraženi antiinflamatorni efekat. Takva terapeutska procedura je i LLLT koja je primenjena u ovom istraživanju.

Zaključak

Na osnovu primenjene metodologije i dobijenih rezultata, uočeno je značajnije smanjenje inflamacije gingive kod dijabetesnih bolesnika primenom kombinovane terapije laserima male snage i bazične terapije parodontopatije, u odnosu na smanjenje inflamacije gingive primenom samo bazične terapije parodontopatije.

Laseri male snage pokazali su se efikasnim u eliminaciji inflamacije gingive i preporučuje se njihova primena kao dodatak bazičnoj terapiji parodontopatije dijabetesnih bolesnika.

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Kliničke manifestacije atopije kod dece u prve dve godine života

Clinical manifestations of atopy in children up to two years of age

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Apstrakt

Uvod/Cilj. Atopijske bolesti kao što su atopijski dermatitis, alergijski rinitis i astma tokom poslednjih decenija imaju povećanu prevalenciju i sada se u ekonomski razvijenim zemljama javljaju kod svakog trećeg deteta. Cilj rada bio je da se utvrdi učestalost i vrsta atopijskih bolesti kod dece u prve dve godine života, kao i dijagnostički – prognostički značaj koncentracije ukupnih IgE antitela. **Metode.** Ispitivanjem je bilo obuhvaćeno 175 dece uzrasta do dve godine. Postojanje *allergy like* simptoma utvrđivano je anketnim ispitivanjem roditelja i iz pedijatrijskog kartona. Imunofluorescentnom metodom (FIA) na *Immunocap* 100 aparatu određene su koncentracije ukupnih IgE antitela i specifičnih IgE antitela multitestom – *Phadiatop infant*. **Rezultati.** Jedan ili više *allergy like* simptoma imalo je 57,7% dece uzrasta do dve godine, a kod 19,4% dokazano je postojanje alergijske bolesti posredovane IgE antitelima. Atopijska bolest najčešće se klinički manifestovala kao atopijski dermatitis (11,4%), vizing/astma posredovani IgE antitelima (10,8%) i alergija na hranu (7,4%), a znatno ređe kao alergijski rinitis (3,4%) i urtikarija (1,7%). Koncentracija ukupnih IgE antitela bila je statistički značajno viša u grupi dece sa *allergy like* simptomima ($p < 0,0005$) (*cut off* 15,15 kU/L, senzitivnost 76,5%, specifičnost 71,6%. **Zaključak.** Dvadeset procenata dece uzrasta do dve godine ima ispoljenu neku od alergijskih bolesti, od kojih se najčešće javljaju atopijski dermatitis i vizing/astma posredovani IgE antitelima. Koncentracija ukupnih IgE može biti koristan dijagnostički marker senzibilizaije kod dece u prve dve godine života.

Ključne reči:
hipersenzibilnost, rana; dermatitis, atopijski; astma; odojče; znaci i simptomi; ige; dijagnoza.

Abstract

Background/Aim. Atopic diseases such as atopic dermatitis, allergic rhinitis and asthma have had increased prevalence during the past decade and nowadays occur in every third child in developed countries. The aim of the study was to determine frequency and type of atopic diseases at the age of two, as well as the importance the total IgE antibodies concentrations have in diagnosis and prognosis of the disease. **Methods.** The study involved 175 children up to two years of age. Allergy-like symptoms were found after surveying their parents and pediatric medical records. Using the fluorescence immunossay (FIA) method, total IgE antibodies concentrations and specific IgE antibodies (Phadiatop infant) were determined on an Immunocap 100 Dyagnostic System. **Results.** One or more allergy-like symptoms accounted for 57.7% of findings in children under the age of two, whilst in 19.4% the existence of IgE-related allergic diseases was found. Atopic diseases usually have clinical manifestations of atopic dermatitis (11.4%), IgE-bound wheezing/asthma (10.8%) and food allergies (7.4%), and to much lesser extent those of allergic rhinitis (3.4%) and urticaria (1.7%). The significantly higher total IgE antibodies concentrations were found in children with allergy-like symptoms ($p < 0.0005$) (cut-off 15.15 kU/L, sensitivity 76.5% specificity 71.6%). **Conclusion.** Almost 20% of two-year-old children have any of clinically manifested allergic diseases, with atopic dermatitis and IgE wheeze/asthma being predominant. The higher total IgE antibodies concentration is a good marker for sensitization in children with allergy-like symptoms.

Key words:
hypersensitivity, immediate; dermatitis, atopic; asthma; signs and symptoms; ige; diagnosis.

Uvod

Atopija je podgrupa alergijskih hipersenzitivnosti koja se definiše kao genetska predispozicija za produkciju IgE imunoglobulina u odgovoru na izlaganje alergenu. Pojava specifičnih IgE antitela na alergene leži u osnovi razvijanja kliničkog poremećaja (fenotipa) atopijske bolesti [1]. Atopijske bolesti kao što su atopijski dermatitis, alergijski rinitis i astma imaju po-

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većanu prevalenciju tokom poslednjih decenija, a u populacionim studijama kumulativna prevalencija alergijskih bolesti u dečjem uzrastu kreće se od 25% do 35% (atopijski dermatitis 15–20%; astma 7–10% i alergijski rinitis 15–20%) [2–4].

Sistemska priroda alergijske bolesti ogleda se u činjenici da jedna osoba može ispoljiti različite oblike alergijske bolesti tokom života [5]. Pod pojmom „alergijski marš" podrazumeva se progresija kliničkih manifestacija atopije kod jednog bolesnika od pojave ekcema/atopijskog dermatitisa, alergije na hranu i vizinga u najranijem uzrastu, preko pojave senzibilizacije na inhalatorne alergene i nastanka alergijskog rinitisa, a u najtežim slučajevima i astme [6]. Evidentno je da atopijski marš predstavlja kliničku progresiju atopijskih bolesti kod jedne osobe, ali je neophodno tačno definisanje pojedinačnih fenotipova u najranijem uzrastu u cilju utvrđivanja prediktivnih parametara za kasniji razvoj alergijskih i komorbiditetnih hroničnih bolesti [7–9].

Objektivno testiranje alergije u najranijem uzrastu neophodno je kako zbog rane identifikacije povećanog rizika od razvoja alergijskih bolesti, tako i zbog optimalnog lečenja već nastale alergijske bolesti, što podrazumeva mere izbegavanja specifičnog alergena, adekvatne farmakoterapije, kao i specifične imunoterapije. Prema preporukama Evropske akademije za pedijatrijsku alergologiju i imunologiju indikacije za testiranje alergije su: recidivantni vizing, gastrointenstinalni (GIT) simptomi (povraćanje, nenapredovanje u telesnoj masi, dijareja, izražene kolike), atopijski dermatitis, akutna urtikarija/angioedem, hronična urtikarija, rinitis, konjunktivitis, reakcije na ujede insekata i anafilaksa [10]. U svakodnevnoj kliničkoj praksi dijagnoza atopijske bolesti postavlja se na osnovu prisustva simptoma i pozitivnog kožnog ili *in vitro* testa za specifična IgE antitela [11, 12]. Veći broj studija ukazao je na neophodnost *in vitro* određivanja specifičnih IgE antitela kod odojčadi i predškolske dece [13, 14]. Naime, pokazano je da se kožni test može koristiti u dijagnostici senzibilizacije i kod dece mlađe od tri godine, ukoliko se kao pozitivan rezultat uzima veličina papule od 3 i više mm, pri čemu senzitivnost i specifičnost kožnog testa zavise od potentnosti i vrste ekstrakta, kao i od pritiska lancete pri izvođenju testa [11], a testiranje limitira i postojanje izraženog dermatitisa ili korišćenje antialergijskih lekova [10]. Sa druge strane, *Phadiatop infant* multitest *in vitro* za detekciju specifičnih IgE antitela, pokazuje visoku senzitivnost (96%) i specifičnost (96%) u detekciji senzibilizacije kod dece uzrasta do tri godine sa pozitivnom prediktivnom vredošću od 94 i negativnim prediktivnim vrednošću od 98% [14].

S obzirom na nedostatak tačnih podataka o vrsti i učestalosti atopijskih bolesti u našoj populaciji, cilj rada bio je da se utvrdi učestalost senzibilizacije posredovane IgE antitelima, učestalost i vrsta kliničkih manifestacija atopijskih bolesti, kao i eventualni dijagnostički značaj koncentracije ukupnih IgE antitela u detekciji senzibilizacije i pojavi klinički manifestne atopijske bolesti kod dece uzrasta do dve godine.

Metode

Ispitivanjem je bilo obuhvaćeno 175 dece uzrasta do dve godine. Ispitivana grupa dobijena je metodom slučajnog uzorka, pri čemu su deca uključivana u studiju pri redovnim sistematskim pregledima, neposredno pre vakcinacije ili revakcinacije. Dobijanje saglasnosti i anketno ispitivanje roditelja, kao i uzimanje uzoraka seruma kod dece sprovođeno je u Centralnom dečjem dispanzeru Doma zdravlja u Kragujevcu. Podaci iz ankete dopunjavani su uvidom u medicinsku dokumentaciju (pedijatrijski karton).

Deca su bila razvrstana u dve grupe: 1) zdrava deca bez ispoljavanja simptoma i 2) deca koja su u periodu od rođenja do trenutka ispitivanja ispoljavala simptome i znakove koji se mogu povezati sa alergijskim bolestima (*allergy like* simptomi) i to: jedna ili više ponavljanih bronhoopstrukcija potvrđenih od strane pedijatra, recidivantni rinitis, gastrointenstinalni simptomi povezani sa uzimanjem hrane (dijareja, povraćanje i nenapredovanje u telesnoj masi), ekcematozne promene po koži, akutna i hronična urtikarija/angioedem, anafilaksa, sistemske reakcije na ujed insekta [10].

In vitro koncentracija ukupnih IgE antitela i specifičnih IgE antitela – *Phadiatop infant* (Phadia AB, Uppsala, Sweden) određivana je fluorescentnom metodom (FIA) na *Immunocap* 100 aparatu (Phadia AB, Uppsala, Sweden). *Phadiatop infant* je multitest za detekciju alergijskih bolesti posredovanih IgE antitelima koji obuhvata detekciju specifičnih IgE antitela na proteine: belanca, kravljeg mleka, kikirikija, račića, dlake mačke i psa, grinje, polena srebrne breze, mačijeg repka, ambrozije i koprive [15]. Uzorak krvi uziman je u jutarnjim časovima, nakon dva sata centrifugiranja, odvajan je serum koji je, potom čuvan na -75°C do testiranja.

Na osnovu rezultata multitesta za određivanje specifičnih IgE antitela deca sa simptomima bila su svrstana u dve podgrupe: senzibilisana (*Phadiatop infant* pozitivna) i deca bez senzibilizacije (*Phadiatop infant* negativna).

Ispitivanje je sprovedeno u skladu sa etičkim standardima Helsinške deklaracije iz 1975. god, revidirane 1983. god. Studiju je odobrio Etički komitet Instituta za javno zdravlje u Kragujevcu i Medicinskog fakulteta u Kragujevcu.

Za ispitivanje zavisnosti kategorijskih promenljivih korišćen je χ^2 test. Ispitivanje zavisnosti numeričkih promenljivih vršeno je pomoću Spirmanovog koeficijenta korelacije. Razlike u srednjim vrednostima promenljive između dveju populacija ispitivane su pomoću nezavisnog *t*-testa i pomoću Man-Vitnijevog testa. Analiza kovarijanse služila je da se neutrališe uticaj nekog parametra na ispitivanje značajnosti razlike srednjih vrednosti promenljive dveju populacija. Kriva ROC upotrebljena je da se proceni da li koncentracija ukupnih IgE antitela može da bude marker za pojavu senzibilizacije.

Rezultati

U ispitivanoj grupi bilo je 175 dece (101 dečak i 74 devojčice) uzrasta od pet meseci do 24 meseca (prosečno životno doba 9,6 meseci).

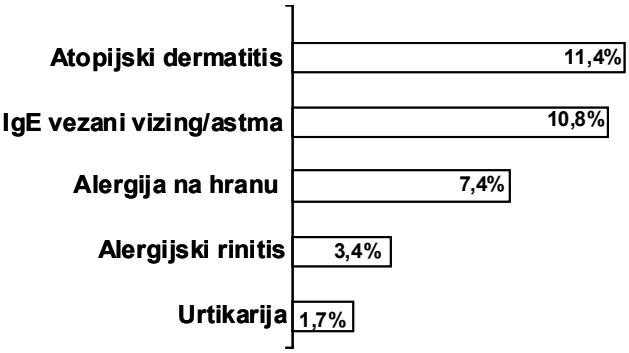
Jedan ili više *allergy like* simptoma imalo je 101 (57,7%) dete. Postojanje alergijske bolesti posredovane IgE antitelima dokazano je kod 34 (19,4%) dece. U grupi dece sa simptomima, senzibilizacija je utvrđena kod 34 (33,7%) dece.

Phadiatop infant pozitivne dece sa *allergy like* simptomima bilo je 34 (21 dečak i 13 devojčica), dok je u grupi *Phadiatop infant* negativne dece simptome (n = 67) imalo 39

dečaka i 28 devojčica. U grupi dece sa simptomima, između senzibilisane i nesenzibilisane dece nije bilo statistički značajne razlike u odnosu na pol ($p = 0,785$).

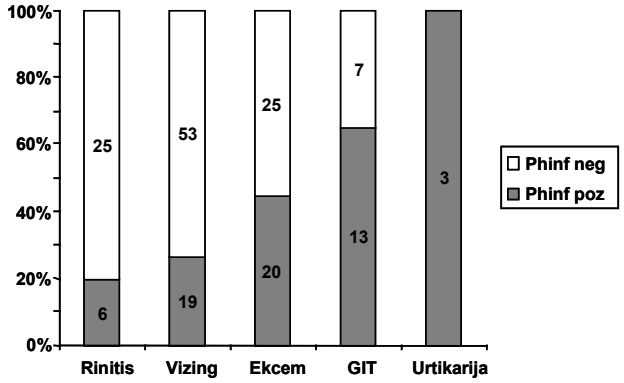
Pozitivnu porodičnu anamnezu (roditelji, brat ili sestra sa dokazanom atopijskom bolešću) imalo je 54 dece, dok je negativnu porodičnu anamnezu imalo 47 dece. U grupi *Phadiatop infant* pozitivnih (n = 34), 17 dece imalo je pozitivnu i 17 negativnu porodičnu anamnezu. U grupi *Phadiatop infant* negativne dece (n = 67) porodična anamneza bila je pozitivna kod 37, a negativna kod 30 dece. U grupi dece sa simptomima, između senzibilisane i nesenzibilisane dece nije bilo statistički značajne razlike u odnosu na porodičnu anamnezu ($p = 0,775$).

Učestalost tipa alergijskih bolesti posredovanih IgE antitelima kod dece uzrasta do dve godine prikazana je na slici 1. U najranijem uzrastu atopijska bolest najčešće se klinički manifestovala kao atopijski dermatitis (n = 20; 11,4%) i vizing/astma posredovani IgE antitelima (n = 19;10,8%).



Sl. 1 – Učestalost Ige vezanih alergijskih bolesti kod dece uzrasta do 2 godine

Kada je analizirano postojanje senzibilizacije u grupi dece sa simptomima, uočeno je da je utrikarija, iako se retko javljala, uvek bila udružena sa pozitivnim *Phadiatop infant* testom. Atopija je, *in vitro* multitestom za specifična IgE antitela, češće bila dokazivana u grupi dece sa GIT simptomima (n = 13/20; 65%) i ekcemom (n = 20/45; 44,4%) u odnosu na opšteprihvaćene simptome IgE vezane alergije u starijim uzrastima – vizing (19/72; n =26,4%) i recidivantni rinitis (n = 6/30; 20%) (slika 2).

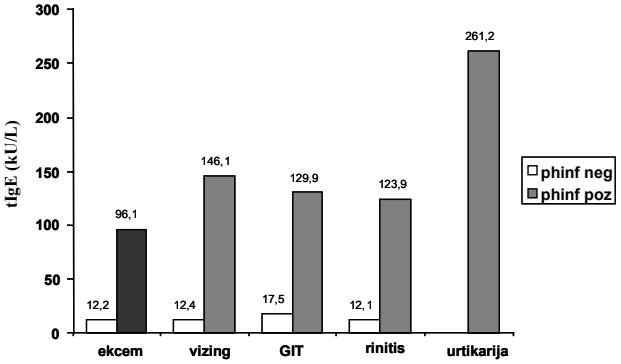


Sl. 2 – Incidencija alergije povezane sa IgE kao uzroka *allergy like* simptoma kod dece uzrasta do 2 godine sa potvrđenom senzibilizacijom (*Phadiatop infant* pozitivna) i bez senzibilizacije (*Phadiatop infant* negativna)

Analizom kombinovanosti *allergy like* simptoma utvrđeno je da postoji zavisnost učestalosti izolovanog vizinga kod *Phadiatop infant* negativne dece ($p = 0,030$). U grupi dece sa izolovanim vizingom 87,5% bilo je *Phadiatop infant* negativno (n = 28/32), dok je u grupi dece sa kombinovanim vizingom to bio slučaj kod 62,5% (n = 25/40). Nije utvrđena statistički značajna povezanost između pojedinačnih i kombinovanih simptoma i senzibilizacije za ekcem ($r = 0,515$), recidivantni rinitis ($r = 0,355$) i gastrointenstinalne simptome ($r = 0,521$).

Analiza koncentracije ukupnih IgE antitela u grupi dece sa simptomima pokazala je statistički značajnu razliku između dece sa atopijom i bez atopije ($p < 0,0005$). Srednja koncentracija ukupnih IgE antitela za *Phadiatop infant* pozitivnu decu bila je značajno veća (108,97 kU/L) u odnosu na *Phadiatop infant* negativnu decu (13,24 kU/L) ($p < 0,0005$).

Srednja vrednost koncentracije ukupnih IgE antitela bila je najveća kod dece sa urtikarijom (261,23 kU/L), a najniža kod dece sa atopijskim dermatitisom (96,06 kU/L) (slika 3).



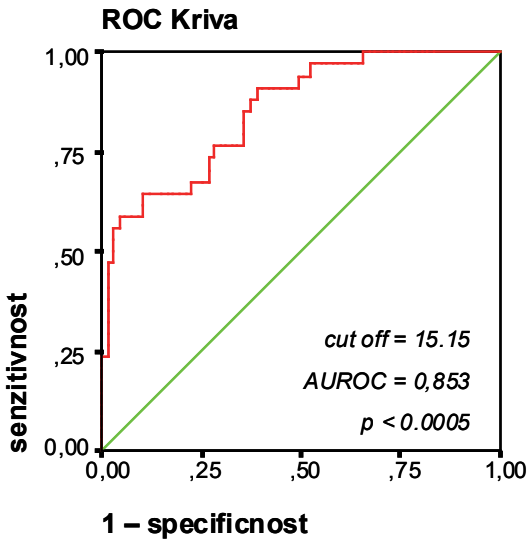
Sl. 3 – Koncentracija ukupnih IgE antitela kod dece sa *allergy like* simptomima

(Phinf neg – *Phadiatop* i *infant* negativna; Phinf poz – Phadiatop infant pozitivna)

U odnosu na pol, nije postojala statistički značajna razlika u koncentraciji ukupnih IgE antitela između *Phadiatop infant* pozitivne i negativne dece ($p = 0,245$), iako su senzibilisani dečaci imali višu srednju koncentraciju ukupnih IgE antitela (124,08 kU/L) u odnosu na devojčice (84,55 kU/L).

Senzibilisana deca sa pozitivnom porodičnom anamnezom imala su višu srednju koncentraciju ukupnih IgE antitela (140,32 kU/L) u odnosu na senzibilisanu decu kod koje u porodici nije bilo alergijskih bolesti (77,61 kU/L). U grupi *Phadiatop infant* negativne dece nije bilo razlike u srednjoj vrednosti koncentracije IgE. Utvrđeno je da porodična anamneza ne utiče na razliku u koncentraciji ukupnih IgE antitela između senzibilisane i nesenzibilisane dece ($p = 0,100$).

Koncentracija ukupnih IgE antitela može biti dobar marker za senzibilizaciju u grupi dece sa *allergy like* simptomima ($p < 0,0005$). Za *cut off* 15,15 kU/L, senzitivnost testa iznosila je 76,5% a specifičnost 71,6% (slika 4).



Sl. 4 – Senzitivnost i specifičnost koncentracije ukupnih IgE antitela kao markera senzibilizacije kod dece sa *allergy like* simptomima

***In vitro* koncentracija ukupnih IgE antitela i specifičnih IgE antitela određena je FIA metodom na Immunocap 100 aparatu. *Cut off*, senzitivnost, specifičnost koncentracije ukupnih IgE u detekciji senzibilizacije određeni su ROC (*Receiver Operating Characteristic*) metodom i prikazani u formi ROC krive.**

Diskusija

Danas više od 300 miliona ljudi u svetu boluje od astme, te se na osnovu ovog podatka može govoriti o postojanju „epidemije" alergijskih bolesti i astme [16]. Rezultati našeg istraživanja su prvi podaci o učestalosti *allergy like* simptoma i atopijskih bolesti na našem podneblju kod dece uzrasta do dve godine.

Ovim istraživanjem utvrđeno je da čak 57,7% dece uzrasta do dve godine ispoljava simptome koji bi se mogli povezati sa alergijom. Pri tome se pedijatri u svakodnevnoj praksi suočavaju sa nizom objektivnih problema u dijagnostici, klasifikovanju, lečenju i prevenciji alergijskih bolesti. Dijagnostika atopije, u našim uslovima uglavnom se oslanja na kožni alergotest, uz niz objektivnih nedostataka u pouzdanosti i reproducibilnosti ovog testa. Neadekvatni rezultati kožnog alergotesta mogu biti posledica korišćenja nestandardizovanih rastvora alergena, neusaglašenog tumačenja i izdavanja rezultata od strane pedijatara, pulmologa, alergologa, pa čak i medicinskih sestara. Sa druge strane, *in vitro* test za određivanje specifičnih IgE antitela na nutritivne i inhalatorne alergene preporučen je za utvrđivanje atopijske konstitucije, kao i precizno dijagnostikovanje bolesti posredovanih IgE antitelima kod dece uzrasta do tri godine [14]. Iako su *in vitro* testovi obuhvaćeni šifarnikom Republičkog zavoda za zdravstveno osiguranje Republike Srbije, predviđena cena je daleko ispod nabavne cene reagenasa, tako da nijedna zdravstvena ustanova nema ove testove na spisku svojih usluga. Zbog svega navedenog, najveći broj dece sa *allergy like* simptomima, u pedijatrijskim kartonima umesto šifre Međunarodne klasifikacije bolesti (MKB) za alergijsku bolest, imaju šifru „neoznačeno" (*non specificata*).

U našem istraživanju, u grupi dece sa ispoljenim simptomima nije postojala statistički značajna razlika u postojanju pozitivne porodične anamneze (alergijske bolesti kod srodnika prvog stepena) između atopičara i neatopičara. Slična studija, sprovedena u Švedskoj, pokazala je, takođe, da ne postoji razlika u postojanju pozitivne porodične anamneze kod srodnika prvog stepena kod atopičara (71%) i neatopičara (61%) [15]. Američki autori [17] u okviru studije PEAK (*Prevention of Early Asthma in Kids*) utvrdili su da su deca sa atopijskim dermatitisom bila češće senzibilisana, bez obzira na postojanje atopije u porodici, od grupe dece koja je imala samo pozitivnu porodičnu anamnezu (66,1% *vs* 51%). Autori smatraju da pozitivna porodična anamneza može biti prognostički faktor, ali samo u grupi starije dece koja nemaju ispoljeni atopijski dermatitis [17].

Iako se u više studija ukazuje na to da je alergijska senzibilizacija češća kod muške dece u odnosu na žensku [18], našim istraživanjem nije utvrđena statistički značajna razlika među polovima u pojavi senzibilizacije.

Analizom vrste simptoma koji se javljaju kod dece i primenom *in vitro* multitesta za detekciju specifičnih IgE antitela došli smo do objektivnih podataka o učestalosti atopije kod dece u najranijem uzrastu. U ispitivanoj grupi 19,43% dece uzrasta do dve godine bilo je senzibilisano na neki od najčešćih nutritivnih ili inhalatornih alergena, zbog čega klinički ispoljavaju neki od simptoma atopijske bolesti.

Ekcem u ranom detinjstvu najčešće je atopijske etiopatogeneze i javlja se kao posledica senzibilizacije na nutritivne ili inhalatorne alergene [19]. U američkoj studiji Tukson utvrđena je učestalost atopijskog dermatitisa u uzrastu do tri godine kod 12% dece [20]. U našoj studiji to je bio slučaj kod 11,43% dece u prve dve godine života, što je bila i najčešća atopijska bolest u ovom uzrastu. U grupi dece sa ekcemom španski autori [18] su istom metodologijom (*Immunocap*) pokazali da je 54,8% dece senzibilisano na najčešće nutritivne i inhalatorne alergene, dok je u našem ispitivanju to bio slučaj sa 44,4% dece sa ekcemom.

Senzibilizacija na hranu, kao uzrok atopijskog dermatitisa, može se javiti kod 60% dece, pri čemu atopijski dermatitis može biti i izolovani simptom alergije na hranu [21]. Rana identifikacija dece sa alergijom na hranu i atopijskim dermatitisom može prevenirati pojavu astme u kasnijem uzrastu [10]. Ajgenman i sar. [22] među prvima su ukazali na značaj *in vitro* testova za utvrđivanje alergije na hranu kao uzroka atopijskog dermatitisa. Problem koji smo uočili tokom prikupljanja podataka, a koji jasno ukazuje na značaj i potrebu *in vitro* određivanja specifičnih IgE, jeste da veliki broj dece sa dijagnozom atopijskog dermatitisa (ekcem+senzibilizacija) u pedijatrijskom kartonu ima opisan simptom kao *eczema infantum*, ali ne i dijagnozu atopijskog ili drugog dermatitisa (kontaktnog iritantnog, seboroičnog i sl). Najčešći problem, koji onemogućava adekvatno klasifikovanje ekcematoznih promena je rasprostranjenost promena po koži, što onemogućava primenu kožnih testova.

U našem istraživanju, alergija na hranu ustanovljena je kod 7,4% dece. I kod ove dijagnoze postoji problem klasifikacije, jer u MKB ne postoji definisana šifra za alergiju na hranu, već se u našim uslovima deca vode pod šiframa E73 (*Intolerantia lactosi*), A09 (*Diarrhoea et gastroenteritis, causa infectionis suspecta*), K59 (*Functio laesa intestini alia*),

K90 (*Malabsorptio intestini*) i slučaju nenapredovanja E44 (*Malnutritio*). U tim slučajevima uglavnom se *ex juvantibus* isključuju kravlje mleko i jaja iz ishrane odojčeta i bez potvrđene senzibilizacije na namirnice.

Vizing, kao jedan od uobičajenih simptoma alergijskih bolesti, koji se javlja kod dece uzrasta do tri godine može se svrstati u tri fenotipa [23, 24]: tranzitorni vizing, koji se najčešće javlja tokom prve godine života, ali se kasnije povlači, a posledica je mehaničkih karakteristika disajnih puteva (mali kalibar disajnih puteva i otpor proticanju vazduha) [23, 24], rođenja pre termina [25], ili izloženosti deteta duvanskom dimu [26]; neatopijski vizing, najčešće prisutan tokom druge i treće godine života, posledica je virusnih infekcija; i atopijski vizing/astma, posredovani IgE antitelima, kod koga genetska predispozicija uslovljava ranu senzibilizaciju na inhalataorne alergene, sa nastankom hronične inflamacije epitela disajnih puteva, što uz ponavljanja oštećenja i posledičnu reparaciju i remodelovanje disajnih puteva, za posledicu ima bronhijalnu hiperreaktivnost i astmu [24].

Dijagnoza astme u najranijem uzrastu nije jednostavna, naročito ukoliko se uzme u obzir i veća učestalost infekcija disajnih puteva kod dece sa atopijskom konstitucijom. Nameće se potreba da se, što je pre moguće, na klinički jednostavan način definiše alergijska, odnosno nealergijska astma kod dece, kako bi se mogla primeniti odgovarajuća terapija [27]. U okviru američke studije Tukson utvrđeno je da najmanje jedan vizing, potvrđen od strane pedijatra u prve tri godine života, ima 53,8% dece, a u našem istraživanju 41,14% dece uzrasta do dve godine (n = 72/175) [20]. Aršad i sar. [28], u kohortnoj studiji na 946 ispitanika uzrasta do dve godine, utvrdili su učestalost astme kod 15,2% [28], dok su Tarig i sar. [19] utvrdili nešto nižu učestalost astme (8,7%) u periodu odojčeta. Naše ispitivanje pokazalo je da je učestalost vizinga/astme povezanog sa IgE antitelima u našoj populaciji gotovo 11%. Astmu posredovanim IgE, sa tri i više vizinga potvrđenih od strane pedijatra, imalo je 4% dece, n = 7/175 (petoro dece imalo je pridruženi atopijski dermatitis, a dvoje podatak o postojanju astme majke). Senzibilisanu decu sa 1–2 vizinga povezanog sa IgE, sa visokim rizikom od nastanka astme i uz konsultaciju sa dečijim pulmologom, savetovali primenu preventivnih mera. Sa druge strane, u istraživanjima etiologije ponavljanog vizinga kod dece mora se razmišljati i o visokoj učestalosti nealergijske astme u najmlađem uzrastu, koja se po različitim studijama kreće od 10% do 46,8% [28, 29]. Tako, pokazano je da je u grupi od 468 ispitivane dece uzrasta 0–5 godina samo 32,4% dece sa vizingom senzibilisano na najčešće nutritivne i inhalatorne alergene, dok su ostali (67,6%) imali vizing nealergijskog porekla [18]. Slično, Aršad i sar. [28] ukazuju na činjenicu da je 30–40% slučajeva hroničnih alergijskih bolesti kod dece u ranom detinjstvu primarno vezano za atopiju, ali da kod 60–70% dece uzrok treba tražiti u faktorima specifičnim za organ ili u neimunološkim mehanizmima. Naše ispitivanje dalo je slične rezultate s obzirom na to da, čak, 74% dece sa vizingom nije senzibilisano, pri čemu je tri i više vizinga bez senzibilizacije imalo 6,9% (n = 12/175) dece, tj. 16,6% dece sa vizingom (n = 12/72).

Rinitis, kao jedna od manifestacija atopijske bolesti, prema Rodrigezu i sar. [30], javlja se kod 16,9% slučajeva. Alergijski rinitis, definisan kao recidivantna sekrecija iz nosa uz jutarnje kijanje, u našem istraživanju javio se kod samo 3,4% dece uzrasta do dve godine. Od sve dece sa simptomima recidivantnog rinitisa samo je njih šestoro (19%) bilo *Phadiatop infant* pozitivno, pri čemu je kod petoro dece postojala udruženost sa vizingom, tako da se o recidivantnom rinitisu ne može govoriti kao o primarnom simtomu atopije u ovom uzrastu.

Urtikarija kao simptom javila se samo kod tri deteta i kod svih bila je povezana sa postojanjem atopije. Nisu zabeleženi hronična urtikarija/angioedem, anafilaksa, niti sistemske reakcije na ujed insekta, te i ove simptome možemo svrstati u grupu nekarakterističnih za atopijske bolesti kod dece u prve dve godine života.

Povišene koncentracije ukupnih IgE antitela u grupi senzibilisane dece utvrđene su u više studija. Nemački autori [31] kod 103 deteta (srednji uzrast 36 meseci) sa atopijskim dermatitisom utvrdili su više vrednosti koncentracije ukupnih IgE u odnosu na grupu dece bez senzibilizacije (224 kU/L *vs* 25,2 kU/L). Sampson [21] ukazuje na podatak da 80–90% dece sa atopijskim dermatitisom ima povišen nivo ukupnih IgE antitela. Kineski autori [29], takođe, navode koncentraciju ukupnih IgE u prve četiri godine života kao jedan od prediktivnih faktora nastanka kasnije senzibilizacije na inhalatorne alergene [29]. I naše istraživanje pokazalo je postojanje statistički značajne razlike u koncentraciji ukupnih IgE antitela između *Phadiatop infant* pozitivne i negativne dece, tako da koncentracije ukupnih IgE antitela iznad *cut off* vrednosti od 15,15 kU/L, sa visokom senzitivnošću (76,5%) i specifičnošću (71,6%) ukazuju na moguću senzibilizaciju. Nasuprot našim rezultatima, Ot i sar. [31] definisali su *cut off* sa značajno višim vrednostima ukupnih IgE (106 kU/L, senzitivnost 68,7% i specifičnost 92,3%). Grupa dece sa prisutnim *allergy like* simptomima, ali bez senzibilizacije, mogla bi se na prvi pogled smatrati neatopičarima. Kineski autori [29] smatraju da bi se deca sa povišenim nivoom ukupnih IgE antitela mogla smatrati potencijalnim atopičarima. Naše istraživanje pokazalo je postojanje podgrupe dece koja imaju povišene vrednosti ukupnih IgE antitela preko navedene *cut off* vrednosti (15,15 kU/L) i *allergy like* simptome (najčešće GIT simptome, a zatim i ekcem). Može se pretpostaviti da u ovoj grupi postoje deca koja su senzibilisana na alergene koje ne obuhvata multitest koji smo mi koristili, te je kod ove dece indikovano dopunsko *in vitro* testiranje specifičnih IgE antitela na namirnice koje nisu obuhvaćene testiranjem *Phadiatop infant*. U svakom slučaju, ovu grupu dece treba opservirati u narednom periodu, kako bi se potvrdila ili eliminisala alergija kao uzrok GIT simptoma i/ili ekcematoznih promena po koži.

Zaključak

Rezultati ovog istraživanja, prema našem saznanju, po prvi put pokazali su učestalost i vrstu kliničkih manifestacija alergijskih bolesti kod dece uzrasta do dve godine. Utvrdili smo da gotovo dvadeset procenata dece ovog uzrasta ima is-

poljenu neku od alergijskih bolesti, od kojih se najčešće javljaju atopijski dermatitis, vizing/astma povezani sa IgE i alergija na hranu. Pored toga, pokazali smo da koncentracija ukupnih IgE može biti koristan dijagnostički marker senzibilizaije kod dece uzrasta do dve godine.

Ovo istraživanje doprineće boljem upoznavanju kliničkih simptoma alergijskih bolesti u najranijem uzrastu što, svakako, utiče kako na postavljanje tačne dijagnoze, a samim tim i primenu optimalne terapije, tako i na pravovremno i adekvatno sprovođenje prevencije alergijskih bolesti.

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CURRENT TOPIC

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Clinical aspects of different types of amblyopia

Klinički aspekti različitih tipova ambliopije

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Key words:
amblyopia; risk factors; diagnosis; treatment outcome.

Ključne reči:
ambliopija; faktori rizika; dijagnoza; lečenje, ishod.

Introduction

Amblyopia is a deficit of vision, principally visual acuity, not immediately correctable by glasses, in the absence of ocular pathology, due to interruption of normal visual development during a sensitive period in childhood. Amblyopia occurs before 6–8 years of age. It may be completely or partially treated by modulation of the visual input during a sensitive period of visual development. The duration of this period varies depending on the cause of amblyopia [1–4].

Amblyopia is the leading cause of visual impairment in children affecting up to 5% of general population [5, 6]. Population prevalence depends upon the fact if there have been any interventions to prevent or treat the condition, or not.

Diagnosis is based on a reduction in best corrected visual acuity by testing visual acuity in each eye separately with a line of symbols with appropriate effect of crowding, and after exclusion of ocular pathology [7–9]. The acuity testing procedure and refraction must be accurate, and the age related norms must be known for the vision test used. Amblyopia is defined in terms of visual acuity, but other visual functions are affected as well. It is important to recognize that a finding of reduced visual acuity in a child is not a diagnosis of amblyopia. Amblyopia is only found in the setting of a causative factor. If no obvious amblyogenic factor is found on examination, then either the reliability of the visual acuity measurement should be questioned or subtle ocular pathology such as optic nerve hypoplasia or macular disease should be considered [10].

Classification

Classification of amblyopia is based on clinical conditions responsible for its development. Etiology is heterogeneous, may be caused by stimulus deprivation, strabismus, refractive error or a combination of these. Amblyopia is usually unilateral, but it may be bilateral in cases of bilateral high refractive error or bilateral ocular pathology, such as cataract.

The commonest risk factors for amblyopia are constant strabismus and different refractive errors in each eye. The child's age when exposed to an amblyopia-inducing condition appears to be the most important determinant for the development of amblyopia [11].

Differences exist in psychophysical functions between the fovea and the retinal periphery in human strabismic amblyopia, on the one hand, and in anisometropic and visual deprivation amblyopia, on the other. There are also differences in the severity and reversibility of the various types of amblyopia. The basic amblyogenic mechanisms are the same even though their contribution to each type of amblyopia varies [10].

Stimulus deprivation amblyopia occurs when a physical obstruction along the line of sight prevents the formation of a well-focused, high-contrast image on the retina. The degree to which amblyopia develops depends on the time of onset and the extent of form deprivation. This type of amblyopia requires early, vigorous treatment. Untreated unilateral form deprivation extending past the first 3 months of age profoundly affects visual acuity development. Untreated bilateral visual form deprivation has a similar effect if it extends past 6 months of age. If treatment for these conditions is not initiated during this critical developmental period, the prognosis for normal vision development is poor. When the onset of the cause of deprivation occurs after the first 6–12 months, the prognosis for vision recovery is improved with early treatment.

Strabismic amblyopia is suspected when a child shows either constant unilateral squint (without alternation of fixation) or a fixation defect with one eye. It is always unilateral. It occurs far more often in esotropes. Anisometropic amblyopia occurs when an interocular difference in spherical or cylindrical refractive error exceeds certain limits. In spherical anisometropia, a minimum difference of 1.25 DS may be significant [2, 4, 7–9, 12].

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Presentation and referral

Constant squint is generally recognized early by the family or general practitioner. A positive family history of squint or amblyopia should alert those in primary care when carrying out routine checks or immunizations. Small angle deviations and anisometropic amblyopia are the primary screening targets [12–14].

Early screening compared with later screening at 3 years of age and at school entry (4–5 years of age) may not reduce the overall prevalence of amblyopia by 7 years of age [15]. The prevalence and severity of amblyopia have declined substantially where screening programmes are in place [16, 17].

Aims of intervention

It is important to detect amblyopia early and to initiate treatment for amblyopia at a stage when treatment is likely to be effective (ideally between 3 and 5 years of age, and under 7 years of age) [8].

The rationale for treatment of amblyopia is to optimize visual function and binocular vision. Severe amblyopia persisting in adulthood is a significant risk factor for blindness in the case of an individual losing sight in the fellow eye [18, 19]. Amblyopia is treated by modulating the visual input into the amblyopic eye. In the case of stimulus deprivation amblyopia, the cause of the visual deprivation needs to be dealt with. Significant refractive errors need to be corrected. Any remaining visual deficit may be treated by obscuring or degrading the visual input to the fellow eye. Commonly used methods are patching (occlusion), instillation of atropine drops and occasionally, occlusive contact lenses. Patching or other amblyopia treatment may be delayed while progressive improvement in visual acuity occurs with refractive correction alone [8, 12]. In addition, vision does not improve in patients not complying with treatment, and can thus be improved with supervised compliance [20].

Treatment issues in amblyopia

Practice still varies widely in the management of strabismic, anisometropic and combined amblyopia: how much patching, start and maintenance, penalization, time of screening?

The results of the some Pediatric Eye Disease Investigator Group (PEDIG) amblyopia studies reported results are in contrast to long experience with treating amblyopes largely based on clinical significance [21, 22].

Is the treatment really similar for different types of amblyopia (excluding deprivation)? Does "slow" treatment waste valuable time during the sensitive period?

Refractive correction with spectacles and occlusion therapy remains the mainstay of amblyopia therapy. Spectacles alone may be enough to improve vision in some patients with late-onset amblyopia [23].

Debate still continues regarding treatment and occlusion modalities. But the reality of amblyopia treatment is that the intensity of patching prescribed is not always the actual amount of patching that is received, even when special monitoring devices have been used [24]. This is one of the reasons, aside from potentially better binocularity outcome, why recent studies have looked at the efficacy of atropine penalization rather than patching in the treatment of amblyopia [25]. Regarding the efficacy of various protocols there have been several reports with conflicting results [21, 26–29]. Treatment duration data have also been contradictory, ranging from no association with treatment effect to both direct and inverse relationships [30, 31]. It has long been known that the "white noise" of unilateral optical defocusing has both monocular and binocular detrimental consequences [32]. Also, recent work has indicated that occlusion enhances binocularity more than penalization [33, 34], and that more intensive patching may be needed in children with better levels of vision in order to re-establish bifoveal fixation [35].

The conclusions indicating that lower intensities of patching are as effective as full time regimens [21] might be too optimistic [36] and have some limitations [37, 38]. In some countries these studies have drawn much attention from the lay press to the point where this publicity appears to play an important role in influencing parent treatment preferences [39]. The evaluation of amblyopia treatment outcome presents a serious challenge. Problems include the design of VA charts, difference in symbols and unequal separation between them on most currently used test charts or projection slides. There is also a problem in analyzing data of children with amblyopia that is not encountered in analyzing acuity data from adults. The VA of children, when tested by the same method, tends to improve with age [25]. Evidence from different studies may be proven to be correct, or will have to be changed as new facts emerge from more tightly monitored controlled clinical trials. Data must be analyzed carefully because nearly all studies suffer from some scientific flaws. For several reasons, including the existence of so few prospective studies, the way in which ophthalmologists move from one to the other, or the way in which we refine some treatment protocols, may also be the right way!

There is a need for good quality trials to be conducted in these areas in the future, to improve the evidence base for the management of amblyopia. Objective electronic compliance monitoring, could be the key to a more evidence- based treatment for amblyopia [40]. It is clear that children do not like patching, and achieving compliance presents a serious challenge [41].

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K A Z U I S T I K A

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Primitivna trigeminalna arterija udružena sa arteriovenskom malformacijom cerebeluma

Primitive trigeminal artery commorbid with aterovenous malformation of cerebellam

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Apstrakt

Uvod. Nalaz perzistentnih karotidobazilarnih anastomoza je redak. Kada je prisutan, često je udružen sa drugim vaskularnim anomalijama i drugim patološkim stanjima. Trigeminalna primitivna arterija najčešća je među njima. Udruženost primitivne trigeminalne arterije sa arteriovenskom malformacijom u cerebelumu je ekstremno retka. **Prikaz bolesnika.** Nakon naglog početka bolesti, 31-godišnja žena sa subarahnoidnom hemoragijom i cerebelarnim hematomom, primljena je u ozbiljnom stanju. Angiografski je prikazana arteriovenska malformacija cerebeluma, primitivna trigeminalna arterija i druge malformacije Willis-ovog kruga. Nakon kompletne hirurške resekcije arteriovenske malformacije bolesnica je otpuštena bez deficita. **Zaključak.** Prikazali smo izuzetno retko stanje udruženosti primitivne perzistentne arterije sa arteriovenskom malformacijom u zadnjoj lobanjskoj jami. Važnost ovakvih nalaza ogleda se, pre svega, u promenama anatomskih i fizioloških uslova moždane cirkulacije, posebno važnim u toku određenih hirurških procedura.

Ključne reči:
centralni nervni sistem, vaskularne malformacije; arterovenske malformacije; mozak, mali; neurohirurške procedure; dijagnoza; lečenje, ishod.

Abstract

Introduction. Persistence of fetal cerebral carotid basilar anastomoses is rare. When it occurs, it is often associated with other vascular malformations, and other pathological conditions. Trigeminal primitive artery persistence is the most often among them. Coincidence of primitive trigeminal artery with cerebellar arteriovenous malformation is extremely rare. **Case report.** We reported a case of a 31-year-old woman with subarachnoid hemorrhage and cerebellar hematoma admitted in serious condition. Angiography demonstrated cerebellar arteriovenous malformation, primitive trigeminal artery and other malformation of Willis circle. After a complete surgical removal of arteriovenous malformation the patient was discharged without neurological or any other deficit. **Conclusion.** We reported an extremely rare condition, which had been reported very few times in the literature. The importance of primitive artery persistence is in changed anatomical and physiological condition of cerebral circulation, that is especially important in surgical procedures.

Key words:
central nervous system vascular malformations; arteriovenous malformations; cerebellum; neurosurgical procedures; diagnosis; treatment outcome.

Uvod

Većina privremenih embrionalnih arterija postoji tokom samo 7 do 10 dana, a potom, usled razvoja odgovarajućih permanentnih arterija dolazi do njihove regresije, dok izvesni njihovi delovi bivaju inkorporirani u definitivni cerebrovaskularni sistem. Međutim, pod nepoznatim uslovima pojedini od ovih privremenih embrionalnih krvnih sudova zaostaju tokom fetalnog i postnatalnog perioda. Perzistentni embrionalni krvni sudovi se, u visokom procentu javljaju udruženo sa ostalim anomalijama krvnih sudova.

Perzistentna primitivna trigeminalna arterija (PPTA) najčešća je perzistentna karotidobazilarna anastomoza. Nju je put opisao Quain 1844. godine, a prvi angiografski prepoznao Sutton, 1950. godine [1–3]. Nastaje u embrionalnom životu kao deo kaudalnog stabla unutrašnje karotidne arterije (ACI) i to u nivou budućeg kavernoznog segmenta karotide. Trigeminalna arterija u stadijumu fetusa od 5 mm, spaja se sa kranijalnim delom longitudinalnih cefaličnih pleksusa i tada

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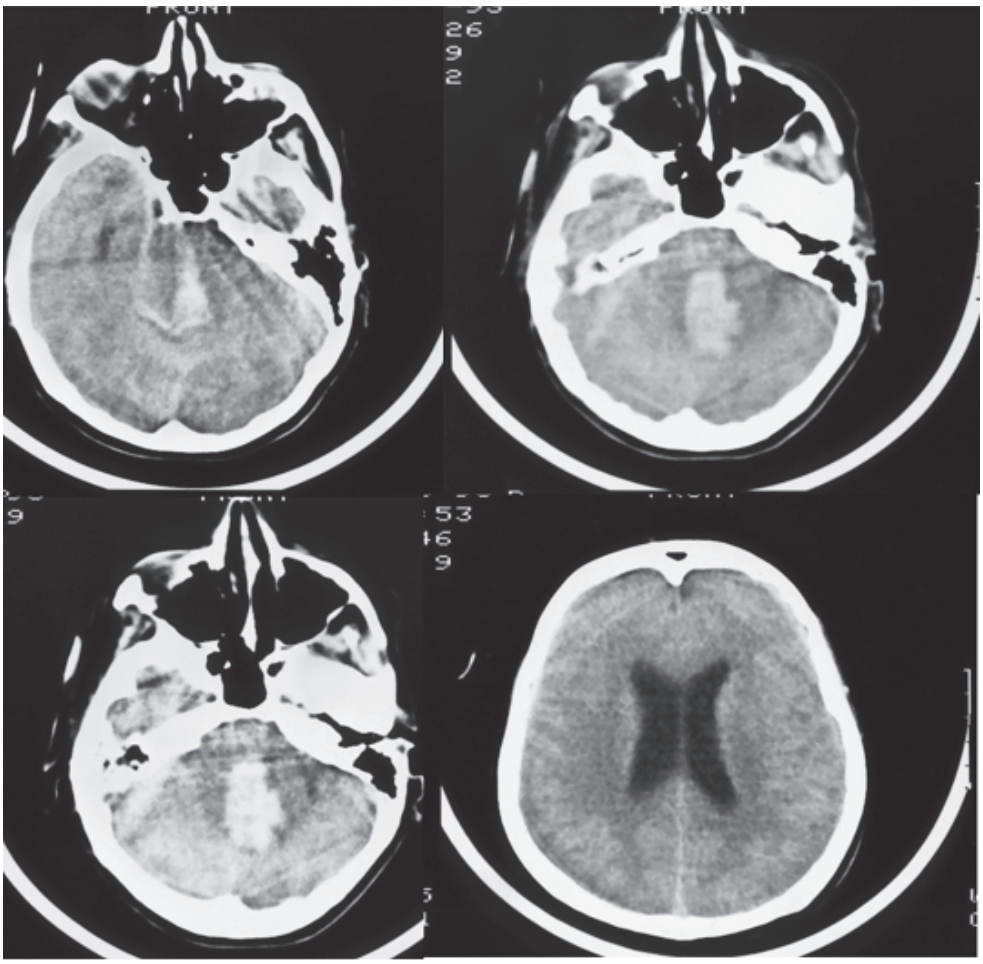
je normalno najjače razvijena. S obzirom na razvoj zadnje komunikantne arterije PPTA gubi svoj značaj i u stadijumu od 8 do 10 mm iščezava. U slučaju perzistencije, PPTA počinje od nivoa intrakavernoznog segmenta ACI u blizini vrha piramide neposredno ispod i medijalno od Gasser-ovog gangliona. U početnom delu može biti ispod dure, kada probija zadnji zid kavernoznog sinusa i prolazi bočnom stranom dorzuma sele nekada čineći žleb [2] da bi se, najčešće u nivou gornje trećine, spojila sa bazilarnom arterijom (BA) ili, ako je ekstraduralna, probija zid kavernoznog sinusa između *n. opthalmicus*-a i *n. oculomotorius*-a i u luku obilazi oko dorzuma sele da bi se spojila sa BA. Dužina joj varira od 1,3 do 2,4 cm, a učestalost od 0,1 do 0,3% [4], dok neki autori navode podatke da se javlja kod 0,1 do 2% slučajeva. Nalaz ove arterije u vrlo visokom procentu udružen je sa drugim anomalijama Willis-ovog kruga.

Shibata i sar. [5] navode 27 slučajeva udruženosti arteriovenskih malformacija (AVM) i perzistentnih karotidobazilarnih anastomoza, prikupljenih iz raznih štampanih izvora navodeći da su Krayenbuhl i Yasargil prvi koji su još 1957. referisali ovu koincidenciju. Od 29 navedenih slučajeva, 22 su bila sa primitivnom trigeminalnom arterijom, pet AVM bile su infratentorijalne, od čega su samo tri bile cerebelarne.

Prikaz bolesnika

Bolesnica, stara 31 godinu, doživela je u ranim popodnevnim časovima nagli gubitak svesti. Posle kratkotrajnog besvesnog stanja bila je konfuzna, somnolentna, sa intenzivnom glavoboljom, više puta je povraćala. Neurološki, bila je bez lateralizacije, nije mogla da stoji, niti da ustane iz postelje, a meningealni znaci bili su pozitivni. Kompjuterizovana tomografija (KT) mozga pokazala je subarahnoidnu hemoragiju i hematom u vermisu, sa kompresijom četvrte moždane komore i umerenom konsekutivnom dilatacijom lateralnih komora (slika 1). Digitalnom suptrakcionom angiografijom verifikvana je arteriovenska malformacija u vermisu (slike 2 i 3). Zbog kompromitovane cirkulacije likvora ugrađen je šant prema ventrikuloperitonealnom tipu desno. Potom je AVM ekstirpirana u celosti.

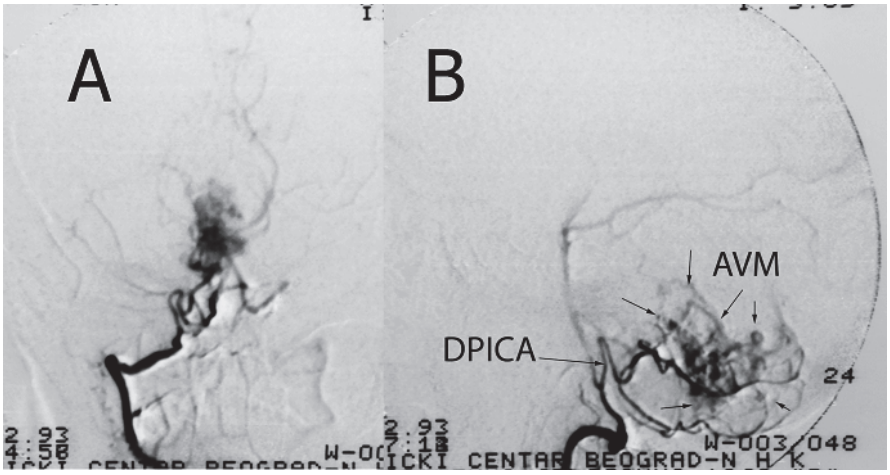
Analizom angiografskog nalaza, dobijen je uvid u krvne sudove koji snabdevaju malformaciju i registrovana je anomalna cirkulacija koja je podrazumevala višestruka odstupanja u cirkulatornoj šemi mozga. Kao prvo, uočena je anastomoza između leve unutrašnje karotidne arterije i bazilarne arterije (slike 3 i 4). Navedena anastomoza identifikovana je kao PPTA. Bio je upečatljiv kalibar ove anastomotičke arte-



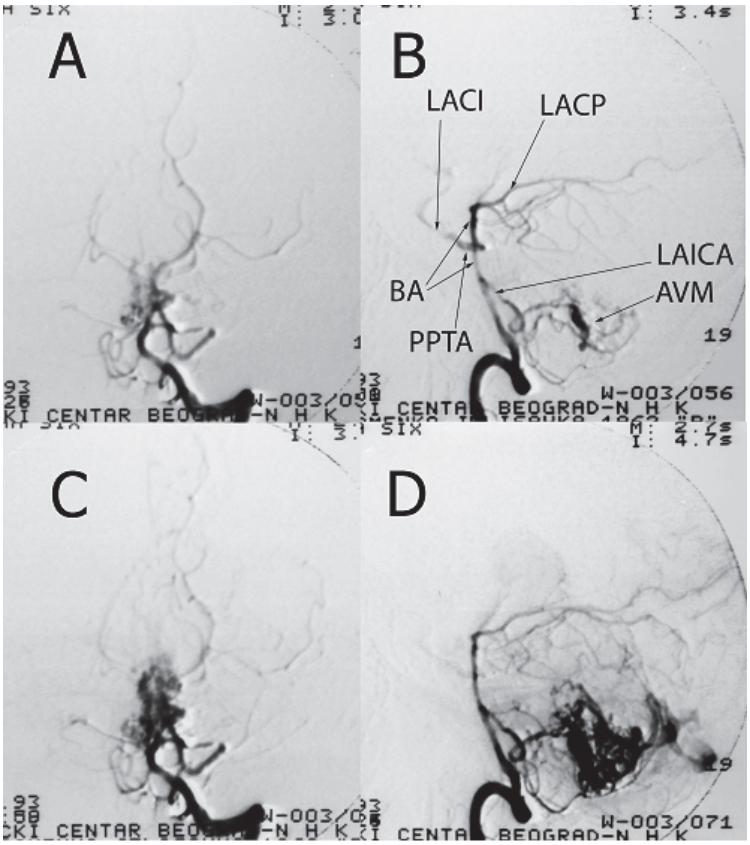
Sl. 1 – Snimci kompjuterizovane tomografije na prijemu: vidi se hematom u vermisu koji komprimuje četvrtu komoru i moždano stablo, kao i konsekutivna dilatacija komornog sistema supratentorijalno

rije koji se mahom punio iz karotidne arterije, a snabdevo gornji deo bazilarne arterije, pretežno levu arteriju *cerebri posterior* (slike 3 i 4). Leva karotidna arterija punila je i neparnu arteriju *cerebri anterior*, koja se nastavljala jednostrukim A2 segmentom, dok su se obe rekurentne arterije punile iz ove jednostruke arterije *cerebri anterior* (slika 4A, C). Desna arterija *cerebri posterior* bila je fetalnog tipa i snabdevala se krvlju iz desne karotidne arterije (slika 5). Istovremeno, uočen je kompletan nedostatak A1 segmenta desne arterije *cerebri anterior* (slika 5). Postoperativni angiogram pokazao je kompletnu resekciju AVM u zadnjoj lobanjskoj jami, uz zaostatak dilatiranih drenažnih vena, ali i drugačiji balans u punjenju arterija. Naime, uspostavljena je ravnoteža punjenja preko trigeminalne arterije, iz vertebralne i iz karotidne arterije (slika 6).

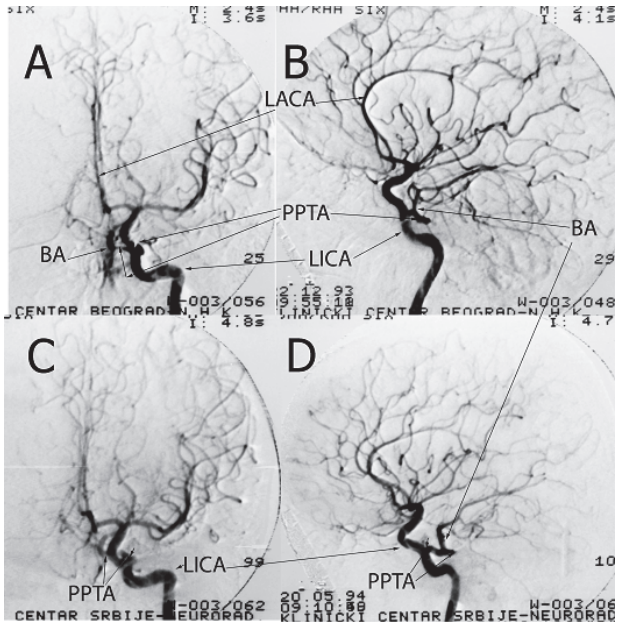
Bolesnica je otpuštena u dobrom stanju i bez sekvela.



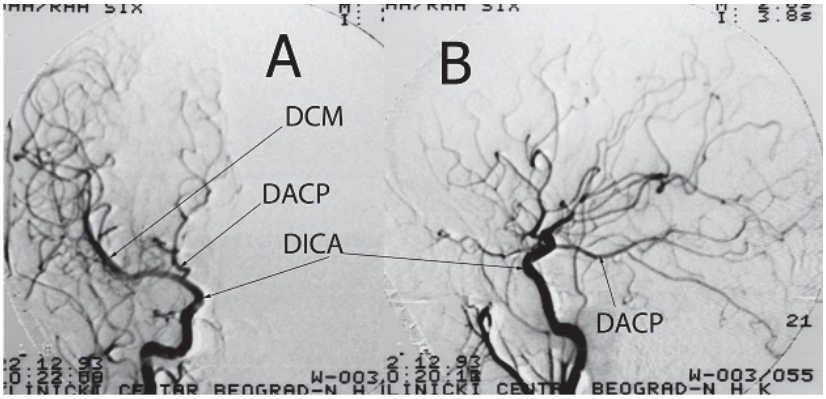
Sl. 2 – Desna vertebralna angiografija, AP (A) i profil (B): vidi se deo cirkulacije arteriovenske malformacije (AVM) iz desne unutrašnje vertebralne arterije (DPICA)



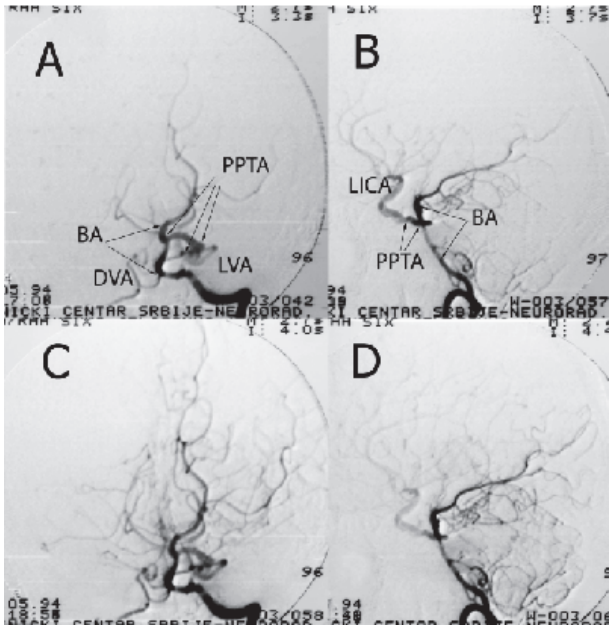
Sl. 3 – Leva vertebralna arterija – AP snimci (A, C) i profilni snimci (B, D): značajniji dotok krvi u AVM putem ove arterije.
Na snimku B uočava se punjenje leve karotidne arterije putem perzistentne primitivne trigeminalne arterije (PPTA), kao i manji kalibar proksimalnog segmenta (hipoplazija) bazilarne arterije (BA) pre spoja sa PPTA. Putem BA puni se samo leva arterija *cerebri posterior* i perforantne grančice vrha BA.
LAICA – leva anterior inferior cerebelarna arterija; LACP – leva arterija *cerebri posterior*;
LACI – leva arterija karotis interna



Sl. 4 – Leva unutrašnja karotidna arterija (LICA) puni neparnu levu arteriju *cerebri anterior* (LACH) iz koje se pune obe rekurentne arterije (vidljivo na A) kao i karotidobazilarna anastomoza peristentna primitivna trigeminalna arterija (PPTA) koja snabdeva bazilarnu arteriju (BA) u gornjoj polovini. Na B se vidi preoperativno potisnuta BA i PPTA prema napred usled pritiska hematoma u vermisu i relaksirano stanje posle operacije na D; PPTA je moćna i krv uglavnom struji iz LKA



Sl. 5 – Desna karotidna angiografija potpuno odsustvo A1 segmenta desno i fetalni tip arterije *cerebri posterior* koja se snabdeva samo iz desne unutrašnje karotidne arterije (DICA) preko zadnje komunikantne arterije.
DACP – desna arterija *cerebri posterior*; DCM – desna arterija cerebri medija



Sl. 6 – Leva vertebralna arterija (LVA) – postoperativni snimci. U svim fazama uočava se i dotok krvi kroz perzistentnu primitivnu trigeminalnu arterija (PPTA) iz zadnjeg sliva. Na slikama A i C vidi se segmentna hipoplazija bazilarne arterije (BA) proksimalno od spoja sa PPTA. PPTA je moćna i krv struji uglavnom iz leve unutrašnje karotidne arterije (LICA).
DVA – desna vertebralna arterija

Diskusija

Nalaz različitih zaostalih primitivnih arterija iz fetalnog perioda i njihov hirurški značaj nije nepoznat [6, 7]. Prepoznavanje perzistentne trigeminalne arterije može biti vrlo važno u slučaju hirurške procedure u regionu kavernoznog sinusa, sfenoidnog sinusa, baze lobanje ili zadnje lobanjske jame, kada može da se spreči povreda PPTA, ili njeno kidanje. Endovaskularne procedure, takođe, moraju poštovati specifičnosti cirkulacije i njenu ranjivost u prisustvu perzistentnih primitivnih anastomoza karotidovertebrobazilarnog sistema.

Iako prava incidencija PPTA u populaciji nije poznata, na osnovu angiografskih serija i obdukcija, procenjuje se da iznosi 0,1–1% [2]. Yilmaz i sar. [8] izveštavaju da je incidencija primitivnih karotidobazilarnih i karotidovertebralnih anastomoza bila 0,14% i 0,023% od 4 400 cerebralnih angiograma, respektivno. Kada se govori o incidenciji ovih malformacija kao izolovanom nalazu ili nasuprot, udruženom sa nekom od, pre svega, vaskularnih patoloških promena, podaci su fascinantni. Incidencija PPTA značajno je veća kod bolesnika sa *moya-moya* bolešću (10,7%) i *quazi-moya-moya* bolešću (60%) [9] i upućuje na razmatranje embrioloških faktora u nastanku ovih oboljenja. U seriji bolesnika sa udruženim kutanim hemangiomima referiše se 17–30% perzistentnih trigeminalnih arterija [10–12].

Kada je udruženost aneurizmi u pitanju, Agnoli [13] referiše 25% aneurizmi i subarahnoidne hemoragije kod bolesnika sa PPTA.

Karakteristike morfologije PPTA su vrlo specifične. Primitivna trigeminalna arterija povezuje kavernozni segment unutrašnje karotidne arterije sa bazilarnom arterijom od polovine visine do početka distalne trećine. Istostrana vertebralna arterija, zadnja komunikantna arterija i ACI mogu biti hipoplastične. Krv obično teče iz ACI ka BA.

Uvažavajući morfološke odlike od kojih je zavisna i teritorija irigacije krvlju, Saltzman [14] navodi tri tipa angiografski karakteristične cirkulacije. Prvi snabdeva obe *a. cerebellaris superior*, gornji deo bazilarne arterije i istostranu *a. cerebri posterior*. Istostrana *a. communicans posterior* je odsutna, a bazilarna arterija pre spoja sa PPTA hipoplastična je, tako da istostrana vertebralna arterija snabdeva samo početni deo BA. Hipoplazija početnog dela BA objašnjava se izostankom stimulusa usled faktora protoka krvi u prisustvu PPTA anastomoze [6]. Ovo je najčešći tip cirkulatorne šeme, a i naša bolesnica pripadala je ovom tipu, stoga i ne čudi činjenica da ova moćna anastomoza jedva učestvuje u krvotoku arteriovenske malformacije. Karakteristično za ovaj tip je da igra važnu ulogu u cirkulaciji krvi cerebeluma i moždanog stabla. Drugi tip podrazumeva snabdevanje obe *a. cerebellaris superior* preko PPTA, dok je treći tip kombinacija prethodna dva.

Vaskularne anomalije poput arteriovenskih malformacija, karotidokavernoznih fistula, *moya-moya* bolesti, drugih perzistentnih anastomoza, arterija i arterijskih anomalija prisutne su kod četvrtine bolesnika sa PPTA, a među njima su daleko najčešće aneurizme i one same daju udruženost sa PPTA od 14% [15]. U seriji koji su objavili Abe i Suzuki [1] koincidencija tumora i PPTA je višestruko nadmašila udruženost sa vaskularnim malformacijama. Međutim, pretražujući literaturu u vezi sa našim nalazom koincidencije primitivne trigeminalne arterije i AVM cerebeluma, zapazili smo da je izrazito retka. U *Pubmed* bazi, pretraživanjem kroz sva tekstualna polja, na upit „trigeminalna arterija“, nađeno je 335 referenci. Unutar te grupe 267 bilo je povezano sa aneurizmama, a samo 13 sa arteriovenskim malformacijama, od čega samo četiri u zadnjoj lobanjskoj jami. Imajući u vidu da su AVM u proseku 7–10 puta ređe od aneurizmi, 20 puta manji broj prikaza nas je podstakao da damo doprinos poznavanju ovih anomalija i prikazom našeg bolesnika. Sva četiri bolesnika iz nama dostupnih izvora (*Pubmed*) potiču iz japanske literature [16–19]. Kada se ovim bolesnicima pridruži i bolesnik koga su prikazali Uzawe i sar. [20], kod koga je verifikovano krvarenje u cerebelumu, broj referisanih bolesnika je i dalje izrazito nizak. Kod ovog bolesnika nije otkriven etiološki faktor. Moguće je da AVM manje od 10 mm ostanu nevidljive nakon krvarenja. Karakteristično za sve slučajeve, pa i za naš, jeste da osim perzistentne embrionalne arterije i AVM postoje i druga odstupanja u funkciji „savršenog anastomotskog“ (Willis-ovog) kruga. Ovakva je cirkulacija ranjiva, sa stanovišta mikrocirkulacije i perforatora u smislu neadekvatnog razvoja kolateralne cirkulacije, pogotovo kod potrebe hirurškog žrtvovanja nekog od krvnih sudova.

Takođe, čini nam se da je nužno pomenuti i činjenicu da su infratentorijalne AVM retke i da čine tek 7–15% svih intrakranijalnih AVM [21, 22]. Ovaj procenat nas asocira na učestalost aneurizmi vertebrobazilarnog sliva, što bi govorilo u prilog činjenice da na oko 10–15% cirkulacije endokranijuma (vertebrobazilarni sliv) otpada i sličan procenat patoloških promena. Razlika je, međutim, izrazitija u načinu prezentacije infratentorijalnih AVM, kod kojih je krvarenje skoro tri puta češće nego kod supratentorijalne AVM i koje [22] imaju veću sklonost ka ponovljenim krvarenjima. Verovatno ove činjenice, u kombinaciji sa specifičnom anatomskom pozicijom, jesu razlog da manje od polovine bolesnika preživljava inicijalno krvarenje [21]. Koliko se rizik povećava u koincidenciji sa retkim stanjima koja menjaju uobičajene fiziološke relacije u cirkulaciji (perzistentne vertebrobazilarne anastomoze), a takođe su u visokom procentu udruženi sa drugim anomalijama, nije poznato. Zato, navedene činjenice obavezuju na najveći oprez, hirurišku agilnost i agresivnost, od kojih, posebno u ovakvim slučajevima, zavise životi bolesnika.

Zaključak

Prikazano je retko stanje udruženosti PPTA sa arteriovenskom malformacijom u zadnjoj lobanjskoj jami. Važnost ovakvih nalaza ogleda se, pre svega, u promenama anatomskih i fizioloških uslova moždane cirkulacije, posebno važnim u toku određenih hirurških procedura.

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Dvanaestogodišnje praćenje bolesnice sa sistemskim eritemskim lupusom i lupus nefritisom

A patient with systemic lupus erythematosus and lupus nephritis: a 12-year follow-up

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Apstrakt

Uvod. Sistemski eritemski lupus (SLE) je hronično imunološko oboljenje koje u mlađoj ženskoj populaciji izaziva značajan morbiditet i mortalitet i koje zahvata mnoge organe, a najčešće bubrege, pa incidencija lupusnog nefritisa (LN) iznosi 60%. **Prikaz bolesnika.** Prikazali smo bolesnicu staru 57 godina, sa SLE dijagnostikovanim 1995. godine. Patohistološka analiza tkiva bubrega pokazala je LN tip V. Lečena je pulsevima kortikosteroida i azatioprinom godinu dana. Postignuta remisija bolesti, održavana je prednizonom, 15 mg/dan. Nefrotski relaps LN javio se 2006, a rebiopsija bubrega pokazala je nedavni infarkt bubrega usled veoma izraženog vaskulitisa. Ubrzo, razvio se cerobravaskularni insult, a na kompjuterizovanoj tomografiji (KT) infarktno ognjište endokranijalno. Bolesnica je lečena bolusima kortikosteroida i ciklofosfamida (ukupno VI mesečnih ciklusa bolusa), a zbog flebotromboze desne noge i niskomolekulskim heparinom, antikoagulantnim i antiagregacionim lekovima. Zbog aktivne imunologije (ANA 1 : 320) i nefrotskog sindroma, posle bolusa dobijala je 20 mg prednizona i 100 mg azatioprina dnevno, a posle šest meseci azatioprin je zamenjen mikofenolat-mofetilom koji se pokazao efikasnim i u indukciji i u održavanju remisije nefrotskog sindroma. **Zaključak.** Cilj lečenja LN jest postizanje i održavanje remisije bolesti, poboljšavanje stope preživljavanja, smanjenje toksičnosti primenjenih protokola i učestalosti relapsa. Mikofenolat-mofetil pokazao se kao efikasan lek u indukciji i održanju remisije nefrotskog sindroma u sklopu LN.

Ključne reči:
nefritis, lupusni; bolest, progresija; lečenje, ishod.

Abstract

Introduction. Systemic lupus erythematosus (SLE) is a chronic immunological disease causing a significant morbidity and mortality in younger women and involving several organs and systems, most often the kidneys, being consequently the incidence of lupus nephritis (LN) about 60%. **Case report.** We reported a 57 year-old patient with the diagnosed SLE in 1995. Pathohistological analysis of kidney biopsy revealed LN type V. The patient was treated with corticosteroid pulses and azathioprine during one year. A remission was achieved and maintained with prednisone, 15 mg daily. Nephrotic relapse was diagnosed in 2006 and the second kidney biopsy revealed recent kidney infarction due to extensive vasculitis. Soon, a cerebrovascul insult developed and CT-scan revealed endocranial infarctus. The patient was treated with corticosteroids and cyclophosphamide pulses (totally VI monthly pulses), and also with low-molecular heparine, anticoagulants and salicylates because of the right leg phlebothrombosis. After the pulses, the patient was adviced to take prednisone 20 mg daily and azothioprine 100 mg daily, and 6 months later mycophenolate mofetil because of persistent active serological immunological findings (ANA 1 : 320) and nephrotic syndrome. Mycophenolate mofetil was efficient in inducing and maintaining remission of nephrotic syndrome. **Conclusion.** The aim of LN treatment is to achieve and maintain remission, improve patients' outcome, reduce the toxicity of immunosuppressive drugs and the incidence of relapses. Mycophenolate mofetil was shown to be efficient in inducing and maintaining remission of nephrotic syndrome in the frame of LN.

Key words:
lupus nephritis; disease progression, treatment outcome.

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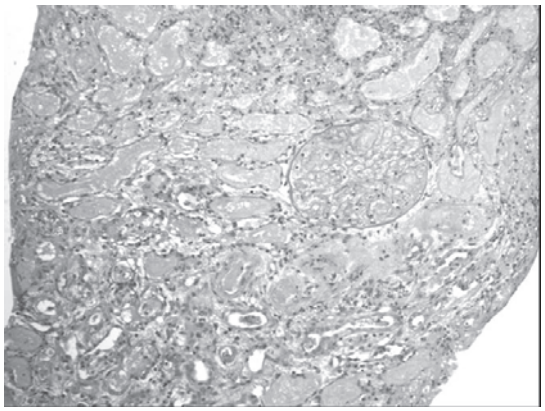
Uvod

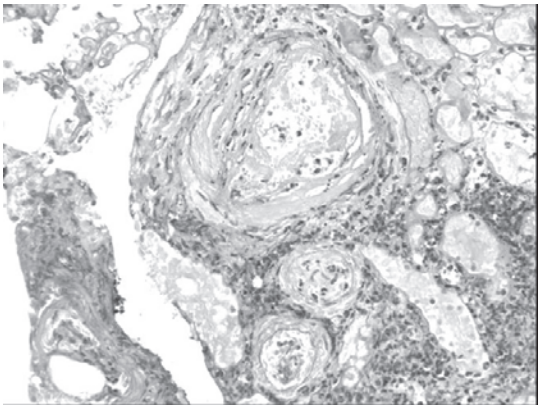
Sistemski eritemski lupus (SLE) je ozbiljno hronično imunološko obolenje koje u mlađoj ženskoj populaciji izaziva značajan morbiditet i mortalitet [1]. Oboljenje je multisistemsko, a najčešće zahvata bubrege. Prema novijim studijama, aktuelna incidencija bubrežne lezije je 14,3 na 100 bolesničkih godina [2]. Prognoza bolesti je bila veoma loša tokom 60-tih godina XX veka. Stope preživljavanja bolesnika i stope očuvanja bubreže funkcije znatno se poboljšavaju poslednjih decenija. Stope preživljavanja bubrega (očuvanje bubrežne funkcije bez potrebe za lečenjem hroničnim dijalizama) kretale su se tokom 90-tih godina od 83 do 92% u toku pet godina i od 74 do 84% tokom 10 godina lečenja [3]. Na prognozu LN utiču kompleksne međusobne interreakcije mnogobrojnih faktora, trajanje oboljenja, pojava i učestalost recidiva. Osim toga, loš prognostički faktor za očuvanje bubrežne funkcije i opšti ishod jeste izostanak remisije glomerulonefritisa i pored primene uobičajenih protokola [4, 5].

Prikaz bolesnika

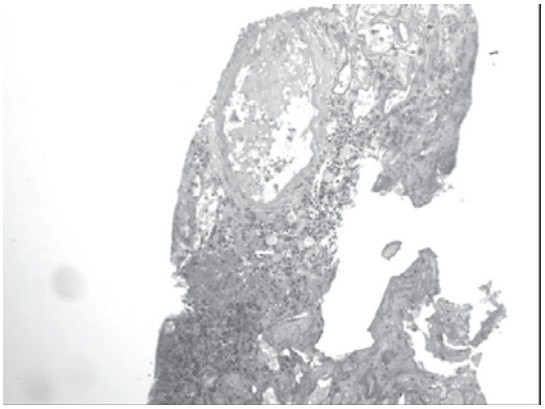
Prikazana je bolesnica, stara 57 godina, sa SLE dijagnostikovanim 1995. koja je kontinuirano praćena 12 godina. U vreme postavljanja dijagnoze imala je povišenu temperaturu, otoke i bolove u sitnim zglobovima šaka i stopala, povišen arterijski pritisak, eritrocituriju i proteinuriju oko 2 g/dan, očuvanu globalnu funkciju bubrega, a u imunološkim analizama anti-dsDNK + i ANA 1 : 160. Patohistološka analiza bioptiranog tkiva bubrega pokazala je lupus nefritis tip V. Bolesnica je dobijala pulseve kortikosteroida i azatioprin tokom godinu dana. Postignuta je remisija bolesti, uz smanjenje titra ANA i smanjenje proteinurije ispod 1 g/dan. Više godina lečena je manjim dozama prednizona, do 15 mg/dan. Do februara 2006. godine nije imala tegoba i bila je u remisiji bolesti (u ponovljenim kontrolama proteinurija ispod 1 g/dan, titar ANA nije prelazio 1 : 80, anti-dsDNK -). U februaru 2006. dijagnostikovan je relaps nefrotskog sindroma sa proteinurijom do 12 g/dan i ozbiljna hipertenzija koja je zahtevala primenu raznih antihipertenziva. Rezultati imunoloških analiza bili su sledeći: ANA + 1 : 640, anti-dsDNK+, C3 na donjoj granici, C4 u granicama normale. Marta 2006. urađena je rebiopsija bubrega, a patohistološka analiza pokazala je da je skoro celokupno tkivo dobijenog uzorka u nekrozi uz prisustvo infiltracije granulocitima i limfocitima duž ivice nekrotičnog područja (slika 1). To je odgovaralo nedavno nastalom infarktu bubrega usled prisutnog vaskulitisa u dve arteriole dobijene punkcijom (slike 2 i 3). Ubrzo posle biopsije bubrega bolesnica je imala cerebrovaskularni insult sa afazijom i desnostranom hemiparezom, a kompjuterizovanom tomografijom (KT) endokranijuma ustanovljeno je infarktno ognjište. Odmah je započeto lečenje kombinovanim bolusima solumedrola po 1,5 g i ciklofosfamida po 1 g (ukupno VI mesečnih ciklusa bolusa). Bolesnica je lečena nekoliko sedmica u Centru za rehabilitaciju. U junu 2006, zbog flebotromboze desne noge, u lečenje je uveden niskomolekulski heparin, zatim antikoagulantna terapija tokom 10 meseci, a potom antiagregaciona terapija. U imunološkim analizama (jul 2006) ANA su bila 1 : 160, a u septembru 1 : 320. U biohemijskim analizama perzistirao je kompletan nefrotski sindrom sa vrlo obilnom proteinurijom i posle primene 6 g ciklofosfamida, te je novembra 2006, uz 20 mg prednizona uvedeno 100 mg dnevno azatioprina. Rezultati kontrolnog imunološkog testiranja (8.12.2006) bili su sledeći: ANA



Sl. 1 – Nekroza glomerula i tubula ukazuje na infarkt bubrega – gornji deo slike pokazuje infiltraciju granulocitima i limfocitima, tj. stvaranje demarkacione linije (bojenje PAS-om, ×200).



Sl. 2 – Vaskulitis: arteriola sa teškom perivaskulnom infiltracijom limfocitima i monocitima koji infiltruju i zid krvnog suda, u samom krvnom sudu nekroza endotela sa naslagama fibrina (bojenje PAS-om, ×200).



Sl. 3 – Manja arterija sa perivaskulnom infiltracijom – u lumenu krvnog suda naslage fibrina ukazuju na početak stvaranja tromba usled vaskulitisa (bojenje PAS-om, ×100).

1 : 40, anti-dsDNK negativan, a u februaru 2007: ANA + 1 : 80. I pored navedenih vaskularnih incidenata, tokom intenzivne imunosupresivne, terapije antikardiolipinska antitela bila su negativna. Zbog perzistiranja izraženog nefrotskog sindroma sa proteinurijom do 6 g/dan, tokom jula 2007, azatioprin je zamenjen mikfenolat-mofetilom. Tokom šest meseci po uvođenju mikofenolat-mofetila u terapiju, registrovano je postepeno smanjivanje proteinurije, koja je sada manja od 0,5 g/dan. Globalna funkcija bubrega bila je i dalje očuvana i ostali biohemijski parametri nefrotskog sindroma su se povukli. Popravljao se i neurološki status, bolesnica je bila osposobljena da hoda i koristi ruke, ali se afazija održavala.

Tokom primene imunosupresivne terapije, bolesnica je u nekoliko navrata lečena zbog leukociturije i bakteriurije. Ovaj problem posledica je upotrebe stalnog urinarnog katetera, koji je bio neophodan zbog ograničene pokretljivosti. Drugih infekcija nije imala.

Tokom juna 2007. kod bolesnice je dijagnostikovana tranzitorna blaga leukopenija (smanjenje broja leukocita na 2 600), pa je četiri nedelje prestala da uzima azotioprin, do normalizacije broja leukocita.

Drugi neželjeni efekti imunosupresivne terapije nisu dijagnostikovani i bolesnica je dobro podnosila primenjene lekove.

Bolesnica je, takođe dobijala suportivnu terapiju: inhibitore angitenzin-konvertujećeg enzima, kao i beta blokatore i antagoniste kalcijujma u cilju regulisanja arterijskog pritiska, zatim statine i gastroprotektivnu terapiju.

Diskusija

Poremećena funkcija B- i T- ćelija, stvaranje autoantitela usmerenih na strukture sopstvenog organizma i poremećeno odstranjivanje apoptotičnih ćelija razlozi su za nastajanje sistemskog eritemskog lupusa. U bubrezima bolest obično primarno zahvata bazalnu membranu i mezangijum glomerula, ali se promene često registruju u tubulointersticijumu i na krvnim sudovima [6]. Histopatološke promene na bubrezima klasifikovane su u VI klasa sa nekoliko podtipova [7], a nalaz različitih aktivnih i hroničnih promena u bioptiranom tkivu bubrega ima prognostički značaj, jer su aktivne promene, kao što su subendotelni depoziti imunih kompleksa i intersticijumska imflamacija, osetljiviji na terapiju od teških lezija kao što su polumeseci ili nekroza tkiva. Ponovljene biopsije bubrega korisne su za praćenje patohistoloških promena kod pojedinih bolesnika tokom vremena [8]. I kod prikazane bolesnice, zahvaljujući ponovljenoj biopsiji bubrega, potvrđena je evolucija bolesti iz stadijuma LN tip V u stadijum gotovo generalizovanog infarkta bubrega sa pratećom infiltracijom tkiva mononuklearima i limfocitima.

Klinička slika lupus nefritisa može varirati od asimptomatskih urinarnih anomalija do brzo progresivnog gubitka bubrežne funkcije [9]. Tok oboljenja prolazi kroz različite faze, koje uključuju i periode kompletne i nekompletne remisije. Studije pokazuju da približno jedna četvrtina bolesnika ostvaruje remisiju, od kojih jedna polovina ostaje u remisiji u proseku šest godina. Naša bolesnica je posle prve serije bolusa kortikosteroida i primene azatioprina uvedena u nekompletnu remisiju, sa proteinurijom nižom od 1g na dan. Oko 72% bolesnika zahteva primenu manjih doza kortikokosterida (≤ 7,5 mg prednizona) tokom deset godina praćenja od postavljanja dijagnoze lupus nefritisa [10]. Treba naglasiti da je kod naše bolesnice remisija trajala deset godina, uz doze prednizona do 15 mg dnevno.

Relapsi su klinički značajni jer zahtevaju dodatnu imunosupresivnu terapiju i loš su prognostički događaj [11]. Kod naše bolesnice težak nefrotski relaps javio se deceniju posle postizanja remisije, a brza dijagnostika patohistoloških promena na bubregu i agresivno lečenje kombinacijom bolusa kortikosteroida i ciklofosfamida pomogli su da se imunološka aktivnost bolesti smiri.

Cilj lečenja LN je postizanje i održavanje remisije bolesti, poboljšavanje stopa preživljavanja, snižavanje toksičnosti primenjenih protokola i učestalosti relapsa [12]. Kortikosteroidi su prvi bili efikasni i još uvek su ključni lekovi u lečenju LN [13]. Kombinacija citotoksičnih lekova – azatioprin, ciklofosfamid i, poslednjih godina, mikofenolat-mofetil, omogućila je smanjenje doza i neželjenih efekata kortikosteroida i znatno poboljšala ishod bolesti [14–19]. Nema konsenzusa o dužini lečenja, ali opšti stav je da intenzivnu imunosupresivnu terapiju treba davati najmanje dve godine posle postizanja remisije. Kod naše bolesnice, posle 10 meseci lečenja nije više bilo imunološke aktivnosti bolesti, ali u tom momentu nije postignuta remisija nefrotskog sindroma. U takvim situacijama proteinurija može da bude posledica hroničnih patohistoloških promena, odnosno glomeruloskleroze, a ne aktivnih imunoloških procesa i treba voditi računa o opasnosti od preteranog izlaganja imunosupresivnim lekovima i njihovim neželjenim efektima. Kod prikazane bolesnice ipak je postignuta i remisija nefrotskog sindroma posle 29 meseci intenzivnog imunosupresivnog lečenja. Primena kombinacije bolusa kortikosteroida i ciklofosfamida nije dovela do toga. Indukcija remisije pokušana je i uspešno postignuta tek po uvođenju mikofenolat-mofetila. Ovaj lek je pokazao efikasnost i u terapiji održavanja.

Zaključak

Poboljšanju ishoda bolesti kod bolesnika sa lupus nefritisom doprinosi intenzivna primena visokih doza kortikosteroida i imunosupresivnih lekova, bolje poznavanje patogeneze bolesti, blagovremena dijagnoza, kao i napredak u suportivnoj terapiji. Mikofenolat-mofetil pokazao se kao efikasan lek u indukciji i održanju remisije nefrotskog sindroma u sklopu LN.

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Optical coherence tomography in late solar retinopathy

Optička koherentna tomografija kod kasne solarne retinopatije

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Abstract

Introduction. Solar retinopathy refers to retinal injury induced by direct or indirect solar viewing. **Case report.** We presented a patient who had observed partial solar eclipse 51 year before. He had bilaterally decreased vision and scar of the macular region at the time of presentation. The basic diagnostic tool applied in the presented patient, optical coherence tomography, showed hyporeflexivity of the outer retina in the segment of retinal pigment epithelial-photoreceptors complex with atrophy and thinning of the foveolar region. **Conclusion.** Optical coherence tomography is a powerful, non-invasive diagnostic tool which can ease the diagnosis and estimate the level and nature of the macular region damage.

Key words:
eye diseases; retinal diseases; sunlight; diagnosis; opthhalmoscopy; tomography, optical coherence.

Apstrakt

Uvod. Solarna retinopatija predstavlja oštećenje retine uzrokovano direktnim ili indirektnim gledanjem u sunce. **Prikaz bolesnika.** Prikazan je bolesnik koji je 51 godinu nakon posmatranja delimičnog pomračenja sunca ispoljio obostrano smanjenje vidne oštrine i ožiljne promene makularne regije. Bazično dijagnostičko oruđe primenjeno kod prikazanog bolesnika, optička koherentna tomografija, pokazala je hiporefleksiju spoljašnje retine u nivou retinalnog pigmentnog epitela-fotoreceptornog kompleksa sa izrazitom atrofijom i istanjenjem fovealne regije. **Zaključak.** Optička koherentna tomografija predstavlja moćno, neinvazivno dijagnostičko oruđe koje može olakšati dijagnozu i procenu stepena i prirode oštećenja makule kod solarne retinopatije.

Ključne reči:
oko, bolesti; retina, bolesti; sunčeva svetlost; dijagnoza; oftalmoskopija; tomografija, optička, koherentna.

Introduction

Solar retinopathy (foveomacular retinitis, photoretinitis, photomaculopathy, eclipse retinopathy) refers to retinal injury induced by direct or indirect solar viewing [1].

Damaging effects of sun viewing are known for centuries. In the beginning, foveomacular retinitis was characterized as a syndrome of bilateral decreased vision and foveal lesions in military persons [2].

Solar retinopathy is associated, besides military service, with religious sun gazing, solar eclipse viewing, watching the sun by telescope, psychiatric disorders, and the use of psychotropic drugs and sunbathing [3].

Sunlight produces retinal damage through photochemical effect, which can be enhanced by raising the temperature of the retinal tissue. Direct solar observation through a 3 mm pupil raises the temperature of retina by 4ºC, however, solar observation through a 7 mm pupil raises the temperature of retina by 22ºC, which causes the photocoagulation of retina [4].

The symptoms usually occur several hours after the exposition to sunlight and include unilateral or bilateral vision loss, metamorphopsia, central or paracentral scotoma, chromatopsy, photophobia, periorbital pain.

The diagnosis is established based on anamnestic data and fundoscopy.

Immediately after exposition, fundus examination reveals small yellow spot surrounded by gray margin in the foveolar or parafoveolar area. Fluorescein angiography may be normal or showing minimal changes. Optical coherence tomography demonstrates outer layer abnormal reflectivity of macular area, fragmentation or disorders of inner high reflectivity layer which represents the junction of inner and outer segments of photoreceptors in late solar retinopathy [5, 6].

Case report

A 73-year-old male patient was admitted to our clinic for operative treatment of decreased vision on the left eye. On the occasion of preoperative evaluation we found pseu-

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dophakia of the right, senile cataract of the left and chorioretinitis of both eyes. The patient said that on October 2, 1959, he had observed solar eclipse for 3 hours through pale sunglasses.

Partial eclipse on the territory of Serbia began on October 2, 1959 at 11.42 h, with maximum at 12.37 h ending at 13.29 h [7].

Immediately after exposition the patient noticed vision loss and dark round spot, which decreased in following period and had finally gone completely.

The patient served in the army a few years later in infantry unit because he was partially capable for military service due to low vision.

We systematically excluded possible risk factors and other diseases of the macular area which can mimic the optical coherence tomography findings of solar retinopathy in the patient.

Two months later the best corrected visual acuity of the right eye was 0.7–0.8, and of the left eye 0.3 (Snellen). The findings on the anterior segment of the right eye were normal. Fundus examination of the right eye revealed oval interpapillomacular atrophic chorioretinal scar with pigmented margins, more pigmented nasally (Figure 1a).

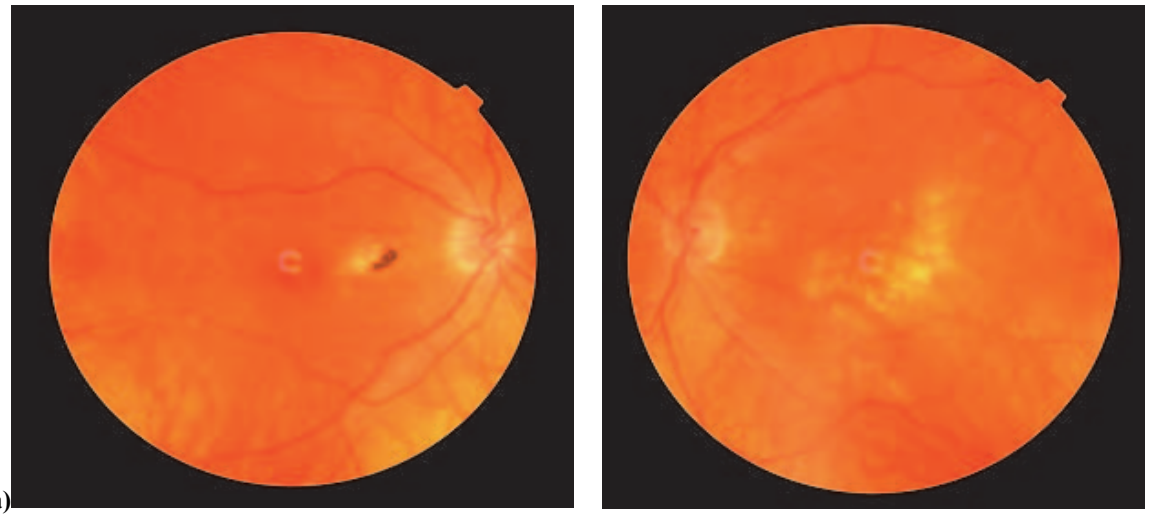
On the left eye, in the macular region, atrophic chorioretinal scar of irregular shape was seen, indistinctively limited without pigmented margins (Figure 1b).

Amsler grid of both eyes showed metamorphopsia.

Optical coherence tomography of both eyes was done (Figure 2) (Topcon 3D OCT-1000) and its findings were as follows.

Right eye: in the foveola a few minor disruptions of the photoreceptors band, nasally from fovea, in the area of clinically significant alteration, a complete absence of photoreceptor band and several disruptions of the retinal pigment epithelium band were seen. Likewise, hyperreflective alteration was seen in the level of RPE-photoreceptor complex which corresponded with clinically seen hyperpigmentation (Figure 2, left). Central foveal thickness was 176 μm and total capacity 6.69 mm^3 (according to Normative Database, Caucasian, Age group: 40–70 years Courtesy of Rotterdam Study).

Left eye: a few micro ablations of pigment retinal epithelium was present. In whole, foveal area major deficiencies and thinning of photoreceptor band with highly hyporeflectivity of that layer were registered. Central foveal thickness was 91 μm and total capacity 6.69 mm^3 (Figure 2,



a) **b)**

Fig. 1 – Fundus examination: (a) – on the right eye oval interpapillomacular atrophic chorioretinal scar with pigmented margins; (b) – atrophic chorioretinal scar of irregular shape in the macular region on the left eye

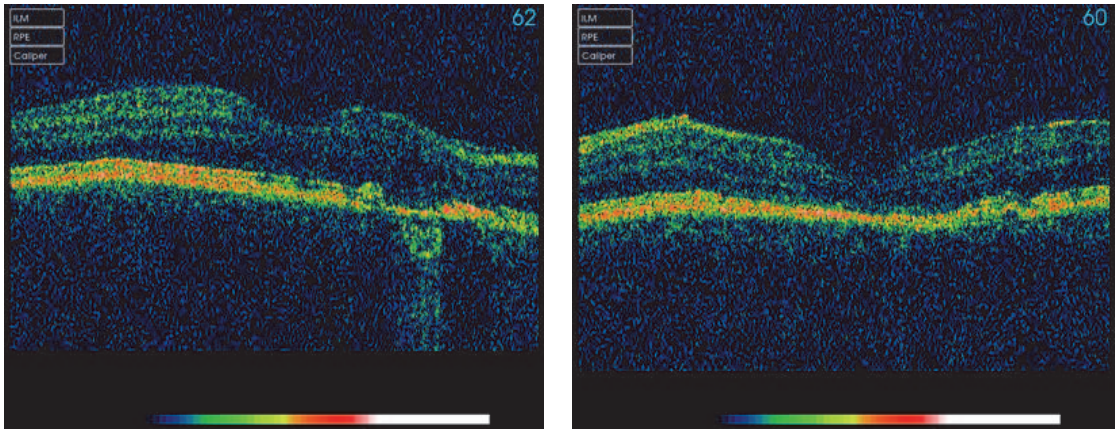


Fig. 2 – Optical coherence tomography of both eyes

right).

Discussion

Solar retinopathy refers to damage of outer retinal layers, retinal pigment epithelium and photoreceptor layer. Three types of retinal damage from intense light have been described: mechanical, thermal and photochemical damage, the last being the most probable mechanism in retinal damage caused by solar eclipse viewing [1, 3].

The clinical symptoms described in the literature include decreased vision, central scotoma, dyschromatopsia, photophobia and metamorphopsia (as in our patient). Although asymmetrical, involvement is usually bilateral. The degree of retinal damage depends directly on the intensity of light, duration of exposure, pupillary size (higher in case of total solar eclipse), transparency of ocular media (kids and younger persons are more liable), refractive state, with increased risk in emmetropees and low hyperemmetrope because the light is focused sharply on the macula [5].

No specific therapy exists for solar or eclipse retinopathy. Direct sun and eclipse viewing should be discouraged unless there is adequate use of the proper protective eyewear [1].

Optical coherence tomography findings in the presented patient with hypo-reflectivity of outer retina in the level of RPE-photoreceptor complex with peculiar atrophy and thinning of the foveal region, which is seen many years after solar eclipse viewing, corresponds to findings of other researchers [6, 8].

Conclusion

Optical coherence tomography findings may facilitate the diagnosis and estimate the degree and nature of macular damage in solar retinopathy. It can help in better diagnostic of solar retinopathy in correlation with anamnestic data and clinical symptoms and signs, especially in atypical cases.

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C A S E R E P O R T

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Simultaneous stenting of the left main coronary stem and internal carotid artery in a hemodynamically unstable patient

Istovremeno rešavanje stenoze glavnog stabla leve koronarne arterije i unutrašnje karotidne arterije stentovima kod hemodinamski nestabilnog bolesnika

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Abstract

Introduction. Combined endovascular interventions on carotid and coronary arteries are rare. Stenting of the unprotected coronary left main stem is a high risk procedure. We presented hemodynamically unstable patient with combined carotid artery and left main stem coronary artery stenting. **Case report.** A 78-year-old female patient was admitted to our institution for right carotid endaterectomy. The patient had 80% stenosis of the right carotid artery and occlusion of the left carotid artery. Coronary angiography revealed 70% ostial left main stenosis, occlusion of the right coronary artery and the left circumflex artery, and 80% stenosis of the left anterior descending artery. Simultaneous carotid artery endaterectomy and coronary artery by-pass grafting were considered. Due to high perioperative risk, surgery was rejected, and the patient was treated endovascularly with stenting of arteries occluded. The procedure was completed without complications and the patient was hemodynamically stabilised. **Conclusion.** This report illustrates simultaneous coronary and carotid stenting as a successfull lifesaving procedure.

Key words:
coronary disease; carotid artery diseases; stents; treatment outcome.

Apstrakt

Uvod. Kombinovane endovaskularne procedure na karotidnim i koronarnim krvnim sudovima u istom aktu su retke. Postavljanje stenta u glavno stablo leve koronarne arterije je procedura sa povišenim rizikom. Prikazana je hemodinamski nestabilna bolesnica kod koje je u istom aktu postavljen stent u glavno stablo leve koronarne arterije i karotidnu arteriju. **Prikaz bolesnika.** Bolesnica, stara 78 godina, hospitalizovana je zbog endaterektomije desne karotidne arterije koja je bila sužena 80%, dok je leva bila okludirana. Koronarografija je otkrila ostijalno suženje glavnog stabla leve koronarne arterije od 70%, okluziju arterije cirkumflekse i desne koronarne arterije, kao i visokostepeno suženje prednje descedentne koronarne arterije. Razmotreno je istovremeno hirurško lečenje koronarne i karotidne bolesti, ali je zbog visokog rizika odlučeno da se bolesnica tretira endovaskularno postavljanjem stenta u okludirane arterije. Procedura je protekla bez komplikacija i bolesnica je hemodinamski stabilizovana. **Zaključak.** Kombinovane endovaskurane koronarne i karotidne procedure u istom aktu mogu biti uspešne kod bolesnika koji su visokorizični za hirurško lečenje.

Ključne reči:
koronarna bolest; a. carotis, bolesti; stentovi; lečenje, ishod.

Introduction

Simultaneous endovascular interventions on carotid and other extracarotid arteries still do not count into routine procedures, although few reports favor them as feasible, relatively safe and cost-effective [1]. Left main (LM) stem percutaneous coronary intervention (PCR) is for itself a procedure with elevated risk. In the era of drug eluting stents, it is performed more often, especially in those patients who are at high surgical risk [2]. We reported a case of combined stenting of internal carotid artery and LM stem coronary artery being only the second case reported in literature up till now [3].

Case report

A 78-year-old female patient was admitted to our institution for right carotid endaterectomy. The patients cardiovascular risk factors included hypertension, diabetes

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(treated with oral hypoglycemics) and hyperlipidemia (type IIb). Her cerebrovascular symptoms were dizziness and vertigo, and she had stable angina. The patient had no previous history of myocardial infarctions or cerebrovascular insults. Her physical and neurological examinations were unremarkable. Color Doppler sonography of the neck arteries revealed 80% stenosis of the right internal carotid artery and occlusion of the left internal carotid artery. During hospitalization, the patient developed symptoms of unstable angina (with 3 mm ST segment depression and negative T wave in D1, D2, aVL and all precordial leads) and underwent coronary angiography which revealed diffuse coronary atheromatosis, with 70% ostial LM coronary artery stenosis, occlusion of the right coronary artery and left circumflex artery, and 80% stenosis of the medial left anterior descending (LAD) artery (Figures 1–3).

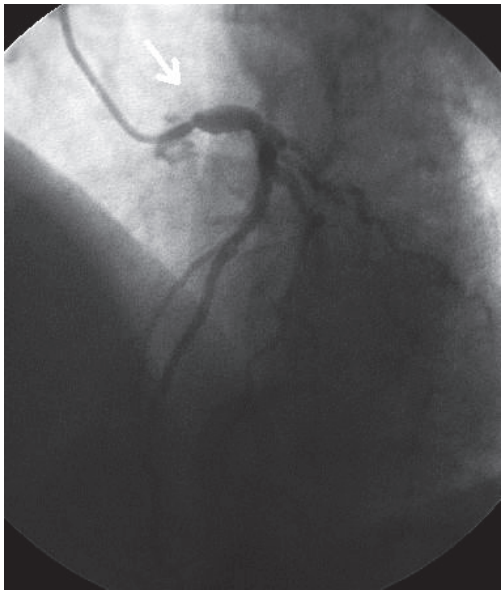

Fig. 1 – Ostial coronary left main stem stenosis, arrow

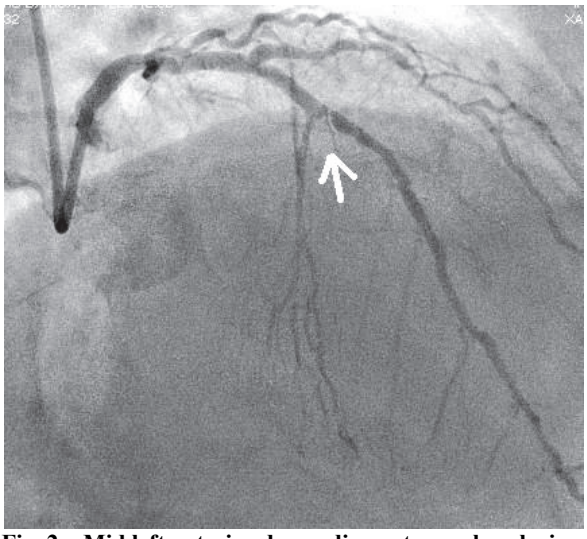

Fig. 2 – Mid left anterior descending artery subocclusion, arrow

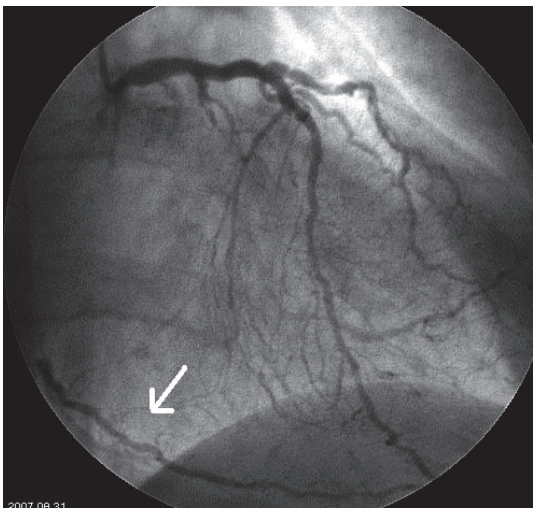

Fig. 3 – Right coronary artery occlusion, arrow pointing towards distal right coronary artery supplied by collaterals from the left coronary artery (catheter in the right upper corner in left main stem).

Echocardiographic control showed that the left ventricular ejection fraction was 40%–45%, with wall hypertrophy and akinesia of basal and mid third of the inferior and the entire posterior wall of the left ventricle, and also moderate mitral regurgitation into mildly enlarged left atrium. Brain computerized tomography findings included old infarct lesions of cortex and subcortical white brain matter localized temporally left, in temporal operculum and frontally precentraly right, and also involutive changes such as cortical reduction and diffuse scarcity of deep white matter periventricularly. The patient was treated with ACE inhibitors, beta blockers, statins and aspirin, and after the patient developed unstable angina, intravenous infusion of unfractionated heparin and nitroglycerin were added.

In later course, the patient became hemodynamically unstable, hypotensive (80/50 mmHg) and received adequate inotropic support (dopamine 5.6 μg/kg/min). Because of contralateral occlusion of internal carotid artery and concomitant high degree LM coronary artery stenosis, simultaneous carotid artery endaterectomy and coronary artery bypass grafting (CABG) were considered. Due to unacceptably high perioperative risk and hemodynamic instability (with an in-hospital onset), surgery was rejected, and the patient underwent simultaneous stenting of the right internal carotid artery and the LM stem coronary artery and medial LAD.

After the patient was premedicated with loading dose of clopidogrel (8 × 75 mg) and after ACT was measured and found to be in working range, PCI was performed using the right femoral approach (6F femoral sheath, guiding catheter 3.75 6F Launcher Medtronic, ATW floppy guide wire – Cordis J&J) LM trunk was directly stented with Tsunami-Terumo 4 mm × 17 mm bare metal stent at 16 ATM, and then the mid LAD artery was directly stented with Tsunami-Terumo 3.5 mm × 16 mm BMS at 16 ATM. LM stem was afterwards post-dilated with NC Sprinter 4.5 mm × 15 mm balloon at 20 ATM (Figures 4 and 5).

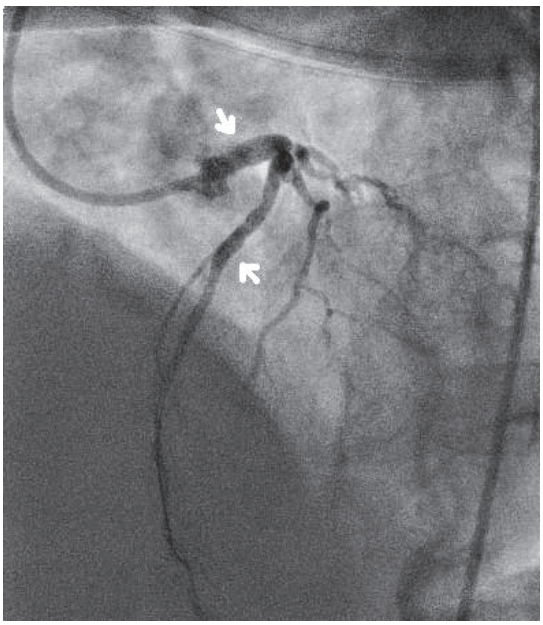

Fig. 4 – Stents in coronary left main stem and left anterior descending artery, arrows

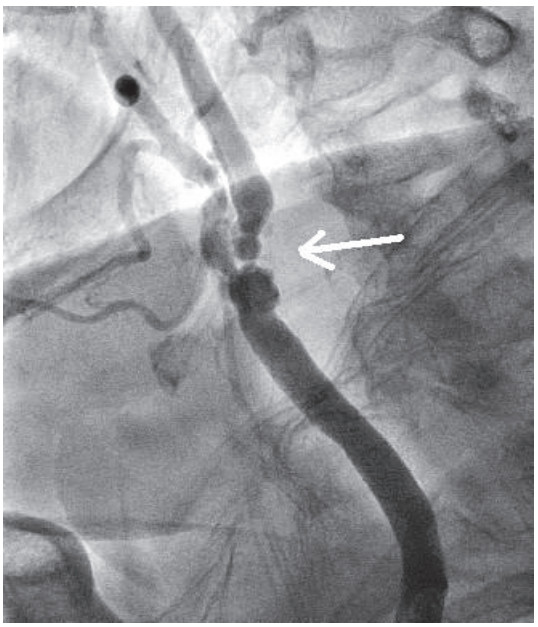

Fig. 6 – Preprocedural angiogram, high degree right internal carotid artery stenosis, arrow

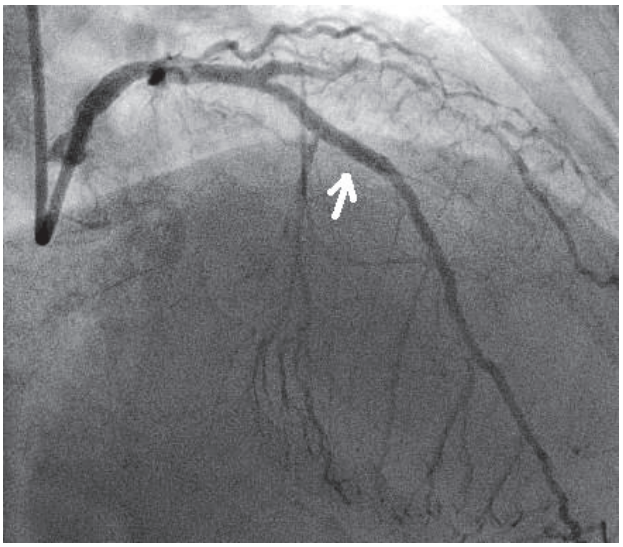

Fig. 5 – Postprocedural left anterior descending artery, arrow

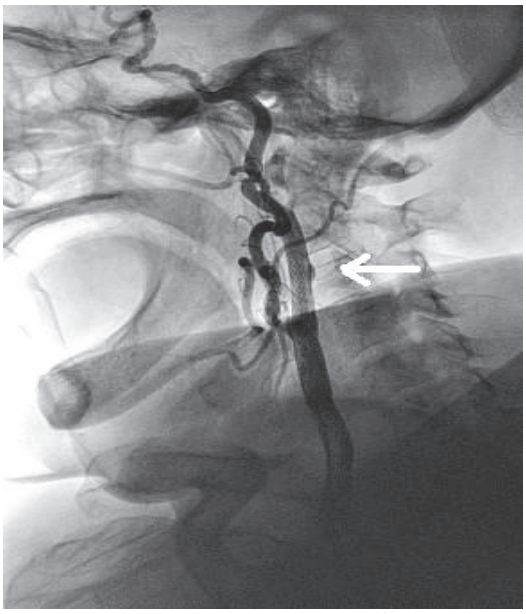

Fig. 7 – Postprocedural angiogram, right internal carotid artery, arrow

After PCI, stenting of the right internal carotid artery was done. After canulating the right internal carotid artery (JR coronary diagnostic catheter, Storq guide-wire – Cordis J&J, guiding sheath 8F 90 cm), distal protection (EPI Filter, Boston Scientific) was placed into the right internal carotid artery, which was then directly stented with carotid stent (Wallstent 9 mm × 40 mm, Boston Scientific), and post-dilated with carotid balloon 7 mm × 20 mm (Amia, Cordis J&J) (Figures 6 and 7).

Intra-aortic balloon pump was not applied during the procedure for several reasons: although the patient was hypotensive, there were no signs or symptoms of pulmonary oedema (as in late stage cardiogenic shock), left ventricular systolic function was relatively preserved, and the patient adquatelly responded to inotropic therapy. In our case, intra-aortic balloon pump insertion would require bilateral femoral artery puncture, and by that increase the posiblity of bleeding complications.

The procedure was completed without complications. After the procedure the patient regained hemodynamic stability and after adequate hydration, the inotropic drug was withdrawn. The patient was released from the Institute after two days in good general condition, and on 1 month and 3-month control her condition remained unchanged.

Discussion

Main indications for carotid artery stenting are surgically unapproachable lesions (beyond the level of C2 or subclavian), carotid stenosis caused by radiation, and previous ipsilateral neck dissection, previous carotid endarterectomy (restenosis) [4]. A high incidence of significant coronary disease is detected among patients who undergo carotid stenting [5]. Long term outcome of carotid stenting is dependent on coronary artery disease presence. Adverse cardiovascular events and total mortality are more frequent in patients with concomitant coronary and carotid disease, although adverse neurological events are equally present in patients with simultaenous coronary disease and in patients with isolated carotid disease [6]. In patients who undergo carotid stenting, coronary artery disease is an independent mortality predictor. On the other hand, stroke is a devastating complication of CABG. It increases mortality and morbidity of surgical procedure, prolongs hospitalization and dramatically decreases postoperative life quality. Although CABG-related stroke is multicausal, extracranial carotid disease is the most important and the most frequent cause. Simultaneous surgical approach is the most widely apllied strategy for treating patient with concomitant coronary and carotid disease, although in present day no treatement consensus exists. A new therapeutic strategy with promising results, consisting of hybrid revascularization by carotid artery stenting (CAS), immediately followed by on-pump CABG has been recently proposed for patients with coexisting carotid artery disease and percutaneously unsolvable coronary artery disease [7].

Carotid artery stenting in patients who also have coronary artery disease is feasible, safe, and shows good results with a prospective follow-up [8, 9]. Carotid artery stenting combined with simultaneous interventions on other central arteries can be applied with high rate of success and with relatively few complications [10]. Advantages of this simultaneous approach compared with phase stenting include: higher comfort of the patient, and reduced incidence of complications related to puncture site, because femoral puncture is performed only once, and also cost-effectiveness because there is no need for repeated hospitalization [1]. Results of recently published multicentric, prospective, unrandomised study on 659 patients with coexistent coronary and carotid artery disease, strongly favor endovascular approach in treating these patients [11]. Adverse event rate (death, stroke or myocardial infarction) within thirty days was significantly lower among patients treated endovasculary in comparison with patients undergoing surgical or hybrid procedures. Nevertheless, experience in simultaneous percutaneous menagement of coronary and carotid disease remains limited in this study (35 patients).

Conclusion

One of the important conclusions of this paper is that simultaneous endovascular coronary and carotid procedures are burdended by higher incidence of bleeding and acute renal failure; therefore simultaneous percutaneous treatement should be limited to unstable clinical presentation, refractory to medical therapy. In our case, considering contralateral occlusion of the internal carotid artery and hemodynamic instability caused by severe form of coronary disease, applied combined endovascular procedure was fully justified.

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Razvoj zdravstvene kulture u Srbiji u prvoj polovini 19.veka

Development of health culture in Serbia in the first half of the 19th century

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Ključne reči:
zdravstvena kultura; istorija, 19 vek, srbija.

Key words:
health education; history, 19th century; serbia.

Počeci razvoja saniteta u oslobođenoj Srbiji

Posle završetka Drugog srpskog ustanka 1815. godine i nakon dobijanja kakve-takve autonomije od strane turskih vlasti, u Kneževini Srbiji započeti su prvi koraci za izgradnju srpske nacionalne privrede, kulture, obrazovanja i zdravstva. To nije bilo lako ostvariti jer je Srbija posle austro-turskog rata (1788–1791), kao i posle Prvog i Drugog srpskog ustanka bila u dobroj meri razrušena i opustošena [1].

U toku obnove Srbije pojavila se potreba za razvojem i normalnim funkcionisanjem svih delatnosti potrebnih za seosko i gradsko stanovništvo u Srbiji [2]. Stabilizacija političkih i privrednih prilika stvorila je pogodan teren za razvitak zdravstvenih prilika u Kneževini Srbiji. U prvim godinama posle završetka Drugog srpskog ustanka zdravstvena prosvećenost i zdravstvena kultura naroda bile su na veoma niskom stepenu. Narod se lečio kako je znao i umeo koristeći svoje „narodne lekare“, vidare i vračare. Uopšteno govoreći, shvatanja o lečenju u Srbiji bila su u to vreme vrlo primitivna i u mnogo čemu su odgovarala uslovima koji su u Srbiji bili zatečeni iz vremena turske uprave. Kako u Srbiji sve do 1819. godine nije bilo nijednog diplomiranog lekara, postojali su uslovi za delovanje vidara i samoukih narodnih lekara. Pišući o zdravstvenim prilikama u Srbiji u prvim godinama posle Drugog srpskog ustanka Lindenmajer ističe da su u to vreme lekarske poslove po srpskim varošima i selima obavljali: berberi, ećimi, samouki lekari i narodni vidari i vidarice. Berberskim zanatom su se uglavnom bavili samo Turci. Oni su uz svoj berberski zanat obavljali i raznorazne lekarske usluge: puštanje krvi, vađenje zuba, sitne hiruške intervencije itd [3]. Ećimi su uglavnom bili Grci, poreklom iz Epira, koji su svoje znanje sticali u porodici kao strogo čuvanu nasleđenu tajnu koja se generacijama prenosila sa kolena na koleno. Uglavnom su se nastanjivali u varošima i većim mestima, a neretko su bili lični lekari pojedinih turskih velikodostojnika. Jedan od najpoznatijih ećima bio je hećim Toma-Kostić-Konstantinović, koga je Srpski sovjet još 1806. godine uzeo za vojnog lekara. Hećim Toma je svoje lekarske sposobnosti pokazao godinu dana kasnije kada je uspešno izlečio kneza Miloša od rane dobijene u sukobu sa turskom vojskom [4]. Samouki lekari su po narodnosti bili Srbi, Grci i Turci. Oni su znanje iz lekarske struke sticali na razne načine: posmatranjem, iskustvom, kontaktima sa školovanim lekarima, pa, čak, i slučajno. Od Srba samoukih lekara tog vremena izdvajaju se Sava Jovanović iz Drenovca kod Šapca i Gojko Marković rodom iz sela Dobrinje, iz Požarevačkog okruga. Jovanović je kao mladić došao u Beograd i boravio u kući doktora Bartolomea Kuniberta. Tu je naučio italijanski jezik i, takođe, stekao osnovno znanje iz medicine koje mu je kasnije omogućilo da se samostalno bavi lekarskom praksom. Iako samouk lekar, bio je službeno angažovan od strane srpskih vlasti koje su mu davale mesečnu platu u iznosu od 120 groša. Svoje znanje iz medicine Gojko Marković je stekao u Smirni i Aleksandriji, gde se našao posle sloma Prvog srpskog ustanka, kada su ga Turci zajedno sa velikim brojem srpske nejači odveli u roblje. Vrativši se kasnije u Srbiju, Gojko se posvetio lečenju zaraznih bolesti, naročito sifilisa, u čemu je imao punu podršku srpskih vlasti. Od samoukih lekara grčke narodnosti, koji su u to vreme obavljali lekarsku praksu u Srbiji, treba pomenuti Antonija Delinija iz Smirne i Kaparisa iz Epira, a od turskih lekara Havaz-Memet hodžu iz Beograda i Hasana Mustafića iz Smedereva [4]. U to vreme u većem broju dolazili su u Srbiju priučeni, samouki „lekari“ i to uglavnom iz evropskih provincija turskog carstva [5]. Njima treba dodati i znatan broj berbera koji su vadili zube i obavljali razne druge „lekarske usluge“. Početkom tridesetih godina 19. veka na prostoru Kneževine Srbije počinju da se koriste pijavice u medicinske svrhe. Upotreba pijavica je za kratko vreme postala veoma omiljeno sredstvo lečenja, ne samo u prvim bolnicama i u privatnoj praksi, već je svoju svakodnevnu primenu našla i u širokim narodnim masama. Glavni „posao“ sa pijavicama obično su obavljali berberi koji su radili sa 5–10 pijavica, a u određenim slučajevima i do 30 odjednom. Oni su ovaj posao

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obavljali po naredbi i uputstvu lekara ili na osnovu svog iskustva i procene stanja bolesnika [6]. Kako u to vreme nije bilo apoteka, lekovi su se kupovali u bakalnicama i njima sličnim radnjama. Mnogi trgovci su u svojim radnjama držali lekove, kao i sirovine za spravljanje lekova, koje su se bez ikakve kontrole prodavale svima koji su imali novca da ih kupe. Početkom tridesetih godina 19. veka ova pojava je uzela toliko maha da su skoro sve trgovačke radnje u Srbiji prodavale lekove i sirovine za spravljanje lekova. Zbog nepostojanja stručne kontrole, trgovci su neretko nabavljali „lekove" koji su po svojim svojstvima nanosili mnogo više štete nego koristi onima koji su ih koristili. Lekovi i sirovine za spravljanje lekova uglavom su se nabavljali (neretko krijumčarili) iz Turske i Austrije [7]. Uz lekove su se, u to vreme, sve češće prodavali i otrovi. Oni su se često zloupotrebljavali, što je predstavljalo veliki problem državnim vlastima u Srbiji. Pored toga, trgovci koji su prodavali lekove i otrove nisu znali ništa o njihovom bezbednom čuvanju i transportu, što je predstavljalo dodatne poteškoće. Uvidevši da trgovci zbog profita prodaju sve i svašta, državne vlasti odlučile su da se sa ovim zloupotrebama mora prekinuti. Tako je u proleće 1845. godine doneta odluka da se sve radnje u Kneževini koje prodaju lekove, sirovine za lekove i otrove, detaljno pregledaju, i da se popišu svi farmaceutski preparati nađeni u njima. Ova naredba je predviđala da svako okružno načelstvo sastavi jednu komisiju koja bi se sastojala od okružnog lekara i policijskog činovnika, a njen zadatak je bio da vrši strogi nadzor nad trgovačkim radnjama u kojima su se prodavali lekovi. Naredba iz 1845. god. strogo je zabranjivala prodaju pilula, tinktura i ostalih lekova u trgovačkim radnjama, tako da je ceo posao vezan za trgovinu lekovima vrlo brzo bio prebačen na praviteljstvene apoteke koje su tih godina počele da se otvaraju u Srbiji [6].

Dolazak školovanih lekara u Srbiju

Kada se govori o razvoju zdravstvene kulture u Srbiji u prvoj polovini 19. veka, mora se istaći da najveće zasluge za to pripadaju Milošu Obrenoviću. U želji da zaostalu i nerazvijenu Srbiju izvede na put što bržeg napretka, knez Miloš je par godina posle završetka Prvog srpskog ustanka počeo da dovodi školovane lekare koji su svoje medicinsko obrazovanje sticali na zapadnoevropskim univerzitetima. Prvi školovani lekar koji je po pozivu kneza Miloša došao u Srbiju bio je Konstantin Aleksandridi. On je u Srbiju došao 1819. i postao lični lekar kneza Miloša. Nije se dugo zadržao u Srbiji pa je na njegovo mesto došao lekar Vittorio Romita rodom iz Italije. Romita je bio Napolitanac i član revolucionarnog karbonarskog udruženja koje se borilo za oslobođenje i ujedinjenje Italije. On je u Srbiji ostao do 1826. godine, kada je na njegovo mesto došao njegov zemljak dr Bartolomeo Kunibert, inače zet dr Romitija. Od stranih lekara koji su u to vreme boravili u Kneževini Srbiji treba pomenuti i dr Karla Paceka i dr Emerika Lindenmajera. Pacek, po narodnosti Slovak, rođen u Ugarskoj (Mađarskoj), u Srbiju je došao 1833. godine i bio je prvi načelnik saniteta Kneževine Srbije. Dr Lindenmajer je svoje medicinsko obrazovanje stekao na univerzitetima u Beču i Pešti, a 1835. godine došao je u Srbiju gde je bio lični lekar kneza Miloša i njegove porodice. Lindenmajer je učinio dosta na razvoju saniteta u Kneževini Srbiji. Organizovao je vojno sanitetsku službu, mobilisao je srpsku javnost u borbi protiv velikih boginja i kolere koje su u to vreme harale ovim prostorima, a kao šef saniteta uspeo je da od državnih vlasti izdejstvuje naredbu kojom je nadrilekarima i vidarima bilo zabranjeno da leče bolesnike. Lindenmajer izabran je za prvog šefa vojnog saniteta 1839. godine, a potom je od 1845–1859 obavljao funkciju načelnika i vojnog i civilnog saniteta [5]. Stabilizacija ekonomskih i političkih prilika u Srbiji stvorila je povoljnije uslove za razvitak zdravstva u zemlji, a samim tim i za obavljanje lekarske prakse. Tako već 1836. godine u Srbiju dolazi dr Herman Majnert iz Lajtmerica u Češkoj, zatim 1838. godine dr Đorđe Mušicki iz Đurđeva, kao i dr Josif Rebić iz Hrvatske, dr Karlo Beloni iz Ugarske, dr Konstantin Mihailović, dr Komnenović, dr Vartijades, dr Jovan Compo i drugi. Iako su lekari dobijali novac iz državne kase, nije postojao propis koji je precizno određivao način na koji su lekari primali svoje plate. Novčane nagrade su, uglavnom, bile različite i iznosile su od 200 do 500 talira u srebru i zavisile su od naklonosti koji su dotični lekari uživali od kneza Miloša [5]. Lekari koji su u to vreme dolazili u Srbiju stupali su, najčešće, u ličnu službu kod kneza Miloša i njegove porodice. Kako su uslovi za život i rad ovih lekara bili relativno loši, oni su se kratko zadržavali u Kneževini i brzo su se vraćali nazad u Austriju, odakle su, uglavnom, i dolazili [5].

Borba protiv epidemija

Sredinom tridesetih godina 19. veka u Srbiji se poklanja sve veća pažnja zaštiti narodnog zdravlja. Razlog za to se delimično nalazio i u strahu od zaraznih bolesti, uglavnom kolere i kuge koje su povremeno izbijale i ostavljale stravične posledice po stanovništvo. I dok se u Austriji i u ostalim zapadnim zemljama vodilo računa o zaraznim bolestima, u Turskoj je situacija bila potpuno drugačija. Usled odsustva ikakve svesti o opasnostima koje su nosile zarazne bolesti, kao i usled loših higijensko-sanitarnih uslova, bolesti su se nesmetano širile i desetkovale čitave oblasti [8]. Takva je bila epidemija kuge koja je zahvatila Bosnu, Hercegovinu, Srbiju, Staru Srbiju i Makedoniju, koja je bez prekida trajala od 1814. do 1816. godine. Lično se uverivši u stravične posledice ove epidemije, knez Miloš je rešio da učini sve kako bi Srbiju što bolje zaštitio od ovakvih epidemija. Kada je 1831. zavladala epidemija kolere u Mađarskoj, srpske vlasti su zatvorle granicu i uvele karantin, istovremeno naredivši „da se održava čistota i izbegava sve što može bolest raširiti". Sredinom 1836. godine u Bosni i Hercegovini je izbila velika epidemija kolere koja je i pored svih preduzetih mera zahvatila i Podrinje i zapadne krajeve Srbije. Smrtnost obolelog stanovništva bila je velika, tako da je samo u Loznici umiralo od 10 do 15 ljudi dnevno. Srpske vlasti su brzo reagovale. Po putevima su bile postavljene straže koje su držale u blokadi sva mesta pogođena epidemijom. Po naređenju Kneza Miloša u Podrinje je bio poslat dr Lindenmajer, a u Valjevo dr Nikolić. Mere usmerene na lokalizovanje epidemije u borbi protiv bolesti dale su rezultate tako da je krajem leta epidemija prestala [4]. Otprilike u vreme kada je epidemija kolere popustila, u Turskoj i oko srpsko-turske granice izbila je velika epidemija kuge koja je trajala od 1836. do 1838. Po na-

ređenju kneza Miloša podignuti su bili novi karantini u kojima su zadržavani svi putnici koji su iz Turske dolazili u Srbiju. U proleće 1837. godine podignute su poljske bolnice u Aleksincu (za Carigradski drum), u Radojevcu (prema Vidinu i Bugarskoj) i u Mokroj Gori, Ljuboviji i Rači (prema Staroj Srbiji i Bosni). Takođe, bio je stvoren poseban sanitarno-policijski kordon sa karantinima i rastelima, „sastancima" uz odgovarajući kontumatski period od 3 dana do 6 nedelja prema stepenu opasnosti. Duž cele granice bile su postavljene vojničke straže kojima su upravljali oficiri. Na svakom stražarskom mestu nalazila su se po 2–3 vojnika i isto toliko seljaka nad kojima je bio po jedan buljubaša [4]. Srbija je na ovaj način bila potpuno izolovana od Turske, tako da je njena granica brojnim šančevima bila omeđena, i prelaz iz Turske u Srbiju bio je moguć samo preko određenih mesta. To su bili: Aleksinac, Radojevac, Rača, Ljubovija, Mokra Gora, Vasilina česma, Pandiralo, Gramada, Vrška Čuka i Raška [8]. Međutim, i pored sprovedenih mera, epidemija kuge je, zahvaljujući nemaru turskih vlasti, u leto 1837. godine uspela da zahvati delove Srbije. Centar epidemije bio je u Jagodini, odakle se kuga raširila na Ražanj i Valjevo. Za sanitetskog nadzornika bio je postavljen dr Nađ koji je u cilju suzbijanja zaraze dobio široka ovlašćenja nad civilnim, vojnim i policijskim vlastima. Zahvaljujući velikom zalaganju dr Nađa, kao i angažovanju upravno-policijskih i vojnih vlasti, epidemija kuge bila je zaustavljena [6].

Razvoj medicinske biohemije

Ne želeći da srpsko zdravstvo zavisi od hirovitih austrijskih podanika duže nego što .je to potrebno, knez Miloš je početkom tridesetih godina 19. veka pokrenuo inicijativu koja je predviđala školovanje srpskih pitomaca na visokoobrazovnim medicinskim ustanovama u zapadnoj Evropi. Zanimljivo je da je novčanu pomoć srpskim pitomcima koji su u inostranstvu studirali medicinu, pored kneza Miloša, davao i njegov brat Jevrem. Razlozi za to ležali su u činjenici da je on bio slabog zdravlja zbog čega je želeo da ima svog ličnog lekara što je, u godinama kada je Srbija oskudevala u školovanim lekarima, bilo praktično nemoguće [6]. Sredinom tridesetih godina 19. veka novčanu pomoć od strane Srpskog praviteljstva primalo je nekoliko srpskih studenata koji su svoje obrazovanje sticali na medicinskim fakultetima u Beču i Pešti. To su bili: Jakov Užarević, Antonije Nedeljković, Aleksadar Budimirović, Pavle Ilić i Stefan Budimirović [3]. Pavle Ilić, student farmacije na Peštanskom univerzitetu, odmah se, po završetku svoga školovanja 1835. godine, shodno obavezama koje je imao prema srpskoj vladi, vratio u Srbiju. Odmah po dolasku u Kneževinu Ilić je po naređenju kneza Miloša osnovao prvu državnu apoteku u Kragujevcu [7].

Razvoj medicinske biohemije u Srbiji u 19. veku bio je vezan za apoteke. Tako se u Zakonu za apotekare iz 1865. godine, u članu 24 izričito navodi da su apotekari i njihovi zastupnici obavezni da vrše svaku hemijsku analizu koju od njih zatraže državni organi ili privatna lica [9]. Hemijske analize, obavljane u to vreme, uglavnom su se odnosile na laboratorijska ispitivanja alkoholnog pića, mineralne vode, lekova, olova, baruta. Svaka apoteka bila je dužna da ima svoj „laboratorijum" koji je morao da bude „dovoljno prostran i vidan". Od pribora i aparata koji su se koristili u ovim laboratorijama „bile su obavezne: furune za kuvanje; astal za labortisanje sa potrebnim posuđem i spravama; numeričke sprave za destilisanje i razgrađivanje, ceđenje (filtriranje), topljenje, varenje, presovanje, merenje, ispitivanje i analiziranje čistoće lekova; avani za tucanje; gvozdena i kamena sita raznih veličina česti i ređi, i platna za alkoholiziranje za razne potrebe" [10]. Početkom sedamdesetih godina 19. veka širom Kneževine počela se koristiti i stručna medicinska literatura koja je uglavnom dolazila iz Austrije. Od knjiga koje su u to vreme mogle da se nađu kod srpskih farmaceuta i lekara najprisutnija je bila studija „Hemijske analize" od Vila [11]. Otvaranjem Praviteljstvene apoteke u Kragujevcu, Pavle Ilić kao knjaževski apotekar bio je finansiran od strane srpske državne uprave. Kako je apoteka u Kragujevcu počela sa radom u poslednjoj dekadi 1835. godine, Ilić je od srpske vlade dobio skromnu nadoknadu u iznosu od 300 čaršijskih groša [12]. Naredne 1836. godine za platu državnog apotekara Pavla Ilića bila je određena suma od 1 200 čaršijskih groša [13], dok je 1837. godine za svoj rad Pavle Ilić bio nagrađen sa 3 000 poreskih groša [14]. Te iste 1837. odlukom Državnog saveta bilo je rešeno da se knjaževskom apotekaru Pavlu Iliću dodele dva pomoćnika i to: jedan praktikant (Đoka Bogdanović) i jedan laborant (Đorđe Ristić). Oni su za svoje angažovanje bili nagrađeni godišnjom platom od 720, odnosno 660 poreskih groša [15]. Kako su ova novčana primanja bila i više nego skromna, odlukom Državnog Saveta iz 1846. godine rešeno je da se godišnje plate pomoćnika u državnoj apoteci uvećaju na sumu od 72 talira [16].

Početkom 1849. godine srpska vlada donela je odluku kojom je preuređen rad Praviteljstvene apoteke u Kragujevcu. Tada je rešeno da se za pomoćnike Pavla Ilića postave tri nova laboranta od kojih bi najmanje jedan „morao znati čitati i pisati srpski, a od časti latinski i nemački" [17]. Laboranti su sklapali ugovor na pet godina. Prve dve godine službe njihova godišnja plata iznosila je 72 talira da bi se, potom, po odluci vlade, njihova plata povećala na 100 talira. Po završetku četvrte godine službe, oni su morali da polažu „ispit o naučenom zanatu" koji bi im, ukoliko bi ga sa uspehom završili, stvorio mogućnost da ponovo budu birani u državnu službu [18].

Krajem 1856. godine apotekar Pavle Ilić uputio je vladi predlog za osnivanje jedne posebne hemijske državne laboratorije. Tom prilikom Ilić je predložio da Srbija krene putem razvijenih zapadnoevropskih zemalja i iškoluje nekoliko pitomaca koji bi se specijalizovali za izučavanje analitičke hemije. Ovi pitomci, po preporuci Pavla Ilića, trebalo je najpre da završe opšte obrazovanje u Srbiji, zatim neophodno medicinsko obrazovanje, a potom stručno usavršavanje na univerzitetu u Berlinu ili Minhenu [19]. Kako srpska Vlada nije bila zainteresovana za realizaciju Ilićeve ideje, otvaranje državne hemijske laboratorije moralo je da pričeka do septembra 1859. godine, kada je Ministarstvo unutrašnjih poslova pokrenulo inicijativu da se u Sanitetskom odeljenju pri Ministarstvu policije ustanovi hemijska laboratorija kojom bi upravljao državni hemičar [20]. Knez Miloš Obrenović uvažio je ovaj predlog, pa je početkom novembra 1859. godine Držav-

na hemijska laboratorija otpočela sa radom. Za prvog upravitelja ove laboratorije bio je postavljen Pavle Ilić čija je plata iznosila 600 talira godišnje [21]. U Državnoj hemijskoj laboratoriji, pored hemijsko-toksikoloških, obavljale su se i pojedine medicinsko-biohemijske analize (npr. krvi, mokraće i pljuvačke). Pavle Ilić nalazio se na čelu Državne hemijske laboratorije sve do 1871. godine, kada je preminuo [9].

Zaključak

Stabilizacija političkih i privrednih prilika u Kneževini Srbiji stvorila je pogodan teren za unapređenje i razvitak saniteta. I pored zdravstvene neprosvećenosti, u prvim godinama posle Prvog srpskog ustanka, knez Miloš uspeo je da podigne nivo zdravstvene kulture, u čemu je imao veliku pomoć od stranih lekara koji su u to vreme boravili u Srbiji. U godinama koje su usledile srpska vlada, uprkos mnogobrojnim problemima, uspela je da značajno popravi zdravstvene prilike u zemlji i da stvori obrazovan i sposoban medicinski kadar, koji je udario temelje srpskom sanitetu. Zdravstvene prilike u Srbiji značajno su bile unapređene donošenjem Zakona o sanitetu, 1881. godine, koji je zdravstvo u Srbiji u pojedinim segmentima izjednačio sa sanitetima razvijenih zapadnoevropskih zemalja.

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SERBIAN DIASPORA MEDICAL CONFERENCE 2011

at The White Palace
22 - 24 September 2011

HRH Crown Princess Katherine Foundation and Lifeline Humanitarian Organization in partnership with the Ministry of Health of the Republic of Serbia, Ministry for Diaspora of Republic of Serbia, Faculty of Medicine University of Belgrade, Serbian Medical Association, World Health Organization, Medical Board of Lifeline Humanitarian Organization Chicago, and under the patronage of Their Royal Highnesses Crown Prince Alexander II and Crown Princess Katherine, will organize an important "Serbian Diaspora Medical Conference 2011" at the White Palace, Belgrade, 22-24 September 2011.

This Conference is a continuation of our Conference last year and we would like to invite you to come and share your academic and professional experience with our medical doctors in Serbia. One of the concepts behind the Conference is to establish a network between the medical staff of Serbia and Diaspora.

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HRH Crown Princess Katherine Foundation

11 July 2011

Dear Doctor,

It is a pleasure to inform you that HRH Crown Princess Katherine Foundation and Lifeline Humanitarian Organization in partnership with the Ministry of Health of the Republic of Serbia, Ministry for Diaspora of Republic of Serbia, Faculty of Medicine University of Belgrade, Serbian Medical Association, World Health Organization, Medical Board of Lifeline Humanitarian Organization Chicago, and under the patronage of Their Royal Highnesses Crown Prince Alexander II and Crown Princess Katherine, will organize an important "Serbian Diaspora Medical Conference 2011" at the White Palace, Belgrade, 22-24 September 2011.

We would like to invite you to come and share your academic and professional experience with our medical doctors in Serbia. With your support the medical doctors in Serbia will have the opportunity to share your knowledge and experience with their colleagues, which will benefit the people of Serbia. We thank you in advance for your participation in this Conference and sincerely hope in achieving mutual success. The main idea behind the Conference is to establish a network between the medical staff of Serbia and Diaspora, and as a continuation of our conference last year.

Please fill in the attached form and include a short biography (up to 350 words) and send it as soon as possible to sdmc@pkfond.rs or through the web site www.serbiandiasporamedical.rs. The deadline is 7 September 2011.

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We look forward to hearing from you and to seeing you in September.

Yours sincerely,

Dusan Babac

Director
HRH Crown Princess Katherine Foundation

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Examples of references:

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

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Type each table double-spaced on a separate sheet. Number tables consecutively in the order of their first citation in the text in the upper right corner **(Table 1)** and supply a brief title for each. Place explanatory matter in footnotes, using the following symbols, in this sequence: ***, †, ‡, §, ||, ¶, **, ††, ...** Each table has to be mentioned in the text. If you use data from another source, acknowledge fully.

Illustrations

Figures are submitted in triplicate, and for the final version also on diskette/CD. Photos should be sharp, glossy black and white photographic prints, not larger than 203 × 254 mm. Letters, numbers, and symbols should be clear and even throughout and of sufficient size that when reduced for publication, each item will still be legible. Each figure should have a label on its back indicating the number of the figure, author's name, and top of the figure. If a figure has been published, acknowledge the original source.

Legends for illustrations are typed on a separate page, with arabic numerals corresponding to the illustrations. Identify and explain each one clearly in the legend symbols, arrows, numbers, or letters used to identify parts of the illustrations. Explain the internal scale and identify the method of staining in photomicrographs.

Abbreviations and symbols

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

Detailed Instructions are available at the web site: **www.vma.mod.gov.rs/vsp/download/instructions_to_authors.pdf.**

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