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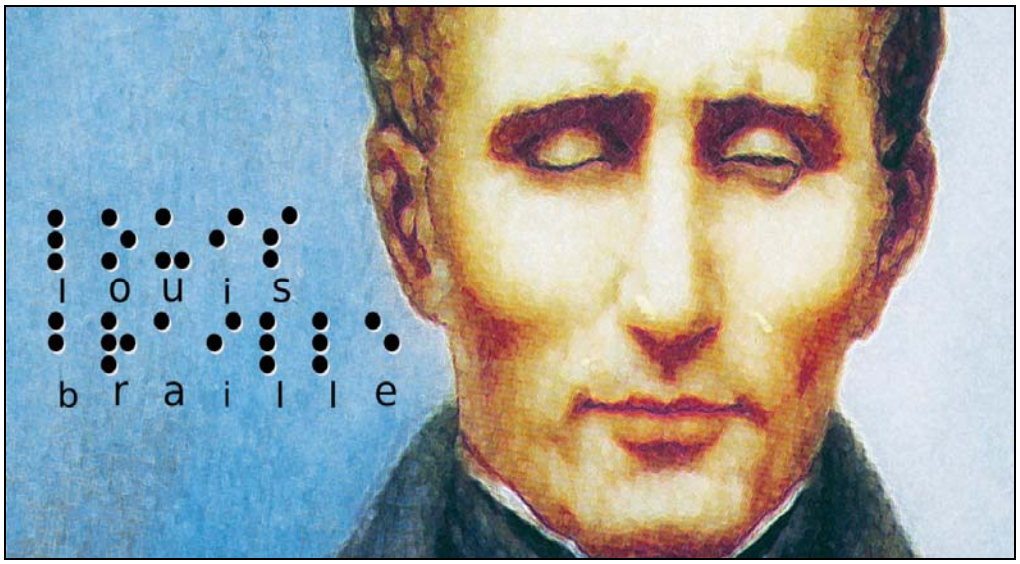
Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2016; January Vol. 73 (No. 1): p. 1–124.



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VOJNOSANITETSKI PREGLED

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

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

IZDAVAČ
Uprava za vojno zdravstvo MO Srbije

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The Journal continues the tradition of *Vojno-sanitetski glasnik* which was published between 1930 and 1941

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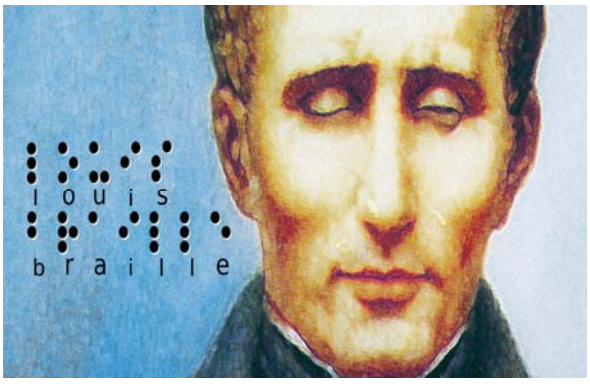

Louis Braille (January 4, 1809 – January 6, 1852), inventor of the reading and writing system (Braille alphabet) that is used by millions of blind and partially sighted people all over the globe providing them with access to the same learning opportunities as the sighted and improving their quality of life.
In gratitude, his birthday (January 4) is celebrated as World Braille Day.

Luj Braj (4. januar 1809 – 6. januar 1852), tvorac sistema za čitanje i pisanje (tzv. Brajov alfabet ili Brajovo pismo) koji koriste milioni slepih i slobovidih osoba širom sveta. Zahvaljujući ovom izumu i te osobe imaju iste mogućnosti za učenje i sticanje znanja kao i osobe koje vide, što im obezbeđuje kvalitetniji i potpuniji život.
U znak zahvalnosti, njegov rođendan (4. januar) proslavlja se kao Svetski dan Brajovog pisma.

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

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We take a look back to make a more successful step forward

Pogled unatrag za uspešniji korak napred

Silva Dobrić

Institute for Scientific Information, Military Medical Academy, Belgrade, Serbia

A new calendar year usually starts with reviewing the work done in the previous year to get the basis for designing a plan of activities to be realised in the approaching time period. This practice has been applied for many years by the Editorial Staff of the Military Medical Journal (Vojnosanitetski pregled, VSP) in order to improve any procedures for receiving scientific papers, their editing, publishing, and, consequently, get a better quality of the Journal. In so doing, we put a special accent on the quality analysis of articles submitted to be published, actualness of their topics, plagiarism/self-plagiarism, authors, institutions papers come from, time taken for peer reviewing, and, in the end, on the dynamics of publishing papers that go through peer reading well and are accepted for publishing.

Considering the abovementioned, then, what was the year 2015 like?

Within 2015, to December 25 inclusive, a total of 400 articles were submitted to the Editorial Office, which is a record number, since earlier, more precisely from 2010, the year when the VSP got its first impact factor (IF), that number used to range from 250 to 350. We suppose that the reason for increasing interest of authors to publish their scholarly papers in the VSP lies in the constant increase in the impact factor value (starting from 0.199 in 2010, 0.179 in 2011, 0.21 in 2012, 0.269 in 2013, and 0.292 in 2014), but also in the fact that for the last two years some papers have been published ahead of print, Online First, having their DOI number, making them accessible and known to scientific community. It means much to authors whose advancement in professional and academic career depends on the number of published papers.

Out of the submitted papers in 2015, a total of 128 were rejected (for not complying with technical requirements of the Journal, unsuitable themes or because being rejected by peer reviewers), 151 successfully went through peer reviewing and accepted for publishing. The remaining 121 scholarly works are under peer reviewing currently, of which 26 have already been sent back to the authors to correct them according to the comments of the peer reviewers). The majority

Uobičajeno je da se na početku svake kalendarske godine izvrši analiza rada u prethodnoj godini koja treba da posluži kao osnova za izradu plana aktivnosti za realizovanje u predstojećem periodu. Već duži niz godina ovu praksu primenjujemo i u radu Redakcije „Vojnosanitetskog pregleda“ (VSP) sa ciljem unapređenja svih aktivnosti oko prijema radova, njihove obrade i objavljivanja, kao i poboljšanja kvaliteta samog časopisa. Pri tome, poseban akcenat stavlja se na analizu kvaliteta pristiglih rukopisa za objavljivanje, aktuelnosti tema koje se u njima obrađuju, pojavama plagijarizma/autoplagijarizma, autorima, odnosno ustanovama iz kojih radovi dolaze, dužini trajanja recenzentskog postupka i, konačno, dinamici objavljivanja radova koji uspešno završe proces recenzije i dobiju saglasnost za objavljivanje.

U skladu sa navedenim, kakva je, dakle, bila 2015. godina?

Tokom 2015. godine (zaključno sa 25. decembrom) u Redakciju VSP-a stiglo je 400 radova, što predstavlja svojevrsni rekord, budući da se u proteklim godinama, tačnije od 2010. godine, kada je VSP dobio svoj prvi impakt faktor (IF), broj pristiglih radova kretao u rasponu od oko 250 do 350. Razlog ovako velikog interesovanja autora da svoje radove ponude za objavljivanje našem časopisu, svakako treba tražiti u konstantnom rastu vrednosti njegovog IF (0,199 za 2010; 0,179 za 2011; 0,21 za 2012; 0,269 za 2013. i 0,292 za 2014), ali i činjenici da se već dve godine, pre objavljivanja u štampanom obliku, pojedini radovi objavljuju elektronski kao Online First sa DOI brojem, čime postaju dostupni stručnoj i naučnoj javnosti, što autorima, kojima napredak u profesionalnoj i akademskoj karijeri zavisi od broja objavljenih radova, i te kako, mnogo znači.

Od pristiglih radova u toku 2015. godine, odbijeno je njih 128 (zbog tehničke neusklađenosti sa zahtevima časopisa, neodgovarajuće teme ili mišljenja recenzenata), 151 rad je uspešno prošao recenzentski postupak i dobio saglasnost za objavljivanje, dok se 121 rad trenutno nalazi u postupku recenziranja (od ovog broja 26 radova je već vraćeno autorima na korekcije prema sugestijama recenzenta). Najveći broj pristiglih radova (preko 60%), kao i prethodnih godina, pripada kategoriji Originalnih članaka, slede Prikazi slučajeva (oko 30%), zatim opšti pregledi i aktuelne teme (do 10%), dok se na poslednjem mestu nalaze radovi iz kategorija

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(more than 60%) of the received papers, as in previous years befall to the category of Original articles, followed by Case reports (abot 30%), then General reviews and Current topics (up to 10%), while the least of them (1-2%) are papers from the category of Short communication, Practical advice for physicians, History of medicine, Letter to the Editor, comments and similar.

Analysis of the received papers as *per* institutions the authors come from shows that the majority (almost 75%) of those papers come from civil academic and scientific institutions, including more than 8% of papers from abroad, primarily from the region (Montenegro, Macedonia, Croatia, Bosnia and Hertzegovina, and Slovenia), and the rest from military medical institutions, mainly from the Military Medical Academy. These data greatly coincide with those from the previous years.

For the past few years it has been our practice to check each paper to plagiarism/self-plagiarism by the use of the CrossCheck Service and its software iThenticate. No paper had that level of coincidence in its major parts with a paper or papers already published to be declared as plagiarism/self-plagiarism within the last year. That is partly because of the notice in the Instructions to the Authors for Preparation Papers for Publishing in the VSP on checking any papers to plagiarism, as well as on imposing a sanction on authors with such dishonest behaviour, if found out.

Analysis of papers published in 2015 shows that a total of 185 papers were published in 12 numbers of the VSP, including 4 book reviews (Table 1) which mainly complies with the number of papers published in the previous years.

seminar praktičnog lekara, istorija medicine, pismo uredniku, komentari i slično, koji čine 1–2% svih pristiglih radova.

Analiza pristiglih radova prema institucijama njihovih autora pokazuje da su najveći broj ovih radova, gotovo 75%, poslali autori iz tzv. civilnih akademskih i naučnih institucija uključujući i nešto više od 8% radova pristiglih od autora iz inostranstva, u prvom redu iz zemalja regiona (Crna Gora, Makedonija, Hrvatska, Bosna i Hercegovina i Slovenija), dok ostatak otpada na radove autora iz vojnozdravstvenih ustanova, u prvom redu iz Vojnomedicinske akademije. Navedeni podaci, uglavnom odgovaraju onima iz prethodnih godina.

Već par godina, svaki pristigli rad prolazi proveru na plagijarizam/autoplagijarizam uz korišćenje sistema *CrossCheck Service* i njegovog softvera *iThenticate*. Od pristiglih radova tokom protekle godine ni kod jednog nije ustanovljen toliki stepen podudarnosti ključnih delova rada sa nekim već objavljenim radom ili radovima da bi se rad proglasio (auto)plagijatom. Ovome, svakako, doprinosi upozorenje, navedeno u Uputstvu o pripremi rukopisa za objavljivanje u VSP- u, o proveri svih podnetih radova na plagijarizam, kao i o načinu sankcionisanja autora takvih nečasnih postupaka, ukoliko se otkriju.

Analiza objavljenih radova u 2015. godini pokazuje da je u 12 brojeva prošle godine objavljeno ukupno 185 radova, uključujući i 4 prikaza knjiga (Tabela 1), što je, u najvećoj meri, u saglasnosti sa brojem objavljenih radova i prethodnih nekoliko godina.

Ponovo, kao i ranije, najveći deo pripada kategoriji Originalni članci (53%) i Prikazi slučajeva (29,2%), što odgovara zastupljenosti ovih kategorija i među primljenim radovima, ne samo u toku protekle, nego i svih prethodnih godina.

Table 1

Categories and the number of articles published in the Vojnosanitetski Pregled in 2015/ Kategorije i broj članaka objavljenih u Vojnosanitetskom pregledu u 2015.

Category / Kategorija	Articles/ Članci	
	n	%
Editorial/ Uvodnik	6	3.2
Original Article/ Originalni članak	98	53
General Review/ Opšti pregled	9	5
Current Topic/ Aktuelna tema	3	1.6
Case Report/ Prikaz slučaja	54	29.2
Preliminary Report/ Prethodno saopštenje	1	0.5
Short Communication/ Kratko saopštenje	4	2.2
Letter to the Editor/Pismo uredniku	2	1
Book Review/ Prikaz knjige	4	2.2
In focus/U fokusu	3	1.6
Personal opinion/Lični stav	1	0.5
Total/ Ukupno	185	100.0

Again, the majority of those papres were Original articles (53%), Case reports (29.2%), corresponding to the frequency of those categories among the received papers, not only in the last year, but in all previous years, as well. It has to be pointed out that in 2015, together with printing, a high number of papers were e-published having DOI number (Online First papers) which were later on printed in the monthly Journal according to the contents of printing issues. At the time of writing this Editorial, namely the end of December, the site of our Journal already had Online First

Treba naglasiti da je tokom 2015. godine, paralelno s objavljivanjem štampanih brojeva časopisa, objavljen i velik broj radova u elektronskom obliku sa DOI brojem, znatno veći nego prethodnih godina (tzv. *Online First* radovi) koji će potom, prema usvojenim sadržajima za štampana izdanja, biti prebačeni u redovne štampane brojeve časopisa. U vreme pisanja ovog Uvodnika (kraj decembra 2015. godine), na sajtu časopisa, kao *Online First* radovi, već su postavljeni članci koji će ući u prva tri broja VSP-a u 2016. godini. Plan Redakcije časopisa jeste da se u narednoj godini još veći broj radova koji dobiju saglasnost za

published papers that will be printed in the first three issues of the 2016 VSP. The Editorial Office plans to make even a higher number of accepted papers accessible in this way to professional and scientific circles and, thus, to maximally cut the time of printing papers counting from the moment of their acceptance for publishing.

When we analyse papers printed in 2015 by the institutions their authors come from, again civil institutions prevail (53%), that together with foreign authors makes 65% out of the total number of papers printed in the VSP in 2015. Papers by the authors from military medical institutions make 16%, and those written in cooperation of authors from military and civil institutions, including those collaborators from abroad make 20%. These data, especially those applicable to foreign authors, are favorable, showing increasing affirmation of the VSP as international journal. That is also implied indirectly by the number of accesses to full texts of papers published in the VSP among the users of EBSCO database. There were almost 16,000 accesses to papers in the VSP only in the first quarter of the year, that is more than 5,000 *per* month, or 175 daily. The highest number of those accesses were made abroad (Spain, USA, China, Canada, Turkey).

There is something, however, we are not quite satisfied with yet. It is the time taken for peer reviewing, which somethimes lasts for months. In some cases it is due to the need for extensive revision of manuscripts, but there are instances when peer reviewers do not submit their opinion in the scheduled term, resulting in a few months waiting for final decision. The majority of peer reviews, however, do this significant job highly responsibly, thus, I want to thank to them in my own and in the name of the whole Editorial Staff eager to go on together in 2016.

Table 2 lists the names of peer reviewers involved in peer reviewing process of papers for publishing in the VSP in 2015.

objavljivanje, na ovaj način učini dostupnim za korišćenje stručnim i naučnim krugovima, i da se time maksimalno skrati vreme od trenutka kada rad dobije saglasnost za objavljivanje do trenutka samog objavljivanja.

Kada se radovi objavljeni u štampanom obliku tokom 2015. godine analiziraju s obzirom na institucije njihovih autora, ponovo dobijamo prevagu domaćih civilnih institucija (53%), što zajedno sa radovima autora iz inostranstva čini oko 65% od ukupnog broja objavljenih radova u štampanom izdanju VSP-a u 2015. godini. Radovi čiji autori dolaze iz institucija vojnog saniteta čine 16%, a oni koji su proizvod zajedničkog rada autora iz vojnih i civilnih institucija, uključujući autore-saradnike iz inostranstva, 20% objavljenih radova. Ovi podaci, pogotovo oni koji se odnose na radove inostranih autora, posebno raduju jer govore o sve većoj afirmaciji VSP-a kao međunarodnog časoposa. O ovome posredno govore i podaci o broju pristupa člancima objavljenim u VSP-u korisnika baze EBSCO, preko koje se ti radovi mogu dobiti u punom tekstu. Samo u prvom kvartalu 2015. godine zabeleženo je gotovo 16 000 pristupa člancima objavljenim u našem časopisu, što iznosi više od 5 000 mesečno, odnosno 175 pristupa dnevno. Pri tome, najveći broj pristupa ostvaren je iz inostranstva (Španija, SAD, Kina, Kanada i Turska).

Ono sa čime još uvek nismo u potpunosti zadovoljni jeste dužina trajanja recenzentskog postupka, koja ponekad iznosi i nekoliko meseci. U pojedinim slučajevima razlog za to je i potreba za većim revizijama rukopisa prema zahtevima recenzenata, ali ima i slučajeva kada recenzenti, iako se prihvate obaveze recenziranja, ne dostave svoje mišljenje u predviđenom roku, što uslovaljava ponekad i višemesečno čekanje na konačnu odluku o (ne)prihvatanju rada. Ipak, većina recenzenata obavlja veoma savesno svoj, za časopis izuzetno značajan posao, i na tome im se u ime Redakcije VSP-a najtoplije zahvaljujem uz nadu da ćemo nastaviti uspešnu saradnju i u 2016. godini.

Imena recenzenata koji su u 2015. godini bili angažovani na recenziranju radova za VSP, navedena su u Tabeli 2.

Table 2

Reviewers of the Vojnosanitetski pregled in 2015 / Recenzenti Vojnosanitetskog pregleda u 2015. godini

Aćimović Slobodan
Adler Isabel
Ajdinović Boris
Aleksić Petar
Alimpijević Tamara
Alma MA
Angelkov Lazar
Antonijević Biljana
Antonijević Nebojša
Argirović Đorđe
Arsenijević Nebojša
Arsić Ivana
Arsović Nenad

Baletić Nenad
Bančević Vladimir
Beleslin Branko
Berisavac Milica
Bezmarević Mihajlo
Bjegović Mikanović Vesna
Bokonjić Dubravko
Brdareski Zorica
Brkić Zlata
Bulat Petar
Bumbaširević Marko

Campa Claudio
Cakmak Altug Huseyin
Carević Momir
Chaer Rabih
Chattopadhyay Saurabh
Chunming Lu
Conte Michael
Cornejo Marco

Čekanac Radovan
Čolić Miodrag
Čurčić Marjana
Čurčić Mirjana
Čvorović Ljiljana
Ćirić Zoran
Ćuk Vladimir

Daković Dragana
Dankuc Dragan
Davidović Lazar
Dedić Gordana
Demeši Drljan Čila
Dimitrijević Brana
Dimitrijević Jovan
Dobrić Silva
Doder Radoje
Dragičević Danijela
Dragojević Simić Viktorija
Dragović Tamara
Džamonja Tamara

Đinđić Boris
Đorđević Brižita
Đorđević Snežana
Đorđević Sofija
Đukanović Ljubica
Đukić Mirjana
Đurić Tatjana
Đurović Aleksandar
Đurović Branka
Đurović Branislav

Filipović Branislav
Freeborn Donna

Ganek H
Glišić Branislava
Grdinić Aleksandra
Grubor Nikica
Gupta Dheeraj
Gvozdenović Ranko

Howell Peter

Ignjatović Mile
Ilić Dragan
Ilić Tihomir
Iliyas Sheikh Mohammed
Ivanović Mirjana
Ivanović Vladimir

Jakšić Vesna
Janković Slavenka
Janković Slobodan
Jauković Ljiljana
Jelić Svetlana
Jeremić Zoran
Jokić Radoica
Jonjić Danijela
Jovanović Dragan
Jovanović Dragana
Jovanović Ida
Jović Jasna
Jović Nebojša
Jović Stošić Jasmina
Jurišić Škevin Aleksandra

Kamp Timothy
Kisić Tepavčević Darja
Kjelland Vivian
Končar Igor
Konstantinović Vitomir
Koraćević Goran
Kostoglov-Athanassiov Ifigenia
Kostov Miloš
Kozarski Jefta
Kozomara Ružica
Kravljanac Ružica
Krivokapić Zoran
Kuburović Nina
Kukić Marković Jelena

Laaser Ulrich
Labudović Borović Milica
Lakić Aneta
Latas Milan
Lazić Miodrag
Lazić Srđan
Lazić Zoran
Lee Jea Seung
Lečić Toševski Dušica
Lepić Toplica
Lepšanović Zorica
Lippman Steven
Lobbezoo Frank

Ljubić Aleksandar

Magić Zvonko
Mahmoudi Zeinab
Maksić Đoko
Mandić Gajić Gordana
Manojlović Nebojša
Manolopoulos G. Vangelis
Marić Nađa
Marjanović Ivan
Marjanović Marjan
Marjanović Slobodan
Marković Dejan
Martić Vesna
Matijević Stevo
Matović Vesna
Medenica Ivan
Micić Dragan
Micić Sava
Mihaljevic Biljana
Mijušković Željko
Mijušković Zoran
Mikić Dragan
Mikov Aleksandra
Milenković Marina
Milenković Saša
Milenković Svetislav
Mileusnić Dušan
Milojević Milanko
Milovanović Dragan
Mirković Darko
Mirković Ljiljana
Mirović Veljko
Mitsnefes Mark
Mladenović Zorica
Mohite M. Prakash
Mulić Rosanda

Nagorni Obradović Ljudmila
Nedok Aleksandar
Nenadić Dane
Nešković Vojislava
Neubauer David
Nežić Duško
Nikolić Branka
Nikolić Gordana
Nikolić Ljubiša
Ninković Milica
Nittis Maria
Novaković Marjan
Nožić Darko

Obradović Dragana
Obradović Slobodan
Osborn David

Paović Anka
Pavlović Miloš
Pavlović Milorad
Peco-Antić Amira
Perić Aleksandar
Perić Predrag
Perišić Živko
Petronijević Milan
Petrović Đorđe
Pieruzzi Paola
Plavšić Ljiljana
Popov Boris
Popović Miroljub
Popović Zoran
Potpara Tatjana
Proročić Milenko
Provenzani Alessio

Rabih Chaer
Rabrenović Milorad
Radak Đorđe
Radaković Sonja
Radcliffe M. Nathan
Radovanović Saša
Rađen Slavica
Radojčić Ljiljana
Radosavljević Vladan
Raičević Ranko
Resan Mirko
Ristanović Elizabeta
Ristić Anđelka
Ristić Branko
Ristić Ljubiša
Roganović Zoran
Romić Predrag
Rozman Damjana
Ruiz-Irastorza Guillermo

Sabo Ana
Savić Miroslav
Savić Slobodan
Sečen Nevena
Sekulović Kandolf Lidija
Sekulović Leposava
Seung Lee Jea
Shafaq Saleem
Shroff Rukshana
Simić Snežana
Slavković Slobodan
Soto-Aviles Omar
Spasić Slavica
Stamatović Dragana
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Vacuum-assisted wound closure in vascular surgery – clinical and cost benefits in a developing country

Zarastanje rane pomoću vakuuma u vaskularnoj hirurgiji – klinička i ekonomska korist u zemlji u razvoju

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Abstract

Background/Aim. Surgical and chronic wounds in vascular patients might contribute to limb loss and death. Vacuum-assisted closure (VAC) – Kinetic Concepts, Inc. (KCI), has been increasingly used in Western Europe and the USA clinical practice for 15 years. Advantages of this method are faster wound healing, wound approximation, lower wound related treatment costs and improved quality of life during treatment. Evidence related to the usage of VAC therapy in vascular patients and cost effectiveness of VAC therapy in a developing country are lacking. The aim of this study was to explore results of VAC therapy in vascular surgery comparing to conventional methods and to test cost effects in a developing country like Serbia. **Methods.** All patients with wound infection or dehiscence operated at the tertiary vascular university clinic in the period from January 2011 – January 2012, were treated with VAC therapy. The primary endpoint was wound closure, while secondary endpoints were hospital stay, the number of weekly dressings, costs of wound care, working time of medical personnel. The patients were divided into groups according to the wound type and location: wound with exposed synthetic vascular implant (25%), laparotomy (13%), foot amputation (29%), major limb amputation (21%), fasciotomy (13%). The results of primary and secondary endpoint were compared with the results of conventional treatment during the previous year. **Results.** There was one death (1/42, 2.38%) and one limb loss (1/12, 2.38%) in the VAC group, and 8 deaths (8/38, 21.05%) and 5 (5/38, 13.15%) limb losses in the patients treated with conventional therapy. In the VAC group there was one groin bleeding (1/12, 2.38%), one groin reinfection (1/12, 2.38%) and one resistance to therapy with a consequent limb loss. Costs of hospital stay ($p < 0.001$) and nursing time ($p < 0.001$) were reduced with VAC therapy in the group with exposed graft. **Conclusion.** VAC therapy is the effective method for care of complicated wounds in vascular surgery. Patients with infection of wound with the exposed synthetic graft significantly benefit form this therapy. Cost effectiveness of VAC therapy is applicable to a developing country scenario, however cautious selection of patients contributes to the effectiveness.

Key words:
surgical wound infection; surgical wound dehiscence; vascular surgical procedures; vacuum; socioeconomic factors; treatment outcome.

Apstrakt

Uvod/Cilj. Rane kod vaskularnih bolesnika, kao i komplikacije hirurških rana nakon vaskularnih procedura doprinose gubitku ekstremiteta i smrtnom ishodu. Zatvaranje rane vakuumom (*vacuum-assisted closure* – VAC) je metoda koja se sve više koristi u zapadnoj Evropi i Americi u poslednjih 15 godina. Prednosti ove metode su brže zarastanje rana, približavanje ivica rane, manji troškovi lečenja vezani za ranu i bolji kvalitet života bolesnika tokom lečenja. Dokazi koji se odnose na upotrebu VAC terapije kod vaskularnih bolesnika i njihovi ekonomski efekti u zemlji u razvoju do sada nisu objavljeni. Cilj ove studije bio je da se uporede rezultati lečenja metodom VAC kod vaskularnih bolesnika u odnosu na dosadašnje metode, kao i da se ispita isplativost ove terapije u specifičnim ekonomskim uslovima u zemlji u razvoju kao što je Srbija. **Metode.** Svi bolesnici sa infekcijom ili dehiscencijom hirurške rane, operisani u tercijalnoj vaskularnoj ustanovi u periodu januar 2011 – januar 2012, lečeni su metodom VAC. Primarni cilj bio je zatvaranje rane, dok su sekundarni ciljevi bili da se utvrdi dužina hospitalizacije, broj previjanja, cena lečenja rane, radno vreme medicinskog osoblja potrošeno za tretman rane. Bolesnici su bili podeljeni u 5 grupa prema tipu lečene rane i njenoj lokaciji: rana sa eksponiranim veštačkim krvnim sudom (25%); laparotomna rana (13%); rana nakon amputacije stopala (29%); rana nakon amputacije ekstremiteta (21%); fasciotomna rana (13%). Rezultati su poređeni sa rezultatima lečenja

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konvencionalnim metodama previjanja i lečenja rana kod bolesnika istih grupa lečenih u 2010. godini. **Rezultati.** U grupi bolesnika lečenih terapijom VAC jedan bolesnik je preminuo (1/42, 2,38%) i jedan je izgubio ekstremitet (1/12, 2,38%), dok je u prethodnoj godini preminulo osam bolesnika (8/38, 21,05%), a pet bolesnika (5/38, 13,15%) je izgubilo ekstremitet tokom lečenja konvencionalnim metodama. U grupi bolesnika lečenih metodom VAC zabeleženo je jedno krvarenje u preponi (1/12, 2.38%), jedna reinfekcija u preponi (1/12, 2,38%) i kod jednog bolesnika je infekcija bila rezistentna na terapiju, što je zahtevalo ekstirpaciju grafta i amputaciju noge. Cena lečenja i vreme koje je medicinsko osoblje provelo u lečenju i nezi rane bili su značajno manji u grupi bolesnika sa eksponiranim veštačkim krvnim sudom tretiranih terapijom VAC. **Zaključak.** Zatvaranje rane metodom VAC je efikasna terapija za lečenje komplikovanih rana kod vaskularnih bolesnika. Bolesnici sa infekcijom rane u kojoj je eksponiran veštački krvni sud imaju značajnu korist od ove metode. Isplativost terapije VAC opravdava primenu i u zemljama u razvoju, ali je pažljiv izbor bolesnika jedini način da se ta ekonomičnost zadrži i poboljša.

Ključne reči:
rana, hirurška, infekcija; rana, hirurška, dehiscencija; hirurgija, vaskularna, procedure; vakuum; socioekonomski faktori; lečenje, ishod.

Introduction

Chronic wounds are great burden to the health care system in every country. Vascular patients with peripheral arterial or vein insufficiency are frequently diagnosed with already developed chronic wounds. On the other side vascular procedures might be complicated with different wound complications. Most complicated wounds are related to usage of synthetic material, infected or dehisced laparotomy after complex procedures, extensive amputation in the malperfused area or advanced foot infections. Vacuum-assisted closure (VAC) – Kinetic Concepts. Inc. (KCI) has been increasingly used in the developed countries since 15 years ago for treatment of different wounds [1–3]. The Serbian National Agency for Drugs and Medical Devices registered this method in 2009. Reported advantages of VAC therapy are related to general and gastrointestinal surgery; plastic and reconstructive surgery burns, trauma and cardiac surgery reporting faster recovery, granulation, and shorter hospital stay with lower costs of the therapy. Cost effects in countries with lower gross domestic product (GDP) might be different due to notably lower costs of hospital stay. Publications related to the usage of VAC in specific vascular patients are lacking as well as cost analysis related to the scenario of a developing country.

The aim of our study was to explore the results of VAC therapy in vascular surgery comparing to conventional methods and to test cost effects of this method in a developing country like Serbia.

Methods

We prospectively followed patients operated at the Clinic for Vascular and Endovascular Surgery of the Serbian Clinical Center in the period from January 2011 – January 2012. Out of 2,154 patients operated, 42 (1.95%) patients with complicated wounds were treated with VAC therapy and included in the study. These data were compared with retrospectively collected data from the history group of patients with complicated wound treated with the conventional methods of wound dressings in the period January – December, 2010.

According to the wound type patients were divided in five groups: the group 1 of groin wound with synthetic vascular implant (25%); the group 2 with infected or dehisced laparotomy wound (13%); the group 3 with wound after foot amputation (29%); the group 4 with wound after major limb amputation (21%); the group 5 with fasciotomy wound (13%).

Primary endpoint of the study was wound closure, while the secondary ones were duration of hospitalization, the number of weekly wound dressings, costs of wound care, working time of medical nurse dedicated to the care of those with complicated wound. A positive primary endpoint was considered the moment of closing wound with secondary suture, or discharging patient with wound in a condition that allows home care. A negative primary endpoint was considered wound bleeding, reoperation, wound reinfection requiring new hospitalization and limb loss. Secondary suture or discharge of patient to home care was based on a decision of operated surgeon.

Groin wound with a synthetic vascular implant (the group 1) was considered as any wound in the groin after vascular reconstruction with a Dacron or polytetrafluorethylene (PTFE) graft that had signs of infection defined by Szilagy (stage I, II or III). Infected laparotomy wound (the group 2) was considered as any wound with signs of infection that requires multiple daily dressings and positive microbacterial wound culture. The treatment strategy was to remove necessary sutures and the application of VAC. Dehisced laparotomy wound was considered as any laparotomy wound complicated with disruption of all layers in the abdominal wall. The patients in good general condition were treated with reoperation and were excluded from the study, while the patients in poor general condition, with previous major surgery and the presence of other cardiorespiratory complications were treated with VAC application. Wound after foot amputation (the group 3) was considered as every foot wound that required further hospital dressings after foot amputation or foot incision due to infection. Wound after limb amputation (the group 4) was considered as any wound after below knee, above knee or hip disarticulation that required intrahospital multiple dressings. Fasciotomy wound (the group 5) was considered as any fasciotomy with signs of ischemia or muscle devitalisation. In these patients, combined musculectomy and VAC application were performed. Clean and well-perfused fasciotomy wounds were excluded from the study and immediately referred to plastic surgeon. Each of the five groups of wounds was treated with VAC therapy in 2011 and with conventional methods (multi-

ple wound dressing) in 2010. The results of the two methods were compared.

Different types of wounds treated with VAC therapy are presented in Figures 1–3.

The number of weekly wound dressings was scored by a treating nurse, as well as working time spent for the wound care. Cost of wound care was counted as a sum of spent gauze [100 m – 2,981 Republic of Serbia Serbian Dinars (RSD), 27 Euro], hydrogen (1,000 mL – 28 RSD, 0.32 Euro), chloramin (1,000 mL – 39 RSD, 0.35 Euro) and Octenisept (1,000 mL – 2,900

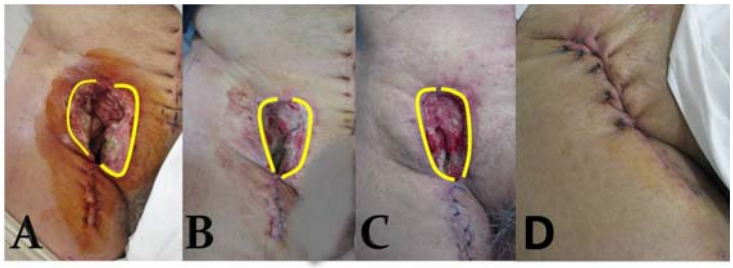

Fig. 1 – Treatment of infection of the polytetrafluoroethylene (PTFE) graft that exposed in the groin. During a two-weeks therapy wound retracted (A–C) with 5 dressings (15 days), and finally secondary suture was possible (D).

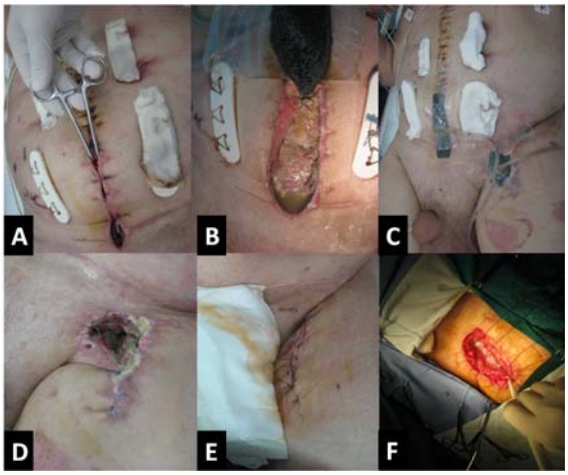

Fig. 2 – Concomitant infections of laparatomy, and groin wound (A, B, D) in a 125 kg weighted patient treated for ruptured abdominal aortic aneurysm. Extensive secretion from both wounds required multiple daily dressings (more than 15 per day), the patient was unmotivated in the Intesive Care Unit. Vacuum-assisted closure dressing was applied to both wounds (C) and with only 3 dressing *per* week the patient's general condition improved, his self esteem improved too and the patient was then motivated for treatment. Finally, the patient was transmitted to the semi-intensive care unit where the patient was prepared for secondary suture of the groin and later of the infraumbilical laparotomy wound (E, F).

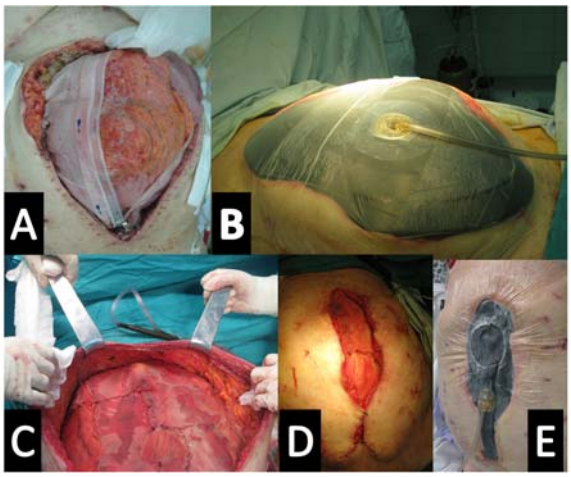

Fig. 3 – Abdominal compartment syndrome after repair of ruptured abdominal aortic repair was treated initially with "zip" abdominal suture (A), and due to dehiscence of the "zip", vacuum-assisted closure (VAC) abdominal set was applied (B), for 15 days when synthetic mash was used to reconstruct abdominal wall (C, D). Later, smaller VAC dressings were used to support healing of the skin and subcutaneous tissue (E). Wound was in such condition that the patient was able to be discharged and treated in the regional hospital. However, the patient suffered sudden death due to pulmonary embolism so no final wound image was available.

RSD, 26.60 Euro) for each patient during conservative treatment. For the patients treated with VAC therapy a sum of spent material was considered as wound care costs (small, medium or large wound set and VAC canister). Antibiotic therapy was expressed as the number of days under antibiotic therapy.

All wounds treated with VAC therapy were initially managed by removing all removable necrotic layers and devitalized tissue. Negative pressure of 125 mmHg in continuous fashion was applied. Wounds were dressed every 48–96 h until home care or the feasibility of secondary suture. Foot wounds with surrounding phlegmona were dressed every 24–48 h until the improvement of local state was visible, when dressing was prolonged (48–96 h) since secondary suture or home care was possible. Antibiotic therapy was selected according to wound culture findings and applied until secondary suture or home care.

Each wound in the history group was treated with multiple dressings using hydrogen, chloramin and Octenisept until home care or secondary suture. Antibiotic therapy was selected according to wound culture findings and applied until secondary suture or discharge to home care.

Statistical analysis

All continuous variables are represented with the median (range). The χ^2 was used to determine the association between a categorical outcome and a categorical factor. A $p < 0.05$ was considered as a statistically significant. SPSS Version 12.0 was used for statistical computing.

The study was approved by the local Ethical committee.

Results

Out of 80 patients, there were 42 (52.5%) in the VAC group treated between January 2011 and January 2012, and 38 (47.5%) patients treated with conventional methods in the period January–December, 2010. There were 72 (90%) males and 8 (10%) females, without statistical difference between the groups (39/3 and 34/4 in the VAC and conventional group, respectively). The distribution of wound types in both groups is given in the Table 1.

The patients with groin wound infection were treated due to lymphorhea with conventional or VAC methods in five out of six (83.33%) patients and four out of 12 (33.33%) patients, respectively. Synthetic graft was exposed in only one patient (1/6, 16.66%) treated with conventional treatment, while eight out of 12 (66.66%) patients in VAC group had exposed synthetic graft. Conventional treatment was used only for infected laparotomy wound, while VAC was used for dehisced (2/6, 33.33%) and infected (4/6, 66.66%) laparotomy. In other wound types there was no specific difference between the two groups.

The length of treatment was counted in hospital days and compared between the different wound types (Table 2).

The number of wound dressings *per* week was significantly lower in all but fasciotomy wounds when VAC treatment was applied. The greatest difference was in the group of patients with exposed graft. The number of wound dressings is presented in Table 3. The greater the difference between the number of weekly dressings, the greater the saving of nursing time: 165, 45, 50, 35 and 25 min/week were

Table 1

Distribution of different wound types in the two compared groups

Wound type	Conventional treatment, n (%)	VAC treatment, n (%)	*p* values
Groin with synthetic vascular implant	6 (15.78)	12 (28.57)	0.003
Infected or dehisced laparotomy	4 (10.52)	6 (14.28)	0.077
After foot amputation	14 (36.84)	12 (28.57)	0.254
After major limb amputation	11 (28.94)	9 (21.42)	0.084
Fasciotomy	3 (7.89)	3 (7.14)	0.216
Total	38 (100)	42 (100)	

VAC – vacuum-assisted closure.

Table 2

Length of hospital stay

Wound type	Length of stay (days), median (range)		
	Conventional treatment	VAC treatment	*p* values
Groin with synthetic vascular implant	45.3 (25–60)	25.1 (20–35)	< 0.001
Infected or dehisced laparotomy wound	20 (15–30)	17 (13–32)	0.806
After foot amputation	13.8 (9–20)	7.6 (5–15)	0.191
After major limb amputation	22.2 (15–32)	12.1 (7–22)	0.783
Fasciotomy	12.4 (9–15)	8.3 (4–18)	978

VAC – vacuum-assisted closure.

Table 3

Number of weekly wound dressings and the amount of medical nurse time saved due to the lower number of dressings

Wound type	Wound dressing, number/week			Saved time (minutes/week)
	Conventional treatment	VAC treatment	*p* values	
Groin with synthetic vascular implant	35	3	< 0.001	165
Infected or dehisced laparotomy	12	2	0.065	45
After foot amputation	9	2	0.030	50
After major limb amputation	10	2	0.038	35
Fasciotomy	7	3	0.182	25
Mean	14.6	2.4	0.022	53.33

VAC – vacuum-assisted closure.

saved for groin, laparotomy, foot, limb and fasciotomy wound, respectively.

The total cost of treatment was lower in the VAC group of patients with the greatest difference in patients with exposed synthetic grafts. The costs of patients with laparatomy dehiscence were higher when VAC treatment was used. The costs of treatment are shown in Table 4.

Chronic wounds do not heal satisfactory even after long lasting conventional treatment. Infection, secretion, malperfusion and slow granulation are the usual causes of prolonged healing process due to lower concentration of growth cytokines, increased level of inflammatory cytokines and proteolytic enzymes [4, 5]. Also mechanical forces in the wound bed have been shown to influence the healing process, as well [6]. VAC therapy provides effective influence on both of these factors [1–3]. Continuous evacuation of interstitial fluid and mechanical influence on wound edges shortens, wound healing time significantly. In addition, this vacuum effect improves cell migration, mitosis and microcirculation with consequent better wound perfusion being of significant help in malperfused wounds. The result of all these actions are higher rate of granulation tissue formation. Advantages of VAC therapy are shown in different wound types, however in this study we were focused on vascular patients operated due to different vascular pathology.

Table 4

Cost of hospital treatment included hospital day, antibiotic therapy and dressing material (due to diversity of vascular procedures costs of primary procedure were not calculated)

Wound type	Total cost of treatment, RSD		
	Conventional treatment	VAC treatment	*p* values
Groin with synthetic vascular implant	212,000	145,000	< 0.001
Infected or dehisced laparotomy	38,000	195,000	0.025
After foot amputation	33,000	22,000	0.055
After major limb amputation	58,000	42,000	0.034
Fasciotomy	27,000	18,500	0.047

VAC – vacuum-assisted closure; RSD – Republic of Serbia Dinar.

Primary endpoint in patients treated with VAC

There was one death (1/42, 2.38%) in the VAC group in a patient after foot amputation with severe cardiorespiratory comorbidity. Death was not related to VAC treatment. There was one groin bleeding (1/12, 2.38%) in the patients with groin infection. Bleeding was related to VAC treatment. There was one groin reinfection (1/12, 2.38%) treated with reoperation and extra-anatomical reconstruction. In one patient with a groin infection (1/12, 2.38%) no improvement in local condition was recorded demanding synthetic graft extirpation with consequent limb loss. All the other 38 patients (38/42, 90.47%) were successfully treated with positive primary endpoint.

Primary endpoint in patients treated with conventional therapy

There were 8 deaths (8/38, 21.05%), in 2, 3 and 2 patients with laparotomy, groin and limb amputation wounds, respectively. In 6 out of 8 (75%) patients the cause of death was sepsis. There were 5 (5/38, 13.15%) limb losses in the patients with groin infection (two patients) or foot amputation (3 patients). All the other 25 patients (25/38, 65.38%) were successfully treated with conventional measures in the prolonged and costlier manner as described above.

Discussion

This study shows clinical benefits of VAC therapy compared to conventional everyday dressings of chronic or surgical wounds in vascular patients. A reduced length of hospital stay and cost reduction in the subgroup of the patients with extensive wound secretion was found to be significant comparing to conventional therapy. The results of VAC therapy were prospectively collected and compared with retrospectively analysed data of conventionally treated patients during previous year.

In all kinds of vascular procedures or interventional cardiovascular interventions groin is the most frequent site of access. For these reasons complication in the groin area are most frequent. Complications of percutaneous groin interventions, such as false aneurysms and hemathoma inducing hemodynamic instability of the patients requiring urgent surgical procedure, are more frequent in obese patients. Obesity, emergency procedure and secondary cavity in the wound increase infection rate in the postoperative time. Such a complicated polyvascular disease patients requires fast recovery after complicated treatment and prolonged groin wound healing might postpone treatment of the main disease (mostly coronary).

Groin infection after procedures when synthetic material is used jeopardizes performed procedure and exposes a patient to high risk of bleeding, sepsis and death. Persistent lymphorhea and superficial infections are usually treated with repetitive wound dressings and antibiotic therapy increasing hospital stay and costs. Such a conservative treatment in deeper infections with already exposed synthetic material is not justified. It requires aggressive surgical treatment with replacement of synthetic graft with autologous or with a new extra-anatomical reconstruction. These procedures are extensive and bare significant risks especially in patients with already performed extensive surgery. In our study, among the patients from the history group, treated with conventional methods,

there were only two patients with such extensive infection and due to severe comorbidity they were not treated aggressively as described. Both of them died during the conservative treatment due to bleeding or sepsis. Other patients with more superficial infection and lymhorhea were treated with significantly longer hospitalization time and cost comparing to the group of patients treated with VAC therapy as shown in our results. In addition, among the patients treated with VAC therapy there was a significant number of them with deep infection and exposed synthetic graft. One of them is presented in Figure 1. There is a low number of reported cases with such extensive infection treated with VAC, however results of other authors are similar to ours [7, 8]. This group of patients has highest benefit from VAC therapy.

Extended aortic vascular procedures require laparotomy that may complicate with infection or dehiscence. These complications are not related to vascular reconstruction however they might jeopardize outcome of treatment, prolong hospital stay, antibiotic therapy. Our study showed benefits in all these attributes when VAC treatment was concerned. The advantage of VAC therapy was immense in patients operated emergently whose postoperative general condition precluded any additional procedures, like patient shown in the Figure 2. There was significantly higher costs of treatment with VAC therapy in this group due to discrepancy between the number of patients and severity of complication in this group. All conservatively treated patients had mild wound infection, while those treated with VAC had abdominal compartment syndrome of wound dehiscency. They were treated with costly abdominal VAC set. There are no publication comparing conservative and VAC treatment of laparatomy wound dehiscence or compartment syndrome due to ethical issues. Abdominal compartment syndrome (ACS) is complication of open or endovascular repair of ruptured abdominal aortic aneurysm. Immediate decompression in some advanced stages is life saving. VAC open abdomen set provides treatment of ACS without intensive care unit stay as shown in Figure 3. Still higher costs of this method are justified due to the life saving results [9].

The main impediment for healing after foot amputation is malperfusion and infection. In our group of patients VAC therapy improved limb salvage in the patients with foot amputation and also significantly reduced hospital stay and treatment costs. Limb salvage was improved in those patients who were admitted with already present extensive foot infection. Radical incision, debridement and subsequent VAC therapy was the strategy that gave obvious effects. In patients with simple amputation of foot finger that had demarcated necrosis VAC therapy decreased time until home care was possible. Still the patients did not recognize that and the majority of them complained on worse quality of care since they were fixed to bad. We did not examine quality of life with standard questionnaires; however we asked patients if they felt any improvement after our care or not. The majority of the patients with foot amputation complained on being fixed to bad. Other patients that experienced much extensive surgery and those with extensive secreting wounds reported a significant improvement and better motivation since the number of daily dressings were reduced and hygienic conditions improved. Vuerstaek et al. [10] found similar results in 2006. In this study the authors found decrease in quality of life during the first week of treatment while at the end of the therapy the quality of life was improved. We did not notice this improvement since in our series the mean treatment length was one week.

Infection and malperfusion after limb amputation is usually found in patients with previous vascular surgery. These patients are frequently found after synthetic graft infection in the groin when extirpation is necessary without an option to restore flow. Atypical reconstruction with viable tissue is made after previous open treatment of stump wound. In both situations, VAC therapy might be used to reduce the number of weekly dressings and improve perfusion. For these reasons, survival in this group of patients was better when VAC is used. With VAC therapy, patients avoid uncomfortable and annoying everyday dressings.

This study has some limitations. It compared the results of VAC therapy with the control history group that might bring some flaws. However, the patients in the history group were treated just one year before. The groups of patients were heterogenous and it was not possible to compare the results between the different wound types. On the other side, all the groups of wound types were present in both VAC and history group. The patients treated with VAC therapy were in worse general condition with more complex complications. For example, groin infections were more superficial in the history group while the graft was more frequently exposed in the VAC group. Laparotomy infections and dehiscence were also in a more advance stage in the VAC group. All these increase the strength of the results favoring VAC therapy.

Conclusion

Vacuum-assisted wound closure is the effective method for care of complicated wounds in vascular surgery providing limb or life salvage, shorter hospital stay and less nursing time to patient. Cost effectiveness of VAC therapy has been confirmed in the scenario of a developing country in particular groups of patients where longer hospital care is necessary. Due to the fact that patients with foot wound might feel less satisfied with VAC therapy outpatient modes of VAC therapy should be considered. VAC treatment of wound with synthetic graft should be considered as a first line therapy especially in superficial infection and high risk patients.

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The expression and significance of p53 protein and Ki-67 protein in pterygium

Ekspresija i značaj proteina p53 i Ki-67 u pterigijumu

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Abstract

Background/Aim. Pterygium is considered to be a degenerative disease of the conjunctiva, however, the presence of tumor markers in pterygium reinforces the hypothesis that this lesion is similar to tumor. Inactivation of p53 function removes an obstacle to increased proliferation. Factors affecting the prevalence of p53 expression in pterygium deserve investigation. The aim of the study was to investigate the expression of p53 and Ki-67 proteins in pterygium and normal conjunctiva, the effects of gender and age on p53 expression, and the relationship between the expression of p53 and Ki-67 proteins. **Methods.** A total of 34 samples of pterygium and 34 samples of the normal conjunctiva were analyzed. The samples were studied by immunohistochemistry using antibodies against p53 and Ki-67. **Results.** Totally 15 (44%) samples of pterygia were p53 positive. Correlations between the expression of p53 protein and sex, and age were not established. The number of Ki-67 positive cells in pterygium (9.74%) was significantly higher than the number of Ki-67 positive cells in the normal conjunctiva (1.74%), ($p = 0.001$). Between the expression of p53 protein and Ki-67 protein in pterygium there was a significant positive correlation ($p = 0.000$). **Conclusion.** The prevalence of p53 positive samples of pterygium was 44%. The influence of sex and age on p53 protein expression in pterygium was not found. The increased proliferative acivity was present in the epithelium of pterygium. The expression of Ki-67 protein is associated with the expression of p53 protein in pterygium. The findings of our study support the thesis of pterygium as tissue growth disorder.

Key words:
pterygium; tumor markers, biological; tumor suppressor protein p53; conjunctiva; age factos; sex; immunohistochemistry.

Apstrakt

Uvod/Cilj. Pterigijum se smatra degenerativnim oboljenjem konjunktive, međutim, nalaz tumorskih markera u pterigijumu pojačava hipotezu da je to lezija slična tumoru. Inaktivacija p53 funkcije uklanja prepreku povećanju proliferacije. Faktori koji utiču na prevalenciju p53 ekspresije u pterigijumu zaslužuju ispitivanje. Cilj rada bio je da se istraži ekspresija p53 i Ki-67 proteina u pterigijumu i normalnoj konjunktivi, uticaj životnog doba i pola na ekspresiju p53 proteina, kao i odnos između ekspresije p53 i Ki-67 proteina. **Metode.** Analizirani su 34 uzorka pterigijuma i 34 uzorka normalne konjunktive. Uzorci su analizirani imunohistohemijskim metodama uz korišćenje antitela za p53 i Ki-67. **Rezultati.** Ukupno 15 (44%) uzoraka bilo je p53 pozitivno. Povezanost između ekspresije p53 proteina i pola, kao i starosti nije utvrđena. Broj Ki-67 pozitivnih ćelija u pterigijumu (9,74%) bio je značajno veći od broja Ki-67 pozitivnih ćelija u normalnoj konjunktivi (1,74%), ($p = 0{,}001$). Između ekspresije proteina p53 i proteina Ki-67 u epitelu pterigijuma nađena je značajna pozitivna korelacija ($p = 0{,}000$). **Zaključak.** Prevalencija p53 pozitivnih uzoraka pterigijuma bila je 44%. Nije utvrđen uticaj pola i životnog doba na ekspresiju proteina p53 u pterigijumu. U epitelu pterigijuma prisutna je povećana proliferativna aktivnost. Ekspresije proteina p53 i proteina Ki-67 u epitelu pterigijuma pozitivno su povezane. Nalazi naše studije podržavaju tezu o pterigijumu kao poremećaju rasta tkiva.

Ključne reči:
pterigijum; tumorski markeri, biološki; protein p53; konjunktiva; životno doba, faktori; pol; imunohistohemija.

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Introduction

Pterygium is a fibrovascular conjunctival lesion and its etiology and pathogenesis are unclear [1–3]. For a long time, it was considered as a degenerative disease. Chronic inflammatory infiltrate, consisting of T lymphocytes, macrophages, plasma cells and mast cells, is present in pterygium [4, 5]. However, finding of tumor markers, such as p53, reinforces the hypothesis that the pterygium respresents a lesion similar to tumor [6–9]. Protein p53 is the guardian of the physical integrity of the cellular genome and inactivation of p53 function eliminates a major barrier for tumorigenesis and increased proliferation. The prevalence of p53 positive samples of pterygium is within a wide range of 7.9% to 100% [10–16]. The cause of the different prevalence of p53 protein expression remains unknown. Race, different parts of pterygium, p53 gene mutation spectrum, gender and age may affect the result of immunohistochemical analysis and should be further investigated [10]. Proliferative cellular activity can be evaluated by the detection of Ki-67 nuclear protein that is essential for the maintenance of the cell cycle. The expressions of p53 and Ki-67 proteins are useful markers of early premalignant lesions [17–19].

The aim of this study was to investigate the expression of p53 protein and Ki-67 protein in pterygium and normal conjunctiva, the influence of age and sex on the expression of p53 protein, and the relationship between the expression of p53 protein and Ki-67 protein in pterygium.

Methods

The study was done on 34 surgically excised pterygia (21 women, 13 men, age from 39 to 82 years) at the Clinical Center, Banjaluka, and 34 samples of normal bulbar conjunctiva (16 woman, 18 men, age from 44 to 82 years) taken during cataract surgery. Excised tissue of pterygium was fixed in 10% buffered formalin and processed for paraffin-embedded sections. The samples were oriented so that the cut was made perpendicular to the epithelium, and longitudinally through the body and the head of pterygium. The 4 μm thick histological sections were deparaffinized and rehydrated.

To detect primary antigens p53 and Ki-67 after standard procedures, histological sections were subjected to immunoperoxidase staining techniques. We used commercial monoclonal mouse anti-human p53 antibody clone DO-7 (Dako®, Code M7001) at 1 : 25 dilutions and monoclonal mouse anti-human Ki-67 antibody clone MIB-1 (Dako®, Code M7240) at 1:100 dilutions. For visualization we used the EnVision system (Dako® Code K4000) and chromogene DAB Liquid (Dako® Code K3467).

The expressions of p53 and Ki-67 proteins in the epithelium and stroma were estimated quantitatively. We counted at least 100 cells of tissue (including immunopositive cells) in three microscopic fields (magnification ×200) from pterygium tissue. The expression of p53 protein was marked as "p53 positive" if the p53 protein was present in more than 10% of tissue cells, and as "p53 negative" if the p53 protein was present in less than 10% of cells (cut-off level was set at 10%).

The number of Ki-67 positive cells was expressed as a percentage.

Examination of histological sections was perfomed using a Leica DM 6000 B microscope equipped with a digital camera Leica Microsystems Camera DFC495. For acquisition and display of digital images the Leica Application Suite software has been used.

The results were analyzed by methods of descriptive and correlative statistics. Statistical analysis was performed using the SPSS software (version 15.0), and the following tests were applied: chi square test, Student's *t*-test, Pearson's test, Spearman's *rho* test.

Results

It was found that the epithelium of pterygium was positive in 15 (44%) samples. Four the samples of pterygium with 4%, 5%, 6% and 9% of p53 positive cells, were taken as p53 negative. Nuclei of epithelial cells positive for p53 protein were mainly located in the basal and parabasal layers of the epithelium (Figure 1). According to the p53 expression in the stroma of pterygium, samples were negative. In the control group, there were no samples positive for p53 protein (Figure 2).

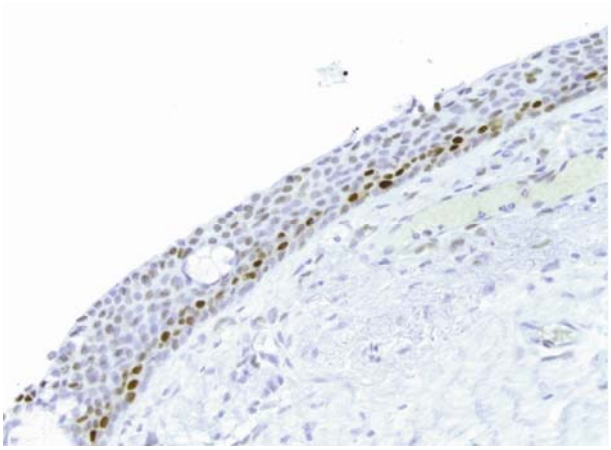

Fig. 1 – p53 positive cells in pterygium (anti-p53, ×200).

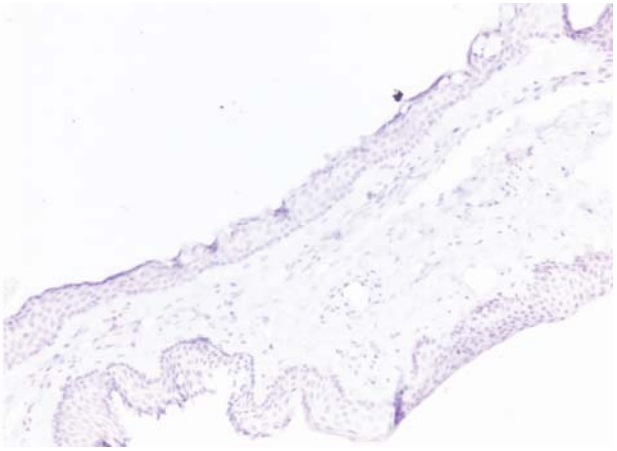

Fig. 2 – The sample of normal conjunctiva is negative for p53 expression (anti-p53, ×100).

Among p53 positive samples of pterygium there were seven men and eight women. No statistically significant difference in frequencies between the groups was found ($\chi 2$ = 0.808, df = 1, p = 0.369). The difference in the expression of p53 protein between men and women was not found.

There was no significant correlation between the positive expression of p53 protein in samples of pterygium and age (r = -0113, p = 0.337).

Nuclei positively stained for nuclear proliferation protein Ki-67 were found in the epithelium of pterygium and normal conjunctiva (Figure 3). Nuclei positive for Ki-67 were located mainly in the basal and parabasal layers of the epithelium (Figure 3).

In the epithelium of pterygium, an association between the expression of p53 protein and Ki-67 protein was found. The parts of the epithelium which showed immunopositivity for p53 were, at the same time, immunopositive for Ki-67 (Figures 1 and 3b, respectively).

There was a statistically significant positive correlation between the expression of p53 protein and Ki-67 protein in the epithelium of pterygium (r = 0.828, p = 0.000).

Discussion

A total of 44% of pterygium samples were p53 positive. In studies the presence of elevated level of stabilized p53 protein which can be detected by immunohistochemistry in the epithelium of pterygium has been found [2, 10–16]. Previous studies found that the prevalence of p53 positive pterygium were inside of a wide range of 7.9% to 100% [10–16]. Using the antibody DO1 Dushku et al. [6] found, immunopositivity for p53 protein in 100% of analyzed pterygia. Onur et al. [12] determined the expression of p53 protein in 7.9% of the analyzed samples of pterygium. Using antibody DO7 and the cut off level set at 10%, Tan et al. [2] found 60%, Weinstein et al. [13] 54%, and Tsai et al. [10, 15] 21.6% p53 positive samples of pterygium. Khalfaoui et al. [16] found positive p53 expression in 100% of analyzed pterygium, of which 75% of samples had the expression higher than 25% positive epithelial cells. Among the factors considered to affect the prevalence of p53 positive cases of pterygium were gender and age.

The difference in the expression of p53 protein between men and women was not found (p = 0.369). The same results have already been published by Khalfaoui et al. [16], Tsai et al. [10], and Perra et al. [14]. The absence of significant correlation between sex and p53 expression is explained by the fact that examined patients, whether men or women, were exposed to the same environmental conditions. Conversely, Ueda et al. [11] found a significantly higher prevalence of p53 expression in males.

Between the positive expression of p53 protein in samples of pterygium and age there was no significant correlation (p = 0.337). The same results have been published by Ueda et al. [11], Tan et al. [2], Tsai et al. [10] and Khalfaoui et al. [16]. This finding does not favor the UV radiation in the etiology of pterygium. Conversely, Perra et al. [14] found the associati-

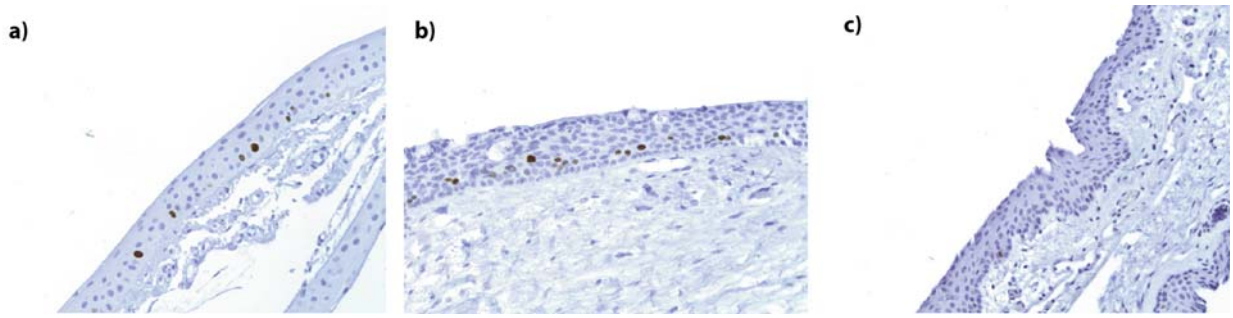

Fig. 3 – Ki-67 positive cells in the epithelium of: a) pterygium head, b) pterygium body; and c) normal conjunctiva (anti-Ki-67, ×200).

All parts of pterygium epithelium were not positive for Ki-67, and the distribution of positive cells was uneven. To determine the number of Ki-67 positive cells in the head and body of the pterygium, parts of the epithelium with the highest density were taken. No positively stained nuclei for Ki-67 were found in goblet cells and stroma of pterygium and normal conjunctiva. The average value of Ki-67 positive cells in the epithelium of pterygium head was 9.74% ± 13.14 (mean ± standard deviation). In the epithelium of pterygium body 4.65% ± 5.99 of Ki-67 positive cells was found (Table 1). The number of Ki-67 positive cells in the head of pterygium was significantly higher as compared to the body of pterygium (t = 2.05, p = 0.044).

Table 1
Ki-67 protein expression in pterygium and normal conjunctiva tissue

Tissue samples	Ki-67 positive cells (%)
	$\bar{x}$ ± SD
Normal conjunctiva	1.74 ± 1.56
Pterygium head	9.74 ± 13.14
Pterygium body	4.65 ± 5.99

In normal conjunctiva the average number of Ki-67 positive cells was 1.74% ± 1.56 (Table 1). The average number of Ki-67 positive cells in the epithelium of pterygium of 9.74% was significantly higher than the average value of Ki-67 positive cells in normal conjunctival epithelium of 1.74% (t = 3.451, p = 0.001).

on between p53 expression and aging. These data are explained by a well-known fact that aging reduces the capacity to repair DNA damage.

The loss of p53 protein function leads to the increased proliferative activity. Nuclear immunopositivity for Ki-67 protein was found in the basal and parabasal layers of the epithelium of pterygium and normal conjunctiva, while it was not found in stroma and goblet cells of the pterygium and normal conjunctiva. A proper orientation of samples and longitudinal sections allow differing parts of pterygium. In the epithelium of pterygium head, the number of Ki-67 positive epithelial cells of 9.74% was significantly higher than the number of Ki-67 positive cells in the epithelium of pterygium body of 4.65% ($p = 0.044$). The number of Ki-67 positive cells in the epithelium of pterygium of 9.74% was significantly higher than the number of Ki-67 positive cells in normal conjunctival epithelium of 1.74% ($p = 0.001$). The same results have been published by Kase et al. [7, 8] and Garfias et al. [9]. They found a relatively high proliferative activity of the epithelial cells of pterygium. In the study of Kase et al. [7], nuclear immunoreactivity for Ki-67 in the head of pterygium was 11.0% of epithelial cells and in the body of pterygium 7.5% epithelial cells, which was significantly higher than immunopositivity in the normal conjunctiva of 2.9%.

Immunohistochemical analysis in our study showed a significant correlation between the expression of p53 protein and Ki-67 protein, indicating increased cell proliferation due to dysfunction of the p53 protein. In pterygium, Shimmura et al. [17] examined the relationship between proliferative cellular activity and p53 protein as guardian of the physical integrity of the cell's genome. They found telomerase activity as a marker of cell proliferation in 58% of analyzed pterygia, but pterygium samples with increased telomerase activity did not have the simultaneous presence of mutation of the p53 gene, which leads to dysfunction, stabilization and increased level of p53 protein. Tsai et al. [15] have immunohistochemically detected the p53 protein in the absence of its mutation and assumed that human papillomavirus participate in the process of stabilization of p53 protein in the pterygium. In actinic keratosis of the skin, it was found that Ki-67 expression was significantly associated with the proapoptotic marker p53 [18].

Conclusion

The prevalence of p53 protein positive samples was 44% in pterygium. The influence of sex and age on the expression of p53 protein in pterygium was not found. In the epithelium of pterygium an increased proliferative acivity was present. In the epithelium of the pterygium head a higher proliferative activity was present when compared to the body of pterygium. The expression of Ki-67 protein positively correlated with the expression of p53 protein in pterygium. The findings of our study indicate the disorder of apoptosis regulation and cellular proliferation in the epithelium of pterygium, and support the thesis of pterygium as tissue growth disorder.

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Fever of unknown origin: Most frequent causes in adults patients

Febrilno stanje nejasne etiologije: najčešći uzroci kod odraslih bolesnika

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Abstract

Background/Aim. The differential diagnosis of fever of unknown origin (FUO) includes more than 200 different diseases and conditions. The aim of this study was to identify the most frequent causes of FUO among adult patients according to gender and age. **Methods.** The study included 74 patients examined from June 2010 to June 2013 at the Infectious Disease Clinic, Clinical Center Kragujevac in Serbia, according to the defined criteria for FUO. The patients were divided according to the diagnosis into four groups: infectious, malignant, rheumatic and "other diseases". A cause of febricity could not be estabilshed in a portion of subjects, and they comprised the group of undiagnosed cases. **Results.** Infectious diseases were dominant in the study, followed by rheumatic diseases, which were most frequently found in women and the elderly. The diseases recognised as the most common causes of febricity were subacute thyroiditis, subacute endocarditis, Still's disease, rheumatic polymyalgia with or without temporal arteritis, and cytomegalovirus infection. In 44% of the patients, the final diagnosis was composed of only six clinical entities. **Conclusion.** The importance of establishing the diagnosis of rheumatic disease is especially emphasised, in line with other authors' research indicating the number of these diseases is on the rise. The diagnostic approach to FUO should always be directed to the known frequency of diseases.

Key words:
fever of unknown origin; diagnosis; diagnosis, differential; age groups; sex.

Apstrakt

Uvod/Cilj. Diferencijalna dijagnoza febrilnih stanja nepoznatog porekla (*fever of unknown origin* – FUO), obuhvata preko 200 različitih bolesti i stanja. Cilj rada bio je da se odrede najčešći uzroci FUO prema polu i životnom dobu. **Metode.** Ispitivanjem su obuhvaćena 74 bolesnika koja su u periodu od juna 2010. do juna 2013. godine bila lečena u Infektivnoj klinici Kliničkog centra u Kragujevcu prema definisanim kriterijumima za FUO. Prema dijagnozi bolesnici su svrstani u četiri grupe: infektivne, maligne, reumatološke i "druge bolesti". Kod dela bolesnika nije utvrđen uzrok febrilnosti i oni su činili grupu sa nedijagnostikovanim bolestima. **Rezultati.** U našem istraživanju dominirale su infektivne bolesti, zatim reumatske, najviše zastupljene kod ženskog pola i starije populacije. Bolesti koje su se izdvojile kao najčešći uzroci febrilnosti bile su subakutni tireoiditis, subakutni endokarditis, Stilova bolest, reumatska polimijalgija sa ili bez temporalnog arteritisa i citomegalovirusna infekcija. Kod 44% febrilnih bolesnika završnu dijagnozu činilo je svega šest kliničkih entiteta. **Zaključak.** Posebno se ističe značaj utvrđivanja dijagnoze reumatskih bolesti, čiji je broj u porastu, što je u skladu sa istraživanjima drugih autora. Dijagnostički pristup FUO treba uvek da bude usmeren prema poznatoj frekvenciji bolesti.

Ključne reči:
febrilnost nejasne etiologije; dijagnoza; dijagnoza, diferencijalna; životno doba, grupe; pol.

Introduction

According to the traditionally accepted definition by Petersdorf and Beeson [1] in 1961, fever of unknown origin (FUO) means a repeated occurrence of fever above 38.3°C in the course of three weeks, the origin of which remains unresolved after three visits to the outpatient doctor or seven days of hospital examination. Thirty years later, with the development of modern diagnostic methods, Durack and Street [2] suggested two significant modifications to the initial definition: differentiation between standard FUO and three other forms of febricity accompanied by neutropenia, nosocomial infections, and human immunodeficiency virus (HIV), and a shorter duration of hospital examinations - three days instead of seven.

FUO represents a great challenge to clinicians because its differential diagnosis is composed of more disorders than in any other medical condition, encompassing both very rare and relatively common entities, classifiable into four groups: infectious, malignant, rheumatic and "other" diseases [3–5]. The published papers indicate that the differential diagnosis of fever

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includes more than 200 diseases and conditions, none of which with an incidence higher than 5% [6]. A significant portion of patients who remain undiagnosed in spite of the rapid development in diagnostic possibilities cannot be neglected, and recently published studies suggest their number is increasing [7].

Many researchers have investigated causes of fever in the elderly compared to the young adult population, and their results suggest that the etiologic factors differ in these populations [8].

The aim of this research was to determine the most common causes of FUO in patients treated at the Infectious Diseases Clinic of the Clinical Center Kragujevac, Serbia, to determine the distribution of various causes of FUO by gender, and to determine the most common causes of FUO in patients younger and older than 65 years.

Methods

The study included 74 adult hospitalized patients examined at the Infectious Diseases Clinic in Clinical Center Kragujevac. All the procedures were approved by the Ethical Committee of the Clinical Center in Kragujevac. Data were retrospectively collected from the medical records in a three-years period, from June 2010 to June 2013. Only the patients with standard FUO, body temperature higher than 38.3°C on several occasions during a three-week period and the origin of fever remaining unknown during three visits to the doctor, or after three days of hospital stay, were included. Patients with neutropenia, nosocomial infections and FUO during HIV infection were excluded from the study. First, all the patients were classified into infectious and non-infectious disease groups. We then divided the patients into the four groups according to the causative disease: infectious, malignancies, rheumatic, and group of "other" diseases not belonging to any of the previously mentioned clinical entities. This group included granulomatous diseases, subacute thyroiditis, inflammatory diseases of the digestive tract as well as drug-induced fever, factitious fever, and habitual hyperthermia. The patients in whom the cause of febricity remained unknown were classified into the group of undiagnosed FUO cases. Classification was then analyzed in relation to patient gender and age. The age limit between the younger and older population was set to 65 years.

The observed data are presented in tabular and graphical form. The results obtained were analysed using the methods of descriptive statistics.

Results

In 28 (37.8%) patients of the 74 examined a disease of infectious origin was diagnosed, while in 43 (58.1%) patients a non-infectious cause of fever was found. In three (4.1%) patients, the cause was not found (Table 1).

Visualizine procedures commonly used for the diagnosis of FUO are shown in Table 2.

In Table 1 it is shown that the majority of patients, 28 (37.8%), had infectious disease followed by 19 patients (25.6%) with rheumatic disease, 13 patients (17.5%) had "other disease", and, finally, 11 patients (14.8%) had malignant disease. The group V was composed of three (4.1%) patients with undiagnosed diseases.

Table 1

Causes of fever of unknown origin (FUO) in total of 74 adults patients

FUO patients' diagnoses	Patients number
Infectious diseases	28 (37.8%)
Cytomegalovirus infection	5
HIV infection	2
Epstein–Barr virus infection	1
staphylococcal sepsis	1
leptospirosis	1
brucellosis	1
ITU	1
Localised Infections	
subacute endocarditis	8
acute cholecystitis	2
tubo-ovarian abscess	1
perinephric abscess	1
pyonephrosis	1
diverticulitis	1
iliopsoas abscesses	1
pulmonary abscess	1
Rheumatic diseases	19 (25.6%)
Still's disease in adults	6
systemic lupus erythematosus	3
Polymyalgia rheumatica	4
temporal arteritis	3
Reiter's syndrome	3
Malignant diseases	11 (14.8%)
colonic neoplasm	3
breast cancer with pleural metastases	1
renal cancer with hepatic and pulmonary metastases	1
lung cancer with thoracic spine metastases	1
prostatic neoplasm	2
pancreatic neoplasm	1
hematologic diseases	
acute myeloid leukaemia	1
Hodgkin's disease	1
Other diseases	13 (17.5%)
subacute thyroiditis	7
Crohn's disease	1
Löfgren syndrome	2
granulomatous hepatitis	1
artificially induced fever	1
drug-induced fever	1
Unknown cause of FUO	3 (4.1%)

Table 2

Visualizing procedures commonly used for the diagnosis of fever of unknown origin

Diagnostic Imaging	Number
Chest X-ray	65
Abdominal ultrasonography	68
Abdominal computed tomography (CT)	27
Thorax computed thomography (CT)	16
Nuclear magnetic resonance (NMR)	7
Positron emission tomography (PET)	2

The diseases recognised as the most common causes of fever were subacute thyroiditis, subacute endocarditis, Still's disease, *polymyalgia rheumatica* and temporal arteritis, and cytomegalovirus infection. The listed diseases were diagnosed in as many as 33 (44%) of the total number of patients

diagnosed with FUO, while the most frequent diagnoses were subacute thyroiditis and subacute endocarditis, together are found to be the cause in every fifth patient with FUO.

Three patients (4.1%) entered the group of unknown causes of FUO. Following discharge from the Infectious Diseases Clinic, an undiagnosed patient experienced spontaneous subsiding of febricity, while maintaining good general condition during the follow-up on the outpatient basis. Autopsy of the second patient revealed malignancy, namely, pancreatic cancer. In the third male patient, three months of febricity accompanied by biohumoral inflammatory syndrome were followed by respiratory failure and death. The diagnosis remained unknown despite exhaustive investigations.

The aim of the study was also to determine gender-wise distribution of different FUO factors. The study included 36 (48.65 %) male and 38 (51.35 %) female patients. The obtained results suggested that in the female population, the most common were rheumatic diseases, 12 (31.5%). They are followed by infections in 11 (28.9%) of the patients, "other diseases" in 8 (21.1%), and malignant diseases in 7 (18.4%) of the patients (Figure 1). All FUO cases in the female patients were diagnosed. Most of the male patients, 17 (47.2%), had infectious disease, followed by significantly lower percentages of rheumatic, 7 (19.4%), "other diseases", 5 (13.8%), and malignancies, 4 (11.1%). Three (8.3%) of the patients entered the group of unknown FUO factors (Figure 1).

Among the patients in our study 48 (64.8%) were under 65 years of age (Figure 2). The most common diagnosis was that of infectious disease, in 21 (43.7%) patients. Viral diseases dominated among them, and were found in eight patients. Cytomegalovirus infection had the highest incidence, verified by serological methods (ELISA test) in five patients. The second most common diagnosis was rheumatic diseases, found in 10 patients (20.8%), especially Still's disease, followed by systemic *lupus erythematosus*. Malignant diseases comprised a significant percentage of conditions in the population under 65, occurring in nine (18.7%) patients. Hematologic diseases were only found within this population, namely acute myeloid leukemia and Hodgkin's disease. Both patients were under 40 years of age.

In the age group over 65 years, comprising 26 (35.1%) patients, the largest number of patients belonged to the group with rheumatic conditions, nine (34.6%) patients. The group was dominated by *polymyalgia rheumatica* and temporal arteritis as separate clinical entities. The group with infectious diseases was the second largest (Figure 2). Localised infections, especially subacute endocarditis, were verified in seven patients in this group. Subacute thyroiditis dominated the "other diseases" group, diagnosed using the radioactive iodine fixation test. Malignancies were confirmed using diagnostic imaging and histopathologic findings in 2 (7.6%) of the patients.

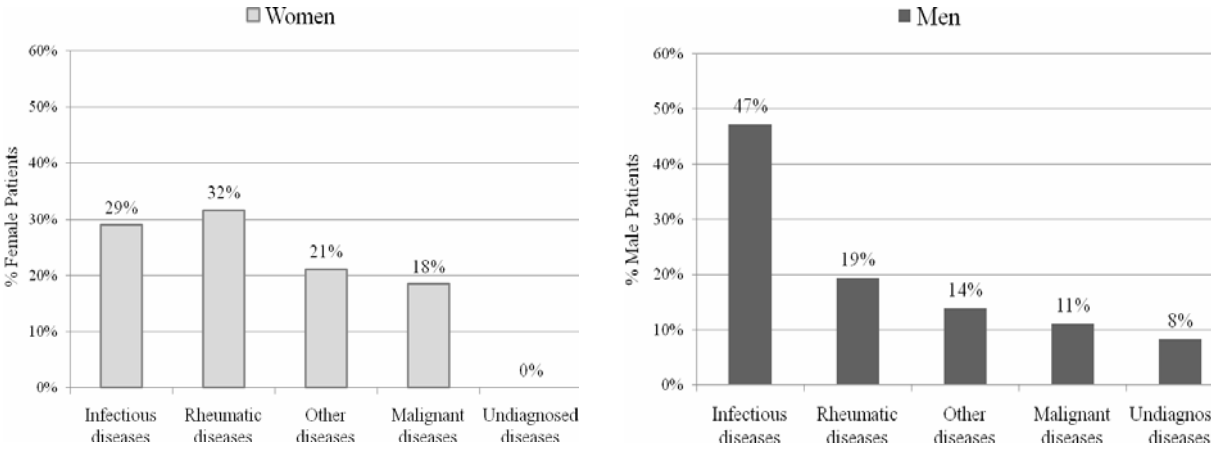

Fig. 1 – Most common etiologies of fever of unknown origin according to gender.

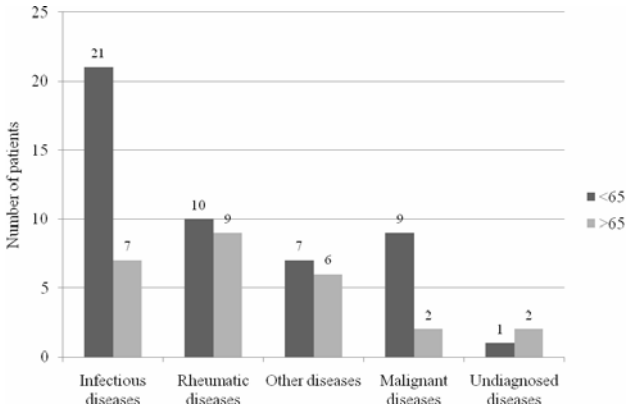

Fig. 2 – Most common etiologies of fever of unknown origin (FUO) according to age ■ – patient with FUO under 65 years old; ■ – patients with FUO above 65 years old.

Discussion

The share of etiologies in the diagnosis of FUO has continuously changed since 1960. In the past two decades, certain authors have suggested a decreased incidence of infectious and malignant diseases, with the increase in the frequency of rheumatic diseases [9, 10]. A recent research continues to pinpoint infections as the leading causes of FUO, which is consistent with our results. In a group of non-infectious FUO, the causes according to other authors [11] are, malignancies, as the most numerous, followed by rheumatic diseases. However, our research indicates that the leading FUO causes among the non-infectious diseases were rheumatic conditions, while malignancies appeared as low as the fourth place.

The development of modern diagnostic procedures continuously modifies the spectrum of diseases with FUO. Introduction of diagnostic imaging, such as echosonography, computed tomography and magnetic resonance imaging, has contributed to the rapid diagnosis of solid tumours and abscesses, which in turn influenced the number of infectious and malignant diseases in our FUO sample [12]. Table 2 shows visualizing procedures commonly used in the diagnostic of FUO patients. The invention and widespread use of serological analysis enabled faster detection of many viral diseases, which reduced their frequency within FUO [11]. Besides serology, in the diagnostic of other infections and infectious diseases we used microbiology tests, as well as polymerase chain reaction.

In our research, subacute endocarditis was the most common disease in the infectious diseases group and was especially dominant in the elderly population. Subacute endocarditis was present in 28% of patients with infectious diseases, a significant percentage given the available diagnostic procedures such as echocardiography. Diagnose determination was based on characteristic clinical findings and findings of vegetations on the hart valvules. Using hemoculture *Streptococcus viridans* was isolated in three patients, *Staphylococcus aureus* in one, while in other patiens no etiologic agent was isolated. It is believed that changes in the heart valves as well as the increased likelihood of sepsis make the elderly population more vulnerable to the development of endocarditis [13].

The obtained results indicate that cytomegalovirus infection is the most common etiologic cause of viral disease. Clinical diagnosis of cytomegalovirus infection can be difficult due to a high prevalence of asymptomatic infections and various clinical presentations of the disease. Increased activity of liver enzymes and splenomegaly, were the main parameters which directed us towards the diagnosis of cytomegalovirus infection. Seroprevalence, ranging from 50% to 80% before 40 years of age, also represents a significant cause of FUO in the middle-aged population.

Among the subjects in the "other diseases" group, subacute thyroiditis was undeniably the most common one. This endocrinologic condition was also dominant among female subjects in our sample. Subacute thyroiditis is a known cause of FUO, although the diagnosis may prove more difficult to make in the absence of typical symptoms, such as frontal neck pain [14].

Some authors set hematological diseases and colorectal cancer apart as the common causes of FUO in the group with malignancies [10]. These diseases were also verified in our sample, especially in the young adult population. Apart from those conditions mentioned above, no other malignancies causing febricity were detected.

According to many authors, the diagnosis of FUO differs between older and younger populations [8], a presumption also confirmed by our study. In the elderly population, the most numerous are rheumatic conditions, while the younger population is dominated by infections, especially viral ones. *Polymyalgia rheumatica* and temporal arteritis, both as joint and separate clinical entities, are most commonly found in the elderly, which is a conclusion drawn by many other authors [15]. For these diseases to appear among the common causes, contributing factors are certainly the subacute course of disease and non-specific symptoms.

In the group of rheumatic patients under the age of 60, Still's disease is the most frequent clinical entity. This multisystem disease can affect joints, skin, eyes, liver and spleen, but not all patients exhibit all symptoms [16]. A review of the available literature revealed that many authors emphasise Still's disease as an important cause of FUO which is the fact that should be given particular consideration because the criteria for the diagnosis require the exclusion of other rheumatic, malignant and infectious diseases [17].

Due to lack of specific tests in the diagnostics of Still's disease and rheumatic polymyalgia, the diagnosis was established using immunological, serological and microbiological analysis by which we excluded other diseases [18].

Conclusion

Our study shows that only six causes account for 44% of final diagnoses. The influence of rheumatic diseases is especially emphasised, particularly in elderly persons, in line with other authors' research indicating that the number of these diseases is on the rise. A diagnostic approach to fever of unknown disease should always be directed to the known frequency of causing diseases. The first step is to rule out the common causes of fever of unknown disease and then review the less know causes.

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ORIGINAL ARTICLE

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Cost effectiveness comparison of dutasteride and finasteride in patients with benign prostatic hyperplasia – The Markov model based on data from Montenegro

Analiza odnosa troškova i efekata finasterida i dutasterida u terapiji benigne hiperplazije prostate – Markovljev model baziran na podacima iz Crne Gore

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Abstract

Background/Aim. Benign prostatic hyperplasia (BPH) is one of the most common disease among males aging 50 years and more. The rise of the prevalence of BPH is related to aging, and since duration of life time period has the tendency of rising the prevalence of BPH will rise as costs of BPH treatment will and its influence on health economic budget. Dutasteride is a new drug similar to finasteride, inhibits enzyme testosterone 5-alpha reductase, diminish symptoms of BPH, reduce risk of the complications and increases quality of life in patients with BPH. But, the use of dutasteride is limited by its high costs. The aim of this study was to compare cost effectiveness of dutasteride and finasteride from the perspective of a purchaser of health care service (Republic Institute for Health Insuranse, Montenegro). **Methods.** We constructed a Markov model to compare cost effectivenss of dutasteride and finasteride using data from the available pharmacoeconomic literature and data about socioeconomic sphere actual in Montenegro. A time horizon was estimated to be 20 years, with the duration of 1 year *per* one cycle. The discount rate was 3%. We performed Monte Carlo simulation for virtual cohort of 1,000 patients with BPH. **Results.** The total costs for one year treatment of BPH with dutasteride were estimated to be 6,458.00 € which was higher comparing with finasteride which were 6,088.56 €. The gain in quality adjusted life years (QALY) were higher with dutasteride (11.97 QALY) than with finasteride (11.19 QALY). The results of our study indicate that treating BPH with dutasteride comparing to finasteride is a cost effective option since the value of incremental cost-effectiveness ratio (ICER) is 1,245.68 €/QALY which is below estimated threshold (1,350.00 € per one gained year of life). **Conclusion.** Dutasteride is a cost effective option for treating BPH comparing to finasteride. The results of this study provide new information for health care decision makers about treatment of BPH in socioeconomic environment which is actual both in Montenegro and other countries with a recent history of socioeconomic transition.

Key words:
economics, pharmaceutical; prostatic hyperplasia; 5-alpha-reductase inhibitors; cost-benefit analysis; montenegro

Astrakt

Uvod/Cilj. Benigna hiperplazija prostate (BHP) jedno je od najčešćih oboljenja kod muškaraca starijih od 50 godina i tesno je povezano sa procesom starenja. S obzirom na to da životni vek ima tendenciju produženja, može se očekivati da će povećanje učestalosti ove bolesti dovesti do povećanja troškova zdravstvene zaštite. Ukoliko se ne leči, BHP ima progresivan tok i dovodi do teških komplikacija. Inhibitori testosterona 5-alfa reduktaze, finasterid i dutasterid, ublažavaju simptome bolesti, povećavaju kvalitet života i snižavaju rizik od komplikacija. Dutasterid u odnosu na finosterid značajno usporava progresiju bolesti i komplikacije, kao što su akutna retencija urina i hirurške intervencije, ali je skuplji od finasterida 2,3 puta. Cilj ove studije bio je da pokaže da li je sa stanovišta odnosa troškova i efikasnosti opravdano finansiranje upotrebe dutasterida od strane Fonda za zdravstveno osiguranje Crne Gore. **Metode.** Studija je sprovedena prema Markovljevom modelu, koji je razvijen na osnovu podataka iz literature o efektivnosti i na osnovu troškova lečenja u Crnoj Gori. Trajanje jednog ciklusa u modelu je jedna godina a vremenski horizont praćenja iznosio je 20 godina. Za troškove i ishode korišćena je perspektiva društva i oni su diskontovani po stopi od 3% godišnje. Urađena je Monte Karlo mikrosimulacija modela sa 1 000 virtuelnih bolesnika. **Rezultati.** Primena dutasterida imala je nešto bolji odnos troškova i kliničke efikas-

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nosti od finasterida (539,51 €/QALY u odnosu 544,11 €/QALY). Jedna dobijena godina života prilagođena za kvalitet upotrebom dutasterida košta Fond za zdravstveno osiguranje Crne Gore 1 245,68 €, što ukazuje na to da je terapija sa dutasteridom farmakoekonomski isplativa. **Zaključak.** Ova studija pokazala je da u terapiji BHP dutasterid ima bolji odnos troškova i kliničke efikasnosti u odnosu na finasterid, pa je finansiranje dutasterida od strane Fonda za zdravstveno osiguranje Crne Gore farmakoekonomski opravdano.

Ključne reči:
farmakoekonomika; prostata, hipertrofija; 5-alfa-reduktaza inhibitori; troškovi-korist, analiza; crna gora.

Introduction

Benign prostatic hyperplasia (BPH) is the most common entity for clinical condition which includes non-cancerous enlargement of epithelial, muscle and stromal tissue of prostatic gland leading to the enlargement of prostatic gland and urinary obstruction [1]. This kind of disease is related to aging [2], and the results of the observational study The Baltimore Longitudinal Study of Aging indicate that the prevalence of BPH rises with aging; the prevalence of BPH is 25%, 50% and 80% in men who are 40–49 years old, 50–59 years old and 70–79 years old, respectively [3]. Since there is the tendency of prolongation of lifetime period [4], the prevalence of BPH will be higher in near future in the USA as well as in European countries and Montenegro, too [5, 6]. The rise of the prevalence of BPH with the tendency of prolongation of life time period will result in higher costs of treatment of BPH and its greater impact on health economic budget in near future. In the USA, BPH is ranked with high prevalence beside other diseases as hypertension, hyperlipidemia etc. among male which indicates the importance of socioeconomic influence of BPH on health economic budget [7].

Clinical features of BPH can reduce quality of life of patients [8], especially if BPH is left untreated when progressive form of BPH can occur with complications as urine retention (acute and complete), urine incontinence, recurrent urinary tract infection, nephrolithiasis, bladder diverticulitis, hematuria and renal insufficiency [1]. The main therapeutic strategy for patients with BPH according to European Association of Urology (EAU) depends on the phase of BPH [9]. In the early stages of disease "watchful waiting" is recommended and in the later progressive form of BPH the main therapeutic strategy is the use of different class of medications: alpha adrenergic blockers which reduce dynamic part of prostatic obstruction and facilitate urination, but do not change the progression of disease, 5-alpha reductase inhibitors which diminish prostatic enlargement, as well as complications of BPH and phytotherapeutics [1]. In the final stage of the disease, the surgical treatments are only therapeutic options since patients in this phase of BPH do not respond to medications and disease has great impact on quality of life of patients.

The effectivness of 5-alpha reductase inhibitors has been proved through the results of numerous clinical studies which indicate that the use of these medications in patients with BPH reduces its symptoms, improves the quality of life of patients, diminishes progression of disease and the rate of serious complications such as urinary retention and development of conditions which need surgical treatment. In Montenegro, two different 5-alpha reductase inhibitors have been registered, finasteride which blocks type 2 isoenzyme of 5-alpha reductase, and dutasteride which inhibits both type 1 and type 2 isoenzymes of 5-alpha reductase. The results of recent clinical trials have shown that dutasteride in comparison to finasteride significantly reduces progression of BPH [10, 11], as well as the rate of severe complications of BPH such as acute urinary retention and development of the late phase of BPH which needs surgical treatment [12]. Yet, the use of dutasteride is limited by its high costs: the costs of dutasteride are 2–3 times higher than the costs of finasteride. Finasteride is a part of the list of drugs which is funded by the Health Insurance Fund of Montenegro while dutasteride is not [13].

The aim of this study was to compare cost-effectiveness of finasteride and dutasteride in patients with BPH in actual socioeconomic environment of Montenegro.

Methods

For the purpose of this research, we conducted cost-effectiveness analysis of dutasteride versus finasteride in patients with BPH, using Tree Age Pro software and constructing Markov model.

The main therapeutic strategies in our model were: oral treatment with finasteride in the dosage regimen of 5 mg/day and oral treatment with dutasteride in the dosage regimen of 0.5 mg/day in patients with BPH. Dose regimens for finasteride and dutasteride were in compliance with actual clinical guidelines for BPH treatment [1]. For both therapeutic options virtual patients were in one of the following health states which represents chronic course of BPH, with the possibilities of moving to another health state at the end of the model cycle: mild BPH, moderate BPH, severe BPH, acute urinary retention (AUR), transurethral prostatic resection (TURP), repeated transurethral prostatic resection (TURP1) and death outcome, like in a study by Ismalia et al. [14] (Figure 1). A time horizon was estimated to be 20 years due to chronic course of BPH, and the duration of one cycle was one year.

All symptoms of severity of BPH in our study were valued according to the International Prostate Score System (IPSS) (Table 1). Acute urinary retention is an acute complication of BPH which needs urgent placement of urinary catheter. A virtual cohort of patient with BPH from every health state in the model can move to the AUR state and if catheterization completes successfully they move into the previous health state, and if catheterization completes unsuccessfully patients need surgical treatment and move to the TURP state, since TURP is the most commonly used surgical treatment. If IPSS does not reduce by 50% and more after

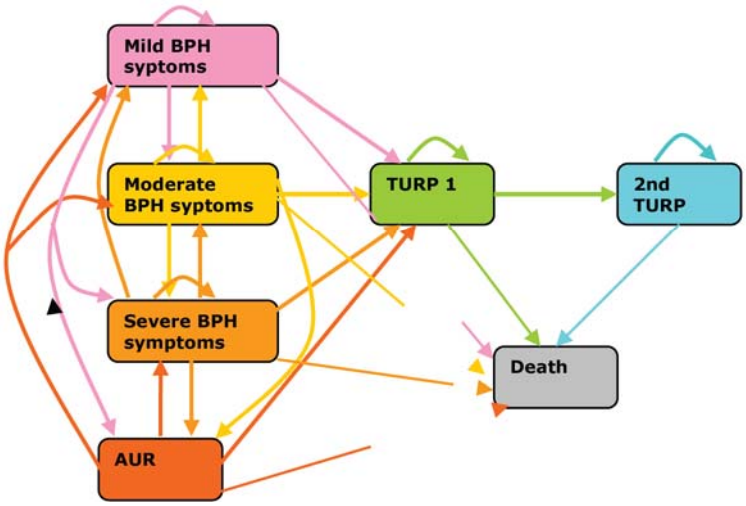

Fig. 1 – Health states in the Markov model for benign prostatic hyperplasia (BPH).
TURP – transuretheral prostatic resection (1, 2 – the number of repeated operations).

Table 1

Symptoms of benign prostatic hyperplasia according to the values of International prostate score system

Score	Symptoms
0–7	Mild
8–19	Moderate
20–35	Severe

performed TURP, virtual patients stay in the TURP state. In our research patient could stay in TURP stay for two cycles.

For every health state of both therapeutic strategies we estimated effectiveness from the available pharmacoeconomic literature. The effectiveness of finasteride and dutasteride was valued through quality adjusted life years (QALY) for every health state in the model, and it was estimated from the available pharmacoeconomic literature [15–18] (Table 2).

chaser of health care service (Republic Institute for Health Insurance of Montenegro). For therapy with finasteride as well for dutasteride in patients with BPH direct and non-medical costs were included in the model – costs of: medications, inpatient and outpatient services (general practice and urology specialist examinations, hospitalizations, laboratory services, diagnostic procedures, surgical procedures, treatment of AUR, treatment in emergency care services, home visiting medical services and patients transport). The afor-

Table 2

Values for quality adjusted life years (QALY) in all health states of the Markov model

Health state	Quality of life		
	Therapy with dutasteride	Therapy with finasteride	Reference
Mild BPH	0.89	0.84	15,16
Moderate BPH	0.76	0.71	15,16
Severe BPH	0.69	0.64	15,16
AUR	0.17	0.17	17
TURP1	0.668	0.668	18
TURP2	0.594	0.594	18

BPH – benign prostate hyperplasia; AUR – acute urinary retention; TURP – transurethral prostatic resection (1, 2 – the number of repeated operations).

Initial and transition probabilities were estimated from the available pharmacoeconomic studies and they are shown in Table 3 [19–31]. For both therapeutic options initial probabilities were the same.

For every health state and for both therapeutic options in the model we estimated costs from the perspective of purmentioned costs of care have been shown to be substantial in prostatic carcinoma and associated disorders [32, 33]. All costs were estimated from randomly chosen patients with BPH, who were treated in General Hospital in Nikšić, Montenegro from January 1, 2012 to December 31, 2012. All costs were expressed in Euros. The costs of medications were estimated

Table 3

Initial and transition probabilities used in the Markov model

Probabilities	Therapy with dunasteride	Therapy with finastride	References
Initial and transition probabilities for the states			
Mild BPH	0.55	0.55	19–24
Moderate BPH	0.35	0.35	19–24
Severe BPH	0.074	0.074	19–24
AUR	0	0	
TURP1	0	0	
TURP2	0	0	
Death	0	0	Data calculated by model
Transition probabilities for model			
Mild BPH → Mild BPH	0.96	0.95	Data calculated by model
Mild BPH → Moderate BPH	0.01	0.012	10, 11, 25
Mild BPH → Severe BPH	0	0	10, 11, 25
Mild BPH → AUR	0.0066	0.0103	12, 26
Mild BPH → TURP1	0.0037	0.009	12, 26
Mild BPH → Death	0.017	0.017	6
Moderate BPH→ Mild BPH	0.27	0.22	10, 11, 25
Moderate BPH→Moderate BPH	0.70	0.74	Data calculated by model
Moderate BPH→ Severe BPH	0.01	0.012	10,11,25
Mild BPH→ AUR	0.0051	0.0079	12, 26
Mild BPH→ TURP1	0.0037	0.009	12, 26
Mild BPH→ Death	0.01	0.01	6
Severe BPH → Moderate BPH	0.07	0.06	10, 11, 25
Severe BPH → Mild BPH	0.16	0.13	10, 11, 25
Severe BPH → Severe BPH	0.75	0.78	Data calculated by model
Severe BPH → AUR	0.0036	0.0057	12, 26
Severe BPH → TURP1	0.0067	0.0164	12, 26
Severe BPH → Death	0.002	0.002	6
AUR → Mild BPH	0.009	0.008	12, 26, 27
AUR → Moderate BPH	0.031	0.027	12, 26, 27
AUR → Severe BPH	0.17	0.15	12, 26, 27
AUR → TURP1	0.649	0.674	12, 26, 27
AUR → Death	0.141	0.141	29
TURP1 → TURP1	0.97	0.97	Data calculated by model
TURP1 → TURP2	0.0195	0.0195	30
TURP1 → Death	0.0065	0.0065	31
TURP2 → TURP2	0.99	0.99	Data calculated by model
TURP2 → Death	0.0065	0.0065	31

BPH – benign prostate hyperplasia; AUR – acute urinary retention; TURP – transurethral prostatic resection (1, 2 – the number of repeated operations).

on maximal drug prices which were valid in Serbia in June 2013 [34], since in Montenegro this kind of document is not available, and costs of medical services were estimated from the Republic Institute for Health Insurance (RIHI) Tariff Book [35]. All costs and effects were discounted for 3% and willingness to pay was estimated on 1,350.00 Euros *per* one gained year of life [36]. We performed Monte Carlo simulation where a randomly chosen patient from virtual cochort of patients with BPH runs through each scenario in the model and the results expressed as incremental cost effectiveness ratio (ICER) in Euro/QALY. For both therapeutic options we calculated mean costs and mean effects and summarized them also as ICER. In order to check robustness of the model results we peroformed one way sensitivity analysis, decreasing the price of dutasteride by 50%.

Results

The total costs of each health state in the model were calculated for both therapeutic options in the model and the results showed the difference in the costs of finasteride and dutasteride (Table 4).

Using the cost effectiveness calculation method we compared total costs *per* QALY for the therapy with dutasteride and the one with finasteride in the patients with BPH. The total costs with dutasteride *per* one year *per* patient was estimated to be 6,458.00 ± 3,726.62 € and for that period total effectiveness with dutasteride was estimated to be 11.97 ± 3.85 QALY while under the same conditions treatment with finasteride required 6,088.56 ± 4,866.8 € *per* 11.19 ± 3.50 QALY (Table 5).

The distribution of ICERs calculated by Monte Carlo simulations (using a cohort of 1,000 virtual patients) for total costs *per* QALY is shown in Figure 2. For therapeutic option dutasteride the calculated ICERs (with finasteride as baseline comparator) for the majority of virtual patients fall on the right side of willingness-to-pay line, which indicates that dutasteride is a cost effective therapeutic option in patients with BPH in socioeconomic enviroment of Montenegro. The

Table 4

Total costs for each health state in the model for finasteride and dutasteride in the patients with benigh prostate hyperplasia (BPH)

Therapeutic strategies → Health states in model ↓	Cost (€)	
	finasteride	dutasteride
Mild BPH	248.01	363.45
Moderate BPH	305.34	403.55
Severe BPH	355.11	466.61
AUR	529.73	564.51
TURP 1	1013.20	1013.20
TURP 2	2026.40	2026.40

AUR – acute urinary retention; TURP – transurethral prostatic resection (1, 2 – the number of repeated operations).

Table 5

Results of Monte Carlo simulation

Parameters	$\bar{x} \pm SD$	Minimum value	Median	Maximum value
Dutasteride				
costs (€)	6,458.00 ± 3,726.62	0	6,328.81	32,420.05
clinical effectiveness	11.97 ± 3.85	0	13.90	14.63
Finasteride				
costs (€)	6,088.56 ± 4,866.81	0	4,374.29	32,420.05
clinical effectiveness	11.19 ± 3.50	0	1.61	13.76

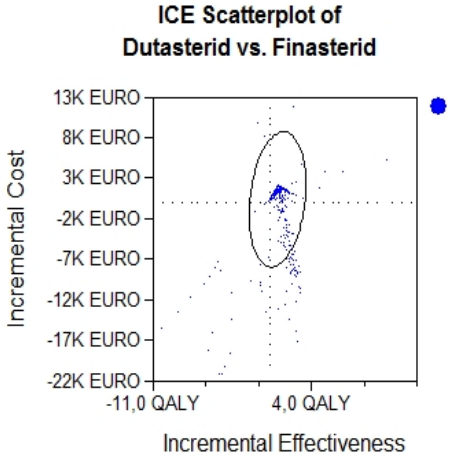

Fig. 2 – The distribution of incremental cost-effectiveness ratios (ICE) for dutasteride comparing to finasteride in the patients with benign prostate hyperplasia.

value of ICER for dutasteride comparing to finasteride in patient with BPH was estimated to be 1,245.68 €/QALY which was below the estimated treshold of 1,350.0 €.

In order to check robustness of our results we decreased the price of dutasteride by 50% performing one-way sensitivity analysis. The results of sensitivity analysis indicate that with the decreasing price of dutasteride by 50% the value of ICER decreases too with the value of 483.72 €/ QALY. Distribution of ICER under the conditions of decreasing price of dutasteride by 50% is shown in Figure 3.

Discussion

The results of our research indicate that the use of dutasteride in the patients with BPH comparing to finasteride requires a slight increase of funding (369.44 €) but provides 11.97 ± 3.85 QALY which is higher comparing with finasteride used under the same conditions providing 11.19 ± 3.5 QALY. The difference between these therapeutic options in costs is minimal (369.44 €), but still lower in the dutasteride group where one QALY requires investment of 539.51 €, while in the finasteride group one QALY requires investment of 544.11 €. In the research that compared dutasteride to placebo and finasteride in socioeconomic environment of Poland [37] dutasteride was a cost-effective therapeutic option, with lower costs providing more gained years of life (1.092 gained years) without complications of BPH as prostatic carcinoma and surgical interventions.

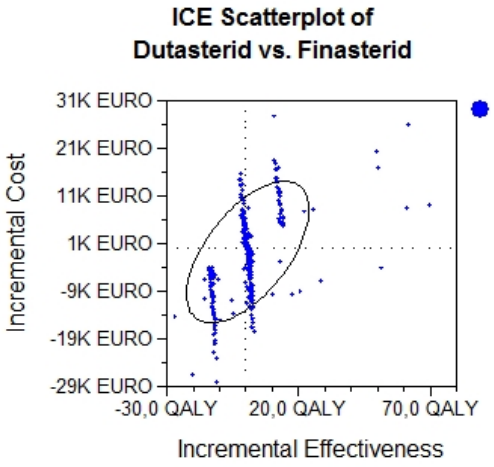

Fig. 3 – Distributions of the incremental cost-effectiveness ratio (ICE) for dutasteride comparing to finasteride in the patients with benign prostate hyperplasia, with the decreasing price of dutasteride for 50%.

We could have expected a better cost-effectiveness position of dutasteride in our research if prices of medical services and drugs in socioeconomic sphere of Montenegro had

been similar to socioeconomic conditions in developed countries in the European Union [38]. In the Balkan region, except in Albania, there is a legacy of the health care system based on socialism and insurance. In the recent period, in the Balkan region there has been a tendency of appearing more integrated strategies for social protection, but very often they have not been carried out to the end, while the monitoring and evaluation of the implementation has been poor. In the context of the economic crisis, conflict and low levels of social security contributions, public spending on social protection are faced with major problems and disadvantages of the funds in the region. Balkan countries fall into the high-middle income countries with the gross national income of $ 3,809 in Albania, to $ 22,169 in Slovenia, in 2012 the age or annually for health care *per* capita stands relatively small amount (in 2012 the age $ 561 in Serbia, Bosnia and Herzegovina, $ 447, $ 1942 Slovenia, Montenegro, $ 493, $ 516 to Bulgaria, Macedonia $ 327, $ 908 Croatia, Poland, $ 854, $ 228 and Albania, Rumania $ 420). Health systems in these countries are state-owned, and the prices of health services are determined and controlled by the state health insurance funds [39].

Since the prices of medical services are determinated by the Republic Institute for Health Insurance of Montenegro and drug prices are controlled by drug producers, the socioeconomic environment of Montenegro is characterized with lower prices of medical services than in the EU and with the similar values for prices of drugs. For example, the TURP state in our model has the highest total costs, and the average price of this procedure in the United Kingdom is 7.5 times higher than in Montenegro (6,128£ or 7,650 €) [40] while the price of finasteride is 14,94 £ (18,64 €) and of dutasteride 29,77 £ (37,14 €) [41] which is approximately 2 to 2,6 higher than in Montenegro. The difference in costs of BPH treating complies also with private practice where costs of surgical treatment of BPH is 2.5 time higher than in state hospitals. All these discrepancies make specific socioeconomic sphere which can blur real cost effectiveness position of drugs as dutasteride is.

On the other side, in the Republics of Serbia and Montenegro the price of dutasteride differs from the price of finasteride (18.13 € and 7.90 €, respectively) which is dissimilar in countries of EU. In Germany total month costs of treatment with finasteride and dutasteride are the same [42], and in Poland a difference between costs of dutasteride and finasteride is lower than in Montenegro [43]. Dutasteride was registred as Avodart® and its generic copies will be available on the drug market in November 2015. After that period we can expect that the price of dutasteride and costs of BPH treating with dutasteride will be lower which has already been shown with finasteride and its generic copies.

According to the World Health Organization a therapeutic option could be considered as cost-effective if its ICER in comparison with the standard therapy (costs *per* quality-adjusted life year gained) is under one, two or three multiples of average gross national income *per capita* for that country [34]. Our results indicate that the value of ICER for dutasteride comparing to finasteride is 1,245.68 € *per* one quality adjusted life year, which is below the estimated threshold of 1,350.00 €, and favors dutasteride as cost-effective therapeutic option comparing to finasteride in patients with BPH in socioeconomic environment of Montenegro. The results of Dardzinski et al. [44] point out that including dutasteride on the list which is financed by the National Institute for Health Insurance in Poland will result in reduction in costs as well as decreasing risk for prostatic cancer and development of complications of BPH which need surgical treatments.

This study has a few limitations. We chose to use data about effectiveness of dutasteride in patients with BPH from the available clinical trials since we had no "real" data from patients in Montenegro. An underlying issue of patient compliance affecting the treatment success rates was difficult to assess due to objective nature of modeling approach and therefore we decided to omit it from further analysis [45]. We chose that patients in our model could undergo only in TURP because it is the most frequently surgical intervention among these patients with frequency estimated from the available literature. Since adverse reactions of dutasteride are minimal and similar to finasteride, we chose not to incorporate them in our model, but we corrected the value of QALY for both therapeutic options with estimated frequency for adverse reactions. This assessment was based on the assumption of patient perceived quality of life [46]. Since the perspective in our study was the one of a purchaser of health care service (Republic Institute for Health Insurance, Montenegro) only the direct costs were included in our model.

Conclusion

Our results indicate that dutasteride is a cost-effective therapeutic option comparing to finasteride in patients with benign prostate hyperplasia (BPH) in socioeconomic environment of Montenegro. Since the differences considering costs and effects between dutasteride and finasteride are minimal, finasteride should still be a part of the list of drugs which is financed by the Republic Institute for Health Insurance. Our results provide new information for health care decision makers about treatment of BPH in socioeconomic environment which is actual both in Montenegro and other countries with recent history of socioeconomic transition.

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Risk factors and the prevalence of anorexia nervosa among female students in Serbia

Faktori rizika i prevalencija anoreksije nervoze među studentkinjama u Srbiji

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Abstract

Background/Aim. The widespread symptoms of anorexia nervosa (AN) in young women require to draw professional attention to this problem in Serbia. In previous research on AN, insecure attachment styles, perfectionism and concerns about body shape were identified as notable risk factors. The aim of this study was to identify the prevalence of AN among female students and assess the importance of these factors in its development. **Methods.** The Eating Attitudes Test (EAT-40), the Experiences in Close Relationships Scale (ECR), the Frost Multidimensional Perfectionism Scale (FMPS) and the Body Shape Questionnaire (BSQ) were applied to a sample of 500 randomly selected female students of the University of Belgrade, the mean age of 22.44 years (min 18, max 35). In addition, Body Mass Index (BMI) was also calculated. **Results.** Although 38 (7.6%) female students displayed symptoms of AN (EAT > 30) and 13 (2.6%) had BMI indicating anorexia nervosa syndrome (BMI ≤ 17.50 kg/m^2), only 1 (0.2%) student fulfilled both criteria. The majority of female students (60.4%) had some type of insecure attachment style. There is a significant influence of attachment styles on symptoms of AN: female students with insecure attachment styles have a significantly higher mean score on the EAT compared to those with secure attachment style ($F = 7.873$; $p < 0.01$). There was a positive correlation between scores on the EAT and FMPS ($r = 0.217$; $p < 0.01$), and scores on the EAT and BSQ ($r = 0.388$; $p < 0.01$). **Conclusions.** The obtained results show the prevalence of AN of 0.2% among female students and indicate the importance of insecure attachment styles, perfectionism and concern about body shape as risk factors. Activities for the prevention of AN in this subpopulation should include internet-based therapy and special counseling services with specific programs focusing on emotion-regulation skills through mindfulness, acceptance and commitment techniques, as well as specific cognitive-behavioral techniques.

Key words:
anorexia nervosa; risk factors; prevalence; students; women; serbia.

Apstrakt

Uvod/Cilj. Rasprostranjenost simptoma anoreksije nervoze (AN) među mladim ženama zahteva skretanje pažnje stručnjaka na ovaj problem u Srbiji. U prethodnim istraživanjima anoreksije nervoze, nesigurni stilovi afektivnog vezivanja, perfekcionizam i zabrinutost povodom oblika tela, identifikovani su kao značajni faktori rizika. Cilj ovog istraživanja bio je da se identifikuje prevalencija AN među studentkinjama i ispita značaj navedenih faktora za razvoj AN. **Metode.** Upitnik stavova o ishrani (*Eating Attitudes* Test – EAT-40), Skala iskustava u bliskim emocionalnim odnosima (*Experiences in Close Relationships Scale* –ECR), Multidimenzionalna skala perfekcionizma (*Frost Multidimensional Perfectionism Scale* – FMPS) i Upitnik doživljaja oblika tela (*Body Shape Questionnaire* – BSQ), primenjeni su na grupi od 500 studentkinja Univerziteta u Beogradu, izabranih metodom slučajnog izbora, prosečnog uzrasta 22,44 godine (min 18, max 35). Takođe, računat je i indeks telesne mase (*Body mass index* – BMI). **Rezultati.** Premda je 38 (7,6%) studentkinja ispoljavalo simptome anoreksije nervoze (EAT > 30), a 13 (2,6%) imalo indeks telesne mase koji upozorava na sindrom poremećaja (BMI ≤ 17,50 kg/m^2), samo je jedna (0,2%) studentkinja ispunjavala oba kriterijuma (EAT > 30, BMI = 15,64 kg/m^2). Većina studentkinja (60,4%) imala je neki od nesigurnih stilova afektivnog vezivanja. Postoji značajan efekat stilova afektivnog vezivanja na izraženost simptoma anoreksije nervoze: studentkinje koje imaju neki od nesigurnih stilova afektivnog vezivanja imaju značajno veći prosečni skor na upitniku EAT u poređenju sa onima sa sigurnim stilom ($F = 7,873$; $p < 0,01$). Postoji pozitivna korelacija između skorova na upitnicima EAT i FMPS ($r = 0,217$; $p < 0,01$), i EAT i BSQ ($r = 0,388$; $p < 0,01$). **Zaključak.** Prevalencija AN među studentkinjama iznosi 0,2%. Rezultati ukazuju na značaj nesigurnih stilova afektivnog vezivanja, perfekcionizma i zabrinutosti povodom oblika tela kao faktora rizika. Prevencija AN u ovoj subpopulaciji treba da se usmeri na izradu internet terapije i specijalizovanih savetovališta sa specifičnim programima usmerenim na veštine regulacije emocija tehnikama pune svesnosti, prihvatanja i posvećenosti, kao i specifičnih kognitivno-bihejvioralnih tehnika.

Ključne reči:
anoreksija nervoza; faktori rizika; prevalenca; studenti; žene; srbija.

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Introduction

Over the recent years, an increasing number of women have been involved in intense exercise and various forms of strict diets. It is estimated that 8–17% of young women show some symptoms of eating disorders [1]. Among undergraduate female students the prevalence of symptoms of eating disorder is 13.5% [2]. Twenty percent of female students at some time in their life have eating disorder, 75% avoid or skip meals while dieting, while 55% know at least one person with eating disorder [3].

Anorexia nervosa (AN) is one of the most common psychiatric disorders among female students [4]. Around the world the prevalence of AN among women is 0.5–1.0% (APA, 2000) [5]. The prevalence of this disorder is 0.5% in the Hungarian sample and 0.2% in the Romanian sample of young women. For subclinical symptoms of AN, the rate in the Hungarian female sample was 1.1 %, and in the Romanian female sample 3.0% [6]. For an explanation of the development of AN, a multidimensional biopsychosocial model that emphasizes the importance of a large number of factors is generally used [7].

A connection between risk factors and symptoms of AN among women who do not have the diagnosis but who express some symptoms of the disorder have been studied in many countries, finding a positive correlation between symptoms of AN and insecure attachment styles, perfectionism and body shape concerns.

Insecure attachment styles represent a general risk factor for the development of AN [8]. There is a positive correlation between insecure attachment styles and AN [9–11]. Generally, patients with AN have a significantly higher prevalence of preoccupied and dismissing attachment styles [11, 12], and significantly lower prevalence of secure attachment styles [13], compared with the control group. In women from non-clinical populations, insecure attachment styles are correlated with higher weight occupancy, body dissatisfaction and attitudes characteristic of eating disorders [14]. While secure attachment style correlates negatively, preoccupied and dismissing attachment styles correlate positively with symptoms of AN in female students [15].

Perfectionism has been identified as a potential risk factor for developing AN, too [16, 17] and represents a discriminative characteristic of the disorder. Patients with AN have significantly higher levels of perfectionism than healthy individuals [18]. They usually have unrealistic standards on physical attractiveness and thinness [19] and interpret normal drawbacks as too upsetting or normal body and body parts characteristics as a sign of imperfection [20]. There is a statistically significant correlation between the different dimensions of perfectionism and symptoms of dysfunctional eating attitudes and behaviour, especially in female students who are preoccupied with exercise and dieting [20, 21].

Weight and appearance concerns, body dissatisfaction, negative self-image and dieting represent significant predictors of eating disorders [22–24]. Body shape is an important feature of body image and body dissatisfaction [25]. Numerous studies have shown a strong correlation between eating disorders and anxiety and preoccupation with body shape [26]. There is a positive correlation between concerns about body shape and pathological eating attitudes and behaviors in female students [25, 27, 28]. Up to 90% of female students are dissatisfied with their appearance and concerned about body shape [29]. There is also a positive correlation ($r = 0.393$) between perfectionism and concerns about body shape ($p < 0.01$) among 45 Serbian female students with dysfunctional eating attitudes and behavior [30].

Eating disorder symptoms may develop in vulnerable young women as a response to life stressors or traumatic events, serving as strategies for coping with overwhelming emotions or circumstances. The problems of a country-in-transition could affect the family through a family discord which can be associated with insecure attachment styles. Then, during their student life, young women in Serbia may experience high levels of stress, primarily due to financial uncertainty and academic competence. They are exposed to parents' high expectations and set themselves high standards, which are sometimes perfectionistic. They also may experience feelings of isolation and intense peer pressure, fear of teasing and mistakes, and of negative assessment by others. Further, they are exposed to unrealistic media images and cultural pressure that women should be slim and good-looking. Therefore, concern about body shape is common.

The aim of this study was to examine the prevalence of the symptoms of AN among female students in Serbia, as well as the relationship between the symptoms of AN and attachment styles, perfectionism and concerns about body shape. These factors, among others, can contribute to the development of the symptoms and syndrome of AN among the population of female students.

Methods

Participants and procedure

A total of 500 randomly selected female students (n = 500) of the University of Belgrade participated in this study. A sample consisted of female students from different faculties, all undertaking undergraduate and postgraduate studies. All fully completed questionnaires were included in the study, regardless the age of the female students. The mean age of the students was 22.44 years (min 18, max 35). The majority (92.8%) of female students were aged between 20 and 25. Testing was conducted at the faculties and in the campus of the University in New Belgrade. Research was conducted with the approval of competent institutions. The participants were given information about research topic and purpose, and their participation was voluntary. They were given a package of 4 questionnaires, and after completion collected. The average time for completing questionnaires was 30 minutes.

Instruments

Eating Attitude Test – EAT [31]. This questionnaire evaluates attitudes, behaviors and traits characteristic of AN and disturbed and extreme eating patterns in the non-clinical population. It is a useful instrument to identify current or initial

cases of anorexia nervosa in the population without diagnosis. It consists of 40 Likert-type statements where respondents indicate their level of agreement with each statement expressed on a 6-point scale: 1 = always, 2 = very often, 3 = often, 4 = sometimes, 5 = rarely, 6 = never. The total score can range from 0 to 120 – a score greater than 30 indicates a preoccupation with food and weight and an increased risk of developing anorexia nervosa. The average score for the control sample of healthy women was 15.6, and for the anorexic patients 58.9. The EAT, applied on our sample, had good internal consistency ($\alpha = 0.83$).

The female students also indicated their height and weight for calculation of body mass index (BMI), an indicator of nutritional status, but also warning of anorexia nervosa syndrome if BMI ≤ 17.5 kg/m^2.

Experiences with the Close Relationships Scale – ECR [32]. This questionnaire examines patterns of emotional ties in adult intimate relationships and is based on a two-dimensional model of individual differences in adult affective bonding – anxiety and avoidance dimensions. Anxiety scale assesses preoccupation and fear of abandonment, and avoidance scale assesses fear of intimacy and discomfort in close relationships with others. These two dimensions thus give four attachment styles: secure, dismissing, preoccupied and fearful. The questionnaire consists of 36 Likert-type statements where respondents indicate their level of agreement with each statement expressed on a 7-point scale: 1 = strongly disagree, 2 = disagree, 3 = partially disagree, 4 = not sure, 5 = partially agree, 6 = agree, 7 = strongly agree. The ECR, applied on our sample, had good internal consistency ($\alpha = 0.89$), with scores on the subscales ranging from 0.84 to 0.89.

Frost Multidimensional Perfectionism Scale – FMPS [33]. This questionnaire assesses the severity of perfectionism. It includes six dimensions of perfectionism: Concern over Mistakes (CM) – negative reactions to mistakes that are considered to be a failure; Personal Standards (PS) – set extremely high standards as criteria of self-evaluation; Parental Expectations (PE) – the belief that parents set high standards and have high expectations; Parental Criticism (PC) – perception of parents as being too condemnatory-minded; Doubts about actions (D) – doubt their abilities and performance; Organization (O) – over-emphasizing the importance of order and tidiness. The questionnaire consists of 35 Likert-type statements and respondents indicate their level of agreement with each statement expressed on a 5-point scale: 1 = completely false, 2 = false, 3 = neither true nor false, 4 = true, 5 = completely true. The total score is the sum of all points and is in the range from 35 to 175. FMPS, applied to our sample, had good internal consistency ($\alpha = 0.88$).

Body Shape Questionnaire – BSQ [34]. This questionnaire measures concern about body shape, especially the experience of the phenomenon of "feeling fat", and refers to the condition of the respondents in the previous four weeks. It consists of 34 Likert-type statements, and respondents indicate their level of agreement with each statement expressed on a 6-point scale: 1 = never, 2 = rarely, 3 = sometimes, 4 = often, 5 = very often, 6 = always. The total score is the sum of points obtained in all claims to the test and is in the range of between 34 and 204. Scores can be classified in 4 categories: not worried about body shape < 81, slightly worried = 81–110, moderately worried = 111–140, extremely worried > 140. The average score for the non-clinical population is 71.9, and for people with eating disorders 136.9. The BSQ has not been used before in Serbia, so a pilot study was conducted on a sample of 50 students of the Faculty of Philosophy, showing good internal consistency ($\alpha = 0.96$). The use of the questionnaire was granted by permission of Prof. Peter Cooper.

Statistical analysis of the data

The collected data were statistically analyzed using SPSS. Descriptive statistics of the obtained data was conducted for all instruments (EAT, ECR, FMPS, BSQ) applied in this study and for the obtained BMI. In order to determine if there was a significant difference in the mean BMI between the female students with symptoms of AN and those who did not display the symptoms, we used the Student *t*-test. The correlations between the symptoms of AN and BMI was examined using correlation analysis. The χ^2-test was used in order to determine if there is a significant difference in the frequency of attachment styles between female students with symptoms of AN and those who do not display such symptoms. A correlation between symptoms of anorexia nervosa and attachment styles was examined using univariate analysis of variance. The correlations between symptoms of AN and perfectionism and body shape concerns were examined using correlation analysis. Multiple regression analysis was used to determine whether we could predict symptoms of AN by the subscales of anxiety and avoidance, perfectionism and concerns about body shape.

Results

Descriptive statistic (Table 1) showed that 38 (7.6%) female students had symptoms of AN according to the cut-off score on EAT (EAT > 30).

According to the BSQ score [34], 358 (71.6%) female students could be considered as not worried about body shape, 87 (17.4%) as slightly worried, 43 (8.6%) as moderately worried and 12 (2.4%) as extremely worried.

Table 2 shows descriptive statistics of BMI for the entire sample (n = 500) and for the subsample of female students with symptoms of AN (n = 38). In the entire sample and in the subsample of female students with symptoms of AN, minimal calculated weight belongs to the category of underweight, while mean weights belong to the category of optimal weight. For the entire sample, the maximum calculated weight belongs to the category of obese class III, while in the subsample with symptoms of AN the maximum calculated weight belongs to the category of overweight.

Based on scores on the EAT and BMI, one (0.2%) female student met both criteria for the diagnosis of AN (EAT > 30; BMI ≤ 17.50 kg/m^2). Three (0.6%) female students were preoccupied and worried about weight and food

Table 1

Minimal, maximal, mean and standard deviation of the data from the Eating Attitude Test (EAT), Experience in Close Relationship (ECR), Frost Multidimensional Perfectionism (FMPS) and Body Shape Questionnaire (BSQ) obtained from the entire sample (n = 500)

Test/Scale	Min	Max	$\bar{x}$	SD	n
EAT	2	87	14.81	10.975	500
ECR anxiety	1	6	2.76	0.987	500
ECR avoidance	1	6	3.11	0.789	500
FMPS	59	154	98.14	15.147	500
BSQ	34	178	70.53	28.436	500

Table 2

Minimal, maximal, mean and standard deviation of body mass index (BMI) for the entire sample (n = 500) and for the subsample of those female students who manifest symptoms of anorexia nervosa (n = 38), and the results of *t*-test for the average of BMI of these two groups of female students

Questionnaire	BMI, kg/m^2						
	min	max	$\bar{x}$	SD	n	*t*-test	*p*
EAT	15.64	54.50	20.84	2.869	500	-1.449	> 0.14
EAT > 30	15.64	29.38	21.48	2.814	38		

EAT = Eating Attitude Test; EAT > 30 = scores that are on EAT scale greater than 30.

(EAT > 30), and belonged to the category of underweight, and thus they were at high risk for developing AN.

Although there might have been expected to be a negative correlation, we did not find statistically significant correlation between scores on the EAT and BMI ($r = 0.067$; $p > 0.13$) on the whole sample (n = 500), as well as on the subsample ($r = 0.028$; $p > 0.86$; n = 38).

In order to identify female students who met the second criterion for AN syndrome, we calculated BMI proposed by the World Health Organization (WHO) in 1995, for those with symptoms of AN (n = 38) according to the cut-off score on EAT (Table 3). Among female students with symptoms of AN on EAT (n = 38), most of them belonged to the category of optimal weight, while the same number of them belonged to the category of underweight and overweight.

Based on scores on dimensions of anxiety and avoidance [32], attachment style for every participant was determined. Using a logarithmic equation, by crossing subscales of anxiety and avoidance, it was determined which attachment style is characteristic for each respondent in the sample. Secure attachment style was characterized by achievement of low scores on both subscales (anxiety and avoidance), while fearful attachment style was characterized by achievement of high scores on both subscales. Preoccupied attachment style was characterized by achieving of high scores on the anxiety subscale and low scores on the avoidance subscale. Dismissing attachment style was characterized by achieving low scores on the anxiety subscale and high scores on the avoidance subscale. The frequency and percentages of attachment styles for the entire sample (n = 500) and for the subsample of female students with symptoms of AN (n = 38). The majority of the respondents were characterized by some insecure attachment style (60.4%), while the secure attachment style was less represented than might be expected given that it was a non-clinical population. In the subsample of 38 female students with symptoms of AN (EAT > 30), the insecure attachment style dominated (81.6%), with the most prevalent fearful and dismissing style, while significantly less prevalent was the secure attachment style (Table 4).

In order to determine if there was a significant difference in the frequency of the attachment styles between the female students with the symptoms of AN (n = 38) and those who did not display them, (n = 462), we used the χ^2-test. There was a statistically significant difference in the

Table 3

Category of body mass index (BMI) in the subsample of female students with symptoms of anorexia nervosa (n = 38)

Questionnaire	Categories of body mass index (BMI), kg/m^2						
	≤ 17.50	< 18.50	18.50–24.99	25.00–29.99	30.00–34.99	35.00–39.99	≥ 40
EAT > 30	1	4	30	4	0	0	0

EAT > 30 = scores that are on Eating Attitude Test greater than 30.

Table 4

Prevalence and frequency of attachment styles for the entire sample (n = 500) and for the subsample of those female students who manifest symptoms of anorexia nervosa (n = 38)

Questionnaire	Attachment styles				n
	Secure	Fearful	Preoccupied	Dismissing	
EAT, n (%)	198 (39.6)	102 (20.4)	32 (6.4)	168 (33.6)	500
EAT > 30, n (%)	7 (18.4)	16 (42.1)	3 (7.9)	12 (31.6)	38

EAT – Eating Attitude Test; EAT > 30 – scores that are on Eating Attitude Test greater than 30.

frequency of categories of attachment styles between the female students who displayed and those who did not display the symptoms of AN (Pearson $\chi^2 = 14.349$; $p < 0.001$; $\bar{x} = 1.07$; SD = 0.265).

Univariate analysis of variance was used to examine the relationship between EAT scores and attachment styles. The female students with secure attachment style had the lowest average score on EAT, while those with some insecure attachment styles had higher mean scores, with the highest value in the female students with preoccupied attachment style (Table 5).

There is a significant difference in mean scores on the EAT between the female students with secure, fearful, preoccupied and dismissing attachment style. Univariate analysis of variance indicated that scores on the EAT were significantly correlated with attachment styles (F = 7.873; $p < 0.001$; df = 3;496). Eta coefficient was 0.202 and indicated was a significant prediction of symptoms of anorexia nervosa based on attachment styles in the amount of 4.1%, respectively these were explained variance scores on the EAT, based on attachment style. There was a statistically significant effect of the attachment styles on severity of symptoms of AN. Scores on Eating attitudes test were significantly different between the groups of female students with secure, fearful, preoccupied and dismissive attachment styles. In order to determine whether there was a significant difference in scores on the Eating Attitude Test between the female students with secure attachment style and the female students with some insecure attachment styles, we used one of the *post hock* tests, analysis of contrast. Based on the results obtained, we could conclude that there was a significant difference in mean scores on the EAT between the female students with secure and fearful attachment styles (value of contrast = 0.1412; $p < 0.001$; df = 496), those with secure and preoccupied attachment styles (value of contrast = 0.1548; $p < 0.003$; df = 496) and those with secure and dismissing attachment styles (value of contrast = 0.0591; $p < 0.036$; df = 496).

Correlation analysis was used to examine the relationship between EAT scores and perfectionism (Table 6). The female students with higher scores on the FMPS achieved higher scores on the EAT. From the six dimensions of perfectionism, four were significantly positively correlated with scores EAT: Concern over mistake, Personal standards, Parental expectations and Doubts about actions. All the correlations were positive and statistically significant. The female students with higher perfectionism expressed a tendency to have unhealthier eating attitudes.

Correlation analysis was used to examine the relationship between scores on the EAT and total scores on the BSQ. Pearson's correlation coefficient ($r = 0.388$; $p < 0.001$) indicated a significant positive correlation between symptoms of anorexia nervosa and concern about body shape. The female students with higher scores on the BSQ achieved higher scores on the EAT.

Multiple regression analysis was used to determine whether we could predict symptoms of AN by subscales of anxiety and avoidance, perfectionism and concerns about body shape. It indicated a significant linear correlation between a set of predictor variables – subscales of anxiety and avoidance, perfectionism and concerns about body shape, and the criterion variable – symptoms of AN (R = 0.409; $p < 0.001$; std. error = 10.056). There was a significant prediction of symptoms of AN based on scores on the subscale of anxiety and avoidance, perfectionism and body shape perception in the amount of 16.8%.

Table 7 shows beta coefficients of the subscales of anxiety and avoidance, perfectionism and concern about body shape as predictors of symptoms of AN.

The results shown in Table 7 indicate that the subscale of avoidance [32], perfectionism [33] and concern about body shape [34] are significant predictors of symptoms of AN. All the three beta coefficients had a positive sign, which indi-

Table 5

Minimal, maximal, mean and standard deviation of EAT scores and attachment styles for the entire sample (n = 500)

Attachment styles	EAT				
	min	max	$\bar{x}$	SD	n
Secure	2	59	12.40	7.782	198
Fearful	2	73	17.90	12.628	102
Preoccupied	6	87	18.13	14.940	32
Dismissing	2	81	15.13	11.649	168

EAT – Eating Attitude Test.

Table 6

Correlations between scores on the EAT and scores on the FMPS for the entire sample (n = 500)

Questionnaire	FMPS	CM	PS	PE	PC	D	O
EAT	0.217**	0.229**	0.195**	0.120**	0.070	0.111*	0.035

EAT – Eating Attitude Test; FMPS – Frost Multidimensional Perfectionism Scale; CM – Concern over Mistakes; PS – Personal Standards; PE – Parental Expectations; PC – Parental Criticism; D – doubts about actions; O – organization; ***Correlation significant at the level of 0.05; ****Correlation significant at the level of 0.01.**

Table 7

Beta coefficients of subscales of anxiety and avoidance, perfectionism and concern about body shape as predictors of symptoms of anorexia nervosa as predictors of symptoms of anorexia nervosa

Predictors	Beta coefficients	Significance	Correlation	Partial correlation
Subscale of anxiety of ECR	-0.004	0.935	0.202	-0.004
Subscale of avoidance of ECR	0.087	0.049*	0.181	0.088
Perfectionism	0.100	0.033*	0.217	0.096
Concern about body shape	0.338	0.000**	0.388	0.317

ECR – Experience in Close Relationship; *Correlation significant at the level 0.05; **Correlation significant at the level of 0.01.

cated that female students with higher scores on the subscale of avoidance, Multidimensional Perfectionism Scale and Body Shape Questionnaire, had higher scores on the Eating Attitudes Test.

Discussion

The mean score for all the female students (n = 500) on the EAT is as expected, compared to an average score for the non-clinical population of 15.6 [31]. The prevalence of female students with symptoms of AN is smaller than findings of other authors – 20% [3] and some results obtained in a similar study [4]. An average result on the FMPS on the student population is higher than results obtained among the American population [33]. The mean score on the BSQ is as expected, compared with the average score for the non-clinical population of 71.9 [34]. Average results on dimensions of ECR are very close to the results on the original sample [32].

The female students with secure attachment style had the lowest average score on the EAT. In the female students who reached the result on EAT above criteria for the diagnosis of AN (n = 38), insecure attachment styles predominated (81.6%), with fearful attachment style (42.1%) as dominant. Our results are in accordance with the data that secure attachment style represented significantly lower in the sample of female students with AN [13] and that insecure attachment styles associated with more severe symptoms of AN [11, 14, 15]. Yet, while some authors report that among women with symptoms of AN the most prevalent are preoccupied and dismissing attachment styles [11, 12], in our sample the most prevalent was the fearful attachment style.

Anorexia nervosa could be, as other self-disorders, the result of chronic parental difficulty in establishing and maintaining an empathic relationship with the child [8]. Disturbed behavior patterns characteristic of eating disorders may occur as a result of failure to develop secure relationships that form the basis of self-regulation and allow a person to gain support [35]. The emphasis is on the failure of development of autonomy, the lack of differentiation in relation to the mother and the search for control that occurs as a result of inability to cope with separation and individualization in adolescence [36]. The period of economic and cultural transition in Serbia might be a reason for the parents, as the figures of attachment, to exhibit high levels of stress, dysfunctional interactions, aggression and denial of the emotional needs of the child, but also the tendency to overprotect the child. These factors may be associated with insecure attachment styles, which are risk factors of anorexia nervosa. Negative self-image can affect the attitudes of a person to achieve a certain standard of beauty and attractive physical appearance and to gain a sense of security and control. Therefore, maladaptive attitudes related to nutrition often represent an attempt to achieve safety and self-esteem in the area where it may seem easiest to gain control over their lives. Furthermore, the lack of self-esteem and self-confidence and lack of confidence in other people can contribute to that person seeking to overcome emotional problems through dysfunctional eating behaviour and attitudes.

The results of this study indicate that more pronounced perfectionism is associated with higher expressed symptoms of AN. As the sample of this study consisted of female students, our results support other similar findings that perfectionism presents a potential risk factor for developing AN [16–18]. Based on this positive correlation between perfectionism and symptoms of AN, we can further assume that perfectionism influence setting unrealistic standards about physical appearance [19, 20]. We found that symptoms of AN were significantly correlated with dimensions of maladaptive perfectionism – concern over mistakes, parental expectations and doubts about actions. It should be noted that the dimension of adaptive perfectionism – personal standards – is also positively associated with symptoms of AN. These results were expected, as other authors already found different correlation between symptoms of dysfunctional eating attitudes and behavior and different dimensions of perfectionism [20, 21].

During student life, young women in Serbia are exposed to many pressures, high expectations, and adapting to a competitive atmosphere. Therefore, female students, influenced by the high standards set in society and the expectations that parents often impose, may develop unrealistic standards for themselves, which are sometimes perfectionistic. Setting very high and inflexible targets is associated with similar behaviour in the area of body image and weight. If they are very strict on themselves, they may exhibit these characteristics in order to achieve their goals in the area of physical attractiveness and to reach their desired weight.

A positive correlation between scores on the EAT and those on the BSQ was expected because many studies reported the same findings [27–29]. Female students who are more dissatisfied with their appearance and more worried about their body shape exhibit more symptoms of AN. Many authors already found that negative self-image represents a po-

tential risk factor for developing AN [22–24]. The results of our study are consistent with these findings, considering that female students who were not concerned about body shape had the lowest average score on the EAT, while those who were extremely worried about body shape had the highest average score on the EAT. Although Lowery et al. [29] find that 90% of female students are concerned about body shape, in our sample that number is much smaller (28.3%), which deserves further research in order to explain such discrepancy.

There is a cultural pressure that women should be slim. The mass media promotes slimness as a symbol of beauty and success – women represented in the media are generally thin which is associated with the ideals of attractiveness and the importance of physical appearance of success. Social comparison with others influences susceptibility to the imposed values in society. Also, there are many commercials on television and articles in women's magazines that are dedicated to effective weight loss. The ideals of thinness are promoted by peers too, and those who seek to depart from such ideals are often subject to teasing. All the above mentioned factors often affect dissatisfaction with the appearance of young women. Further, they can affect the development of maladaptive attitudes and habits regarding diet in order to achieve these ideals.

In order to prevent symptoms and syndrome of AN in female students, it would be useful to organize lectures about eating disorders and educational workshops at universities in Serbia. Taking into account that some female students could be embarrassed to talk openly about their problems, it would be useful to create a website where they can find useful information about AN and professional help through internet-based therapy. The most important goal would be creation and development of specialized counseling services, where young women could find help to cope with problems of anorexia nervosa and eating disorders in general. Specific programs should be focused on enhancing emotion regulation skills, including mindfulness and acceptance and commitment techniques, as well as special tailored forms of cognitive-behavioral techniques for enhancing more realistic, logic and useful attitudes towards eating, body image, themselves in general, others and their surroundings.

Limitations of the study

This study has some limitations, such as non-systematic measurement of respondents' height and weight. Also, although we know that external factors, such as influence of the family, friends, mass media and culture values and pressures are also very important risk factors for developing AN, our study was focused only on internal contributing factors. Further, the obtained findings are applicable to the female students' population and for better understanding AN, it is necessary to conduct research in a heterogeneous or clinical sample. Nevertheless, our results obtained on 500 female students of the University of Belgrade, provide a reliable basis for further research and efforts for improving treatment and prevention of AN symptoms and syndrome.

Conclusion

The majority of female students have a certain form of insecure attachment style. Those students with insecure attachment style and with higher perfectionism exhibit more dysfunctional attitudes and unhealthier behavior towards food. Furthermore, female students who are concerned about body shape manifest more symptoms characteristic of anorexia nervosa. The prevalence of anorexia nervosa was 0.2%, but the symptoms of anorexia nervosa were more frequent and should not be underestimated.

Disturbed eating attitudes and behavior and insecure attachment styles, perfectionism and body shape concerns were identified as risk factors for anorexia nervosa among female students, and may serve as an important basis for targeted interventions in the prevention and treatment of anorexia nervosa in this subpopulation.

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Quality of life of elderly people living in a retirement home

Kvalitet života starih osoba koje žive u gerontološkom centru

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Abstract

Background/Aim. The World Health Organization (WHO) identified four broad domains as being universally relevant to the quality of life, namely physical, and psychological health, social relationships, and environment. The aim of this study was to assess the relationship between sociodemographic characteristics and quality of life of old people. **Methods.** The World Health Organization Quality of Life BREF questionnaire (WHOQOL-BREF) was used to assess quality of life on a random sample of 200 people aged 60 years and over who lived in the Retirement Home in Novi Sad. Items within the questionnaire were organized into four domains: physical, psychological, social relationships and environment. **Results.** The majority of the participants were women (69.8%). The mean age was 79.2 years (SD = 6.6 years). Most of them were widowed (73.4%). More than two thirds of participants (68.8%) reported that they were ill at that moment and almost half of them (48.8%) had cardiovascular, 18.5% musculoskeletal, 9.6% endocrine and 5.9% neurological disease. In the social relations domain scores were lower in males ($t = 2.4$; $p = 0.017$). Scores of other domains did not differ significantly with regard to the age, educational level and the marital status of the participants. Participants who reported the presence of a disease had significantly lower mean scores of physical, psychological and environment domain. **Conclusion.** The presence of disease is a relevant factor for quality of life, whereas age, education and marital status do not reflect on physical health, psychological and environmental domain of quality of life.

Key words:
aged; homes for the aged; quality of life; questionnaires; serbia.

Apstrakt

Uvod/Cilj. Svetska zdravstvena organizacija je identifikovala četiri osnovna domena povezana sa kvalitetom života: fizičko i psihološko zdravlje, socijalne veze i okolina. Cilj rada bio je da se utvrdi povezanost između sociodemografskih karakteristika i kvaliteta života starih osoba. **Metode.** Za procenu kvaliteta života korišćen je upitnik Svetske zdravstvene organizacije o kvalitetu života – kratka verzija (*The World Health Organization Quality of Life* BREF *questionnaire* – WHOQOL-BREF) na slučajnom uzorku od 200 osoba starosti 60 i više godina koje žive u Gerontološkom centru u Novom Sadu. Pitanja u upitniku bila su organizovana u četiri celine: fizičko i psihološko zdravlje, socijalne veze i okolina. **Rezultati.** Većina ispitanika bile su osobe ženskog pola (69,8%). Prosečna starost iznosila je 79,2 godine (SD = 6,6 godina). Najviše je bilo udovaca i udovica (73,4%). Više od dve trećine ispitanika (68,8%) izjavilo je da su u trenutku istraživanja bili bolesni, a skoro polovina njih (48,8%) imala je kardiovaskularnu bolest, 18,5% mišićnokoštanu, 9,6% bolest endokrinih žlezda i 5,9% neurološku bolest. U domenu socijalnih veza skorovi su bili niži kod muškaraca ($t = 2,4$; $p = 0,017$). Drugi skorovi nisu se značajno razlikovali u odnosu na starost, nivo obrazovanja i bračni status ispitanika. Ispitanici koji su izjavili da su bolesni imali su značajno niže srednje vrednosti skora fizičkog i psihološkog zdravlja i domena okoline. **Zaključak.** Prisustvo bolesti je značajan faktor koji utiče na kvalitet života, pri čemu starost, obrazovanje i bračni status ne utiču na domen fizičkog i psihološkog zdravlja i domen okoline kvaliteta života.

Ključne reči:
stare osobe; staračke domovi; kvalitet života; upitnici; srbija.

Introduction

Quality of life (QoL) is not a new concept. Jonathan Swift noted that every man desires to live long, but no man wishes to be old. Isaac Stern had expressed a similar statement when he advised that everyone should die young, but they should delay it as long as possible [1]. The core of the QoL concept is to understand a human being and its needs, from different perspectives, keeping in mind that a human being is in constant interaction with the surroundings, according to the holistic-ecological approach [2]. Quality of life spans a broad range of topics and disciplines. It is made up

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of both positive and negative experiences and affect. It is a dynamic concept, which poses further challenges for measurement [3]. After a long scientific discussion, quality of life is still a concept which is difficult to define. The World Health Organization (WHO) Quality of Life Group developed a definition frequently used in theoretical framework. WHO defines quality of life as an individual's perception of their position in life in the context of the culture and value systems in which they live, and in relation to their goals, expectations, standards and concerns. It is a broad ranging concept, incorporating in a complex way a person's beliefs and relationship to salient features in the environment [4].

Ageing is unprecedented, a process without parallel in the history of humanity. At the world level, the number of older persons is expected to exceed the number of children for the first time in 2045. In the more developed regions, where population ageing is far advanced, the number of children dropped below that of older persons in 1998. It is an enduring process. Since 1950, the proportion of older persons has been rising steadily, passing from 8% in 1950 to 11% in 2009, and is expected to reach 22% in 2050 [5]. People in Europe are older than any other world region. According to the United Nations Population Fund, 2012 in Serbia people over 60 accounted for 20.5% and are expected to increase to 32.2% in 2050 [6]. The ageing of population in Serbia, as well as the whole world population, is the problem which we have to face with.

The elderly in the future will undoubtedly suffer from a variety of diseases leading to disability and reduced quality of life [7]. The interests of the elderly and improving the quality of life in this age, including their health concerns, need to be a priority in the coming years.

Bilgili and Arpacı [8] in a recent study stated that QoL of elderly people needs to be more analyzed, since the majority of recent studies were focused on instrument psychometric characteristics and less on QoL of this population group.

The aim of this study was to assess the relationship between the socio-demographic characteristics and the quality of life of old people living in retirement home.

Methods

The study was conducted in 2009 on a sample of 200 people, representing 25% of the total number of residents of The Retirement Home. Systematic random sample (k = 4) was used in this study. Through random selection, every fourth person from the list of residents of The Retirement Home, which satisfied the criteria, was chosen to participate in this research. The criteria were: aged 60 years or older, able to communicate and oriented in all three directions, the respondent not situated in the stationary part of the home. Data was collected through interviews done by researchers. Ethical approval was obtained from the Faculty of Medicine in Novi Sad. A letter of introduction describing the study was given and a written informed consent was obtained from all the participants before interviewed questioning with the WHOQOL-BREF questionnaire.

The Bosnian-Croatian-Serbian version of WHOQOL-BREF was used in this study and this language version was obtained from The WHOQOL Group. The WHOQOL-BREF is an abbreviated 26-item version of the WHOQOL-100 and it is based on four domain structure (Physical health, Psychological, Social relationships and Environment). Each domain includes three to eight items. Moreover, two questions yield information on the global QoL, and health satisfaction. Each item is based upon self-report and scored on a 5-point Likert scale. The scores are transformed on a scale from 0 to 100 (higher score points to better quality of life). The time frame for responses was the previous two weeks. An additional 6 questions were included concerning sociodemographic characteristics such as age, gender, marital status and educational level, as well as the present health status. The results from 23 countries showed good internal consistency reliability and construct validity for the international WHOQOL-BREF questionnaire [9]. The sensitivity of the questionnaire for assessing quality of life of elderly people who living in the retirement home was tested by examining the validity and reliability. It is a valid and reliable quality of life instrument for older people [10].

Statistical analysis was performed using the statistical package SPSS 14.0 for Windows. Results are given as mean value and proportion. Differences in sample means were tested by Student's *t*-test (to compare means of the two groups) and ANOVA (to test differences between more than two groups). The level of statistical significance was set at $p < 0.05$.

Results

Of the 200 subjects interviewed, 199 were analyzed (one case was deleted with more than 20% missing data). Table 1 shows sociodemographic characteristics as well as the presence of disease in the study group.

Table 1
Distribution of the sociodemographic characteristics and the presence of disease in the study group

Sociodemographic characteristics	n (%)
Sex	
male	60 (30.2)
female	139 (69.8)
Age (years)	
60–69	20 (10.1)
70–79	71 (35.7)
≥ 80	108 (54.3)
Education	
none at all, primary school	80 (40.6)
high school no degree	31 (15.8)
high school degree	49 (24.6)
college degree and above	37 (18.7)
Marital status	
separated, divorced	34 (17.1)
with partner	19 (9.5)
widowed	146 (73.4)
Presence of disease	
yes	137 (68.8)
no	62 (31.2)

The majority of participants were women (69.8%). The highest percentage of respondents was found in the age group 80+ (54.3%). The mean age was 79.2 years (SD = 6.6, range 63–97 years). With regard to education level, 40.6% indicated no education or primary school, 15.8% high school

without degree, 24.6% high school degree and 18.7% college degree and above. Most of them were widowed (73.4%). More than two thirds of participants (68.8%) reported that they were ill at that moment and almost half of them (48.8%) had a cardiovascular disease, 18.5% a musculoskeletal, 9.6% endocrine and 5.9% a neurological disease. The most frequently reported diagnosis was angina pectoris (15.6%).

Scores were lower in males in the social relations domain ($t = 2.4$; $p = 0.017$). The scores of the other three domains (physical health, psychological and environment) as well as total score did not differ significantly with regard to the gender. There was no significant association between age, educational level, marital status of participants and scores of all domains. The participants who reported the presence of a disease had significantly lower mean scores of the physical health ($t = 5.2$; $p = 0.000$), psychological health ($t = 3.1$; $p = 0.002$), and environment domain ($t = 2.2$; $p = 0.029$) and total WHOQOL-BREF score ($t = 3.7$; $p = 0.000$) (Table 2).

Table 2

Mean score of all domains and the World Health Organization Quality of life – BREF questionnaire (WHOQOL-BREF) sociodemographic characteristics, and the presence of disease in the study group

	Domains mean score				
Sociodemographic characteristics	Physical health	Psychological health	Social relations	Environment	WHOQOL-BREF
Sex					
male	70.0	68.5	60.7	71.2	67.6
female	64.7	63.7	67.8	66.4	65.6
t	1.75	1.53	2.4	1.9	0.84
p	0.082	0.126	0.017	0.056	0.401
Age (yars)					
60–69	66.9	66.7	64.8	66.7	66.3
70–79	66.1	64.9	65.1	68.0	65.9
≥ 80	66.4	64.9	66.2	67.9	66.3
F	0.01	0.06	0.08	0.05	0.02
p	0.987	0.937	0.919	0.946	0.978
Education					
none at all, primary school	63.1	62.9	65.2	65.5	64.0
high school no degree	73.8	67.4	71.8	70.4	70.8
high school degree	67.7	65.4	64.8	69.6	66.9
college degree and above	65.8	66.9	62.5	68.2	65.8
F	2.3	0.54	1.4	1.0	1.44
p	0.075	0.655	0.252	0.393	0.231
Marital status					
separated, divorced	67.9	67.8	64.5	68.2	67.1
with partner	64.7	68.2	64.2	70.4	66.9
widowed	66.2	64.1	66.1	67.4	65.9
F	0.17	0.19	0.14	0.3	0.1
p	0.837	0.489	0.869	0.752	0.898
Presence of disease					
yes	61.8	62.2	64.3	66.2	63.5
no	76.4	71.5	68.6	71.6	72.0
t	5.2	3.1	1.4	2.2	3.7
p	0.000	0.002	0.147	0.029	0.000

***t* – Student's *t*-test; F – ANOVA.**

Discussion

Aging causes health and social problems. It means that elderly people have to deal with certain obstacles and difficulties. In addition, there is a lack of everyday activities and the quality of life begins to decline [11]. However, there are studies which reported a higher quality of life in the elderly compared with younger people [12, 13].

Our study examined the quality of life of elderly people living in a retirement home. The study included respondents who use this facility primarily as a residence place and they are capable to take care of themselves independently. However, we should take into account the specific characteristics of life in the community, therefore the findings cannot be completely generalized to the whole population of old people, or it should be done with caution.

In this study one of three respondents considered himself healthy. The most frequently reported diagnoses were from the cardiovascular diseases group. A similar result was obtained in a study performed on elderly people living in rural areas in Turkey. Almost one third of the elderly had no medically diagnosed chronic disease, while the three most frequently occurring chronic diseases were hypertension, rheumatism-related diseases and diabetes [11]. A Taiwan study showed that 10% of the elderly had no medically diagnosed diseases and the most frequent disease were hypertension, stroke, musculo-skeletal diseases and diabetes [14]. Participants who had some kind of disease scored all domains but social relations significantly lower than those who had not.

Considering gender differences, only the social relations domain was significantly lower in men. Scores of other domains were higher in men, but the difference was not significant. Barua et al. [15] revealed that scores of all four domains had not been affected by gender. A study conducted in

Austria on persons aged 57–70 and older than 70 showed that women from a younger age group had higher values of the physical health domain, compared to men, in contrast to women older than 70 years, but in both cases difference was not significant [16]. Other studies confirmed that values of this domain were statistically higher in men [11, 17]. The same result considering psychological health domain was reported in the literature [15, 16, 18]. On the other hand, women had lower values of this domain in the study of Arslantas et al. [11]. Scientists discovered that the loss of physical ability is more expressed in old aged women and this often can lead to depression [19, 20]. How important the gender difference is in quality of life was discussed in a study of Kirchengast and Haslinger [16] who found that older women, especially those aged over 70 years, were more likely to live alone; of these women 47.6% were widowed. In contrast, only 5.4% males same age, like the female group, lived without a partner and only 2.7% were widowed. Besides that, women had significantly less stable employment histories, lower income, and lower pensions than men. All of these factors can cause disorders in the psychological sphere of the quality of life.

Contrast to our results, gender did not affect the social relations domain in several studies [11, 15–17]. Consistent with previous research the environmental domain score did not differ significantly according to gender [15, 16]. Also, environment domain did not show differences between groups concerning other sociodemographic characteristics, probably due to the fact that all participants live in retirement home, therefore they probably have the same living condition, have same opportunity for leisure, similar means of transportation and health services.

There were no statistically significant differences in the average values of the physical health domain according to age in this study, although the opposite could have been expected on the basis of the results of the previous research. Older age is associated with the deterioration of physical abilities that has an affect on the quality of life [11, 15, 17]. Our results suggest that older adults were able to actively adjust the physical changes that appear with aging and kept a positive attitude towards it. On the other hand, the respondents from our study lacked positive feelings, or thought they did not know how to enjoy life. There were no statistically significant differences in mean values of psychological health domain according to the age group even though the youngest group (60–69 years) had the highest value. Perhaps it could be explained by the fact that significant changes in life and psychological adaptation on new situation appear by the age of 65, therefore all later changes are of less importance.

Social factors such as social integration, having a purpose in life and community affiliation were identified as very important factors for the quality of life in older people [21]. Other factors include self-esteem, a sense of their personality and their identity, sense of control, and spiritual well-being. These concepts are important for older people, giving them a positive view of themselves, and have an impact on the relationship with their friends and family in their activities. It is also important to their ability to handle, adapt to change and make sense of their life [22]. Higher-level social companionship was associated with the development of less depression [23]. The social relations domain was represented with only 3 items (personal relationships, social support and sexual activity). The sexual activity item had the lowest response rate in the whole questionnaire (60%), similar to previous research [14, 24]. The average age of participants (79.2 years) could be cause of the low response rate of this item, moreover 73.4% of them were widowed, but cultural and psychological elements also cannot be omitted.

In our study, educational level did not have influence on the quality of life of old people living in a retirement home. The same conclusion was in made the previous research done in geriatric population [15, 25].

Marital status was not associated with significant changes in the quality of life in our study. Hagedoorn et al. [26] deeply explained the role of marriage. It seems that marriage does not protect the elderly from psychological pain, and widows are apparently able to adapt well to their new role as an individual. No doubt that marriage has its advantages (spouse support, friendship and self-esteem), especially if marriage is harmonious, however, these benefits do not explain the higher levels of distress among single people. Singles also have lasting and significant interpersonal relationships from which they can gain the benefit. It can cautiously be concluded that marriage can be harmful if people feel undervalued and dissatisfied in marriage [26].

There are several limitations of the study. It included only the residents of retirement home, not the general population aged 60 and more.

The participants in our study were mostly from the group 80 or older and widowed. But, despite the limitation, the authors wish to emphasize that this topic is less explored in Serbia, therefore, any contribution is a step forward in efforts to improve quality of life of elderly. The results also provide the basis for those wishing to use WHOQOL-BREF instrument to investigate the quality of life of elderly.

Conclusion

The presence of disease is a relevant factor for quality of life, whereas age, education and marital status do not reflect on physical health, psychological health and environmental domain of quality of life.

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Choice of root canal irrigants by Serbian dental practitioners

Izbor rastvora za ispiranje kanala korena od strane stomatologa u Srbiji

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Abstract

Background/Aim. Root canal treatment is considered to be the one of the most important procedures in endodontic treatment. To irrigate the root canal it is most common to use sodium hypochlorite (NaOCl), chlorhexodine, ethylenediaminetetraacetic acid (EDTA), local anesthetic solution, while the most used in Serbia is hydrogen peroxide (H_2O_2). The aim of this survey was to reveal the preferred root canal irrigants used by general dental practitioners in Serbia and to determine the influence of the continuing education program, delivered over the 3-year observation period, on work habits of dental practitioners. This was the first comprehensive survey of this nature carried out in Serbia. **Methods.** The survey was conducted in two instances, a 4-month observation period each, from November 1, 2009 to March 1, 2010 and from November 1, 2012 to March 1, 2013. Internet Web Page Survey was posted on the official web site of the Serbian Association of Private Dentists. In the first survey, 569 completed questionnaires were subjected to analysis. In the next attempt (3 years later), the survey was launched again and 615 completed questionnaires were analyzed using the same criteria. The statistical analysis was carried out with simple descriptive statistics applying the $\chi 2$ test, at a significance level of $p < 0.05$. **Results.** The first survey included 569 dental practitioners, while 3 years later the number of them was 615. Analyzing the questionnaires revealed the number of 10 to 30 interventins on the root canal montly. The most commonly used irrigant solution was H_2O_2 in 2009, while in 2012 it was yet H_2O_2, but also NaOCl, chlorhexodine, and a little less EDTA. **Conclusion.** This study shows significant changes in the irrigation protocol applied in Serbian dental community. After 3 years of observation, NaOCl became widely accepted as the irrigant of choice, whereas H_2O_2 lost its popularity.

Key words:
tooth, root; irrigation; serbia; questionnaires; education, medical.

Apstrakt

Uvod/Cilj. Lečenje korenskog kanala smatra se jednim od najvažnijih koraka u endodontskom lečenju. Za ispiranje korenskog kanala najčešće se koriste natrijum-hipohlorit (NaOCl), hlor-heksidin, etilen-diaminotetrasirćetna kiselina (EDTA), lokalni anestetik, a u Srbiji najčešće vodonik peroksid (H_2O_2). Cilj ovog istraživanja bio je da se odrede primarni irigacioni rastvori koje koriste opšti stomolozi u Srbiji, kao i da se utvrdi mogući uticaj kontinuiranog obrazovanja na svakodnevne procedure endodontske terapije u toku 3-godišnjeg opservacionog perioda. Ovo je prva anketa ove vrste i ovog obima izvedena na teritoriji Srbije. **Metode.** Istraživanje je sprovedeno prikupljanjem podataka u dva četveromesečna perioda, od 1. novembra 2009. godine do 1. marta 2010. godine i od 1. novembra 2012. do 1. marta 2013. godine. Anketni obrazac bio je postavljen na zvanični WEB portal Srpskog udruženja privatnih stomatologa, i sadržao je 90 pitanja vezanih za godine bavljenja praksom, tehnike i instrumente vezane za proceduru, kao i za vrstu korišćenih irigacionih rastvora. U okviru prve ankete analizirano je 569 popunjenih upitnika. Tri godine kasnije, u ponovljenom istraživanju, istim setom kriterijuma analizirano je 615 popunjenih obrazaca. Statistička analiza sprovedena je jednostavnom deskriptivnom statistikom, $\chi 2$- testom, sa nivoom značajnosti od $p < 0.05$. **Rezultati.** U prvoj anketi učestvovalo je 569 stomatologa, a nakon tri godine 615. Analiza upitnika pokazala je da je broj intervencija na korenskom kanalu iznosio od 10 do 30 mesečno. Najčešće korišćen rastvor za irigaciju bio je H_2O_2 u 2009. godini, a 2012. godine on se još uvek često koristio kao rastvor za ispiranje, ali, takođe, korišćeni su u većem obimu i NaOCl, hlorheksidin, a nešto manje i EDTA. **Zaključak.** Studija je ukazala na značajne promene u primeni irigacionog protokola među srpskim stomotolozima. Po isteku 3-godišnjeg opservacionog perioda, NaOCl je postao široko rasprostranjeno, preferentno irigaciono sredstvo, dok je H_2O_2 izgubio na popularnosti.

Ključne reči:
zub, korenski kanal; lavaža; srbija; upitnici; edukacija, medicinska.

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Introduction

Root canal treatment (RCT) is considered to be the essential element of dental services delivered to the population in developed countries. Various investigations were carried out to explore the standards and trends of endodontic treatment performed by general dental practitioners worldwide [1–9]. However, the data on the attitude of general dental practitioners toward various aspects of endodontic treatment in developing countries are still scarce and/or inadequate.

Irrigation of root canal system is recognized as one of the most important steps, and the most critical one during endodontic treatment. Despite modern technologies and equipment, more than one third of the root canal surface can be left uninstrumented [10]. The residuals of necrotic or vital tissue within the root canal space are the main etiological causes of endodontic failures, and therefore the irrigation protocol plays a key role in disinfection of the root canal space. As there is still no ideal root canal irrigant described by Zehnder [11], many kinds of endodontic irrigants have been investigated and none of them has been able to exhibit all the desired properties.

Sodium hypochlorite (NaOCl) seems to be the most popular irrigant, since it has a broad antibacterial spectrum, while also possessing some ability to inactivate endotoxins [1–6, 12]. Therefore, NaOCl remains the irrigant of choice worldwide in spite of its high toxicity, inability to completely remove the smear layer, and very unpleasant taste to patients. However, antibacterial trait of 2% chlorhexidine [13] has made it one of commonly used endodontic irrigants. Ethylenediaminetetraacetic acid (EDTA), a chelating agent that helps in removing the inorganic component of the smear layer [14], is also known as one of frequently applied root canal irrigants [10]. Local anesthetic solution has been reported as routinely used endodontic irrigant amongst some dentists in UK [15], while hydrogen peroxide of 3% was popular and widely used amongst Serbian dentists in Serbia previously [16, 17].

In spite of the progress that academic teaching and endodontic societies have made so far in this field of growing interest, there is a lack of relevant information regarding the attitude of general dental practitioners towards irrigation protocol. What still remains unknown is how far the changes in endodontics have been incorporated into daily practice of private surgeries and public hospitals in Serbia. So far, there have been neither surveys nor researches concerning endodontics standards and general attitude toward root canal treatment in Serbian dental community.

The aim of this survey was to reveal the preferred root canal irrigants used by general dental practitioners in Serbia and to determine the influence of continuing educational program, delivered over the 3-year observation period, on work habits of dental practitioners. This was the first comprehensive survey of this nature carried out in Serbia.

Methods

This longitudinal survey was designed to cover some important aspects of endodontics. The appropriate questionnaire was designed of 90 questions that comprised the following items: main professional activity, years of professional activity, willingness to perform endodontics, reasons not to perform root canal treatment, details on working environment and equipment, use of rubber dam, applied root-canal preparation techniques, choice of instruments, sterilization procedures, choice of root-canal irrigant, utilization and choice of intracanal medication, etc.

For the purpose of this particular part of investigation, only questions related to selection of root canal irrigants were extracted and analyzed.

Internet Web Page Survey was posted on the official web site of the Serbian Association of Private Dentists, easily accessible to all its members (http://www.privstom.org.rs). An introductory cover letter that clearly stated the purpose of the survey was followed by the questionnaire designed to provide reliable answers to the research questions raised. Data were collected during the four-month observation period starting from November 1, 2009 to March 1, 2010 and then from the November 1, 2012 to March 1, 2013. In order to make a more detailed comparison of the data, the sample obtained from both surveys was divided into groups defined by the years of professional experience as follows: group 1 (less than a year of professional experience); group 2 (2–5 years); group 3 (5–10 years); group 4 (10–15 years), group 5 (15–20 years), and group 6 (more than 20 years of clinical practice).

The data were presented by tables and figures. Mean values of endodontic treatments obtained in the two observation periods were calculated using the Students *t*-test. Comparison of RCTs in relation to the type of irrigants was performed with ANOVA test. A difference between the groups was determined by *post hoc* analysis. Comparison of frequency was performed with nonparametric $\chi 2$-test. A correlation between the number of RCTs and years of professional experience was performed by Pearson and Spearman correlation. Statistical analysis was performed with the SPSS (version 18) at a significance level $p < 0.05$.

Results

In the first survey, 569 completed questionnaires were obtained and subjected to analysis. In the next attempt (three years later), the survey was launched again and 615 obtained questionnaires were analyzed using the same criteria. There were 369 repeated respondents who were surveyed in both observation attempts.

Responses were obtained from the different groups of participants clustered by the years of professional experience. It is obvious that the respondents were not evenly distributed in terms of the elapsed professional experience. By observing the obtained statistical sample of 1,184 respondents in total (569 in 2009 and 615 in 2012), it can be noticed that the majority of the respondents had professional expereince from 6–10 years: 45.3% in 2009 and 36.7% in 2012 (Figure 1).

There was a wide variation in the number of root canal treatments (RCTs) performed *per* month ranging from less than 10 to over 30. The average number of RCTs in 2009 was 15.36 ± 5.94, and it was statistically nonsignificant when compared to the avarage number of RCTs in 2012 : 15.12 ± 7.03 ($t = 0.586$; $p = 0.558$). However, there was a significant

correlation between the number of RCTs and years of proffesional experience in 2009 (r = 0.523; $p < 0.001$), and that correlation was almost the same in 2012 (r = 0.302; $p < 0.001$). Almost all experienced dentists stated that they have completed between 10 and 30 root canal treatments each month in 2009, as well as in 2012 (Table 1).

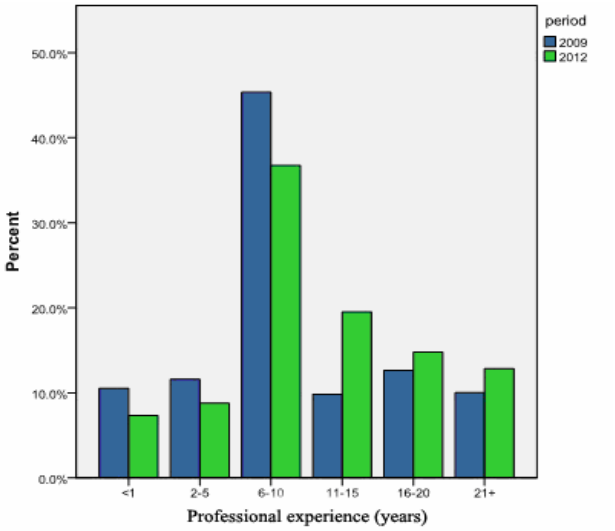

Fig 1 – Distribution of the respondents according to years of professional experience.

The most popular irrigant in the first survey was hydrogen peroxide, and it was used by all respondents regardless of their years of professional experience. Its use was significantly greater than that of any other solution ($p < 0.001$). It was highly significant that in the 3 groups (with professional experience from 6 to 20 years) hydrogen peroxide was the only irrigant for root canal treatment. The most experienced practitioners (group 6), and very young dentists (group 1) used broader variety of solutions in the irrigation protocol than the other groups. The respondents with professional experience of less than one year also used chlorhexidine and EDTA.

NaOCl was used as root canal irrigans only among dentists with 2–5 years of professional experience (25.8%) (Figure 2).

None of the respondents declared the use of either MTAD (a mixture of doxycycline, citric acid and a detergent) or artificial saliva as a root canal irrigant.

However, in the second survey, the ratio between the use of hydrogen peroxide and sodium hypochlorite significantly changed since NaOCl became the most popular irrigant in all the groups ($p < 0.001$). Hydrogen peroxide was still popular among all the respondents, but significantly less than in the previous survey ($p < 0.001$) (Figure 2). The second survey also revealed the increased use of chlorhexidine, especially in the first group comprising young dentists. More than 10% of dentists in the third group started to use chlorhexidine during the

Table 1

Frequency and the number of root canal treatments (RCT) according to years of professional experience

Years of professional activity	RCT/month							
	<1	2–5	6–10	11–15	16–20	21+	Total	*p*
2009, n (%)								
< 10	60 (100.0)	48 (72.7)	36 (14.0)	32 (57.1)	0 (0.0)	0 (0.0)	176 (30.9)	
10–30	0 (0.0)	18 (27.3	222 (86.0)	24 (42.9)	72 (100.0)	57 (100.0)	393 (69.1)	< 0.001
2012, n (%)								
< 10	45 (100.0)	36 (66.7)	30 (13.3)	67 (55.8)	22 (24.2)	0 (0.0)	200 (32.5)	
10–30	0 (0.0)	18 (33.3)	196 (86.7)	53 (44.2)	69 (75.8)	79 (100.0)	415 (67.5)	< 0.001

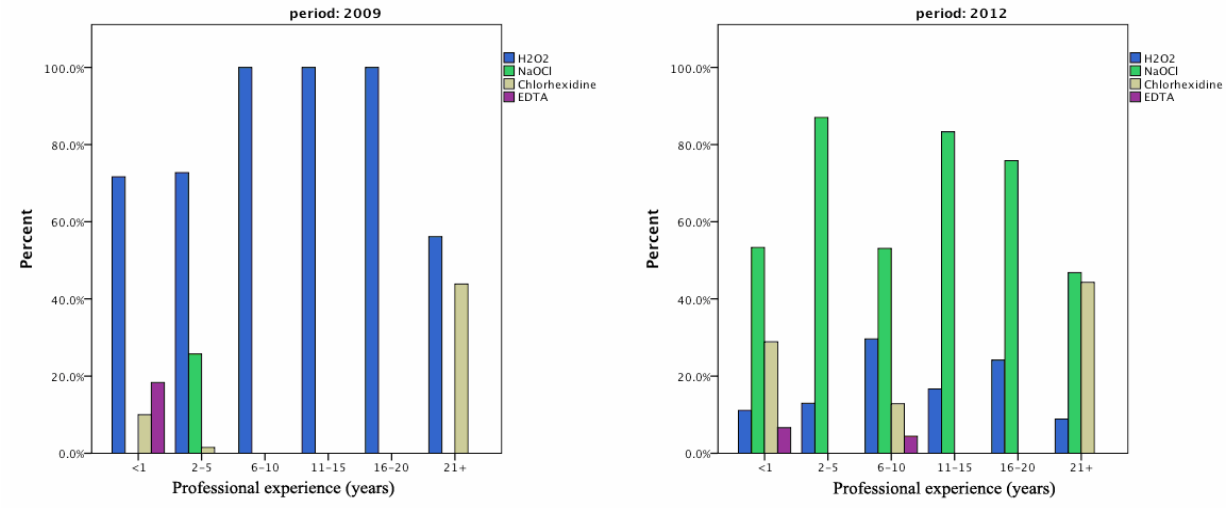

Fig. 2 – Preferred root canal irrigants *per* respondent groups.

observatio period. EDTA did gain minor popularity, but far behind NaOCl.

In the 2009 research, the majority of dentists who used sodium hypochlorite (57%) chose the full-strength concentration (> 5.0%). Three years later, only 6.2% of all the respondents used full-strength NaOCl concentrations, which is an obvious statistically significant difference. (χ^2 =107,9; p < 0,001). In the second survey, the use of concentration of 2.5% was significantly greater than in the previous survey (59% *vs* 18.3%, respectively; $\chi^2 = 35.30$; $p < 0.001$) (Table 2) .

Table 2
Preferred concentration (%) of sodium hypochlorite (NaOCl)

Concentration of NaOCl (%)	Dentists(%)	
	2009	2012
1.5	30.00	34.80
2.5	18.33	59.03
5.0	51.67	6.17

None of the dentists reported using rubber dam routinely. There were 7 (1.23%) respondents who stated that they occasionally used rubber dam for endodontic treatment in 2009. In the repeated survey this number was almost the same, 8 respondents (1.3%). There was no correlation between its use and years of professional experience, and it was an insignificant factor to be correlated with the choice of root canal irrigants.

Discussion

This survey encompassed 569 licensed general dental practitioners in 2009, and 615 general dental practitioners in 2013 and thus provided a large basis for the research (it should be noted that there were less than 6,000 licensed dental practitioners in Serbia during the entire observation period). Thus, the number of respondents in both surveys was at least double compared to other dental communities where similar surveys were previously employed: 602 questioners were analyzed in an endo survey in Turkey [1], 131 replies were collected from an endodontic survey in North Jordan [5], 343 valid responses were processed from questionnaires of endo survey from Hong Kong [3], whereas less than 300 answers were obtained from a Flemish survey [6], and 205 dentists were surveyed in a pilot study from Saudi Arabia [18].

The respondents with professional experience ranging from 6 to 15 years (groups 3 and 4) comprised more than half of total respondents in both the first and the second survey, similarly to the findings of a Turkish endodontic survey from 2012 [1]. The most of the responses came from the group with professional experience from 10 to 15 years. The same trend was noticeable in some other previously mentioned investigations [1, 3]. In the second survey, the number of respondents in this specific group doubled. The prospective reason could be a natural migration of practitioners from the group 3 (as defined in the survey of 2009) to the next one. The number of responses among predefined groups (according to the years of professional experience) was not evenly distributed, and the number of responses in each of the groups might not reflect the present state in the Serbian dental community. It rather highlights the fraction of dental practitioners that regularly take part in continuing education programs, and those with more than average exposure to the modern IT technologies.

The average number of RCTs in both periods was almost the same, and it was quite similar to findings from research conducted in Turkey [1] (15 *vs* 12.8 RCTs *per* month). However, there was a significant correlation between the number of RCTs and years of professional experience in both periods, which is in contrast to findings from the survey mentioned above, where no correlation was found between the number of RCTs and the age of the practitioners. In our study, the practitioners working more than 16 years performed significantly more RCTs than others. It appears that experienced practitioners could be better supplied with modern endodontic instruments and technology (e.g. rotary endo). Moreover, their experience-based self-confidence could play a considerable role in this matter. In the same manner, experienced practitioners could accomplish more specific trainings on endodontics techniques.

The most popular irrigating solution in the first survey from 2009 was H_2O_2. At that time, the usage of hydrogen peroxide was significantly greater than the usage of any other solution ($p < 0.05$). It was revealed that 89.46% of the respondents used hydrogen peroxide, whereas only 8.9% of them chose NaOCl. In the past, hydrogen peroxide was popular amongst European dentists [19], but in recent years it has been seldom applied. It has been reported to be in frequent use only in a survey from North Jordan [5]. In the first observation period we found significant differences in the choice of irrigating solutions employed among the observed groups, which is in contrast to findings from endodontic survey in North Jordan [5], where professional experience was not reported to influence the irrigation habits. There is a considerably large group of Serbian dentists who exclusively used hydrogen peroxide for root canal irrigation. High popularity of H_2O_2 in Serbian dental community cannot be explained by low price, as NaOCl is considerably cheaper. However, it has been the choice of preference amongst general dental practitioners in Serbia for many years now. There is no rational explanation for this finding, and one of the reasons could be the habits from the past and educational inconsistencies of undergraduate curricula in temporal domain.

Three years later, the repeated survey showed an extremely changed choice of irrigants used, and NaOCl became the dominant irrigation solution. These new findings are consistent with findings from similar studies: approximately 70% of dentists used NaOCl in a survey from Saudi Arabia, over 60% of surveyed dentists in Hong Kong used NaOCl, a survey conducted in Australia reported that 94% of endodontists used sodium hypochlorite, and a survey performed in North Jordan reported that 32.9% of general dentist used sodium hypochlorite. In some other studies the selection of irrigant could be associated with the use of rubber dam, as it was found that 70% of rubber dam users among British dentists irrigated with sodium hypochlorite, whilst non-users tended to use local anesthetic solution [15]. Regarding our study, after the

second observation period, we found that the vast majority of our respondents were non-users of rubber dam but all of them used sodium hypochlorite. A similar attitude toward using sodium hypochlorite without using rubber dam for isolation was also reported amongst Flemish dentists [6]. The reasons for such ignoring rubber dam in the Serbian dental community could be addressed primary to insufficient education in the undergraduate teaching curriculum, inadequate skills, and lack of training, following by high cost, extra time, and prospective patient's consent. The use of sodium hypochlorite, especially in full strength concentration, without rubber dam isolation, presents a potentionaly dangerous practice in the use of such irritant irrigation solutions.

In the second observation period, chlorhexidine also gained popularity, but ranked far behind NaOCl. Chlorhexidine was used by very young and very experienced dentists. A greater popularity f chlorhexidine could be explained by its significantly lower toxicity compared to NaOCl, and significantly prolonged shelf life. EDTA is clearly the least applied irrigant in both surveys. That might be due to its relatively high cost and lack of availability on the local market. However, actual reasons for that are yet to be investigated.

The study also revealed a change in the preferred concentration of NaOCl that was applied by the vast majority of dental practitioners in the repeated survey. After the 3-year observation period, the most frequent percentage of NaOCl appeared to be 2.5%, and that might be influenced by continuing educational programs during the 3-year observation period, since that concentration is in accordance with noticed trends worldwide.

This drastic change in preferred irrigant after only 3 years that elapsed between the two studies could be a consequence of delivered programs of continuing education that influenced attendants and brought them closer to contemporary endodontic trends. During the observation period, 237 workshops accredited by the Health Council of Serbia were designed and delivered by the members of teaching staff from dental schools in Serbia. Many of these sessions were repeated a couple of times at different Serbian towns, in attempt to cover all the regions of the country. All meetings were timely announced at Serbian Association of Private Dentists web site (http://www.privstom.org.rs) [20], as well as in electronic and printed media.

Old habits die hard, but persistence in dissemination of progressive approaches obviously yields results and eventually gains success if the curriculum is presented in a proper manner and supported with appropriate evidences. It seems that continuing education programs provide solid basis for experience exchange and motivate open-minded dental practitioners to step outside previously acquired dogmatic standpoints.

The purpose of this survey was to reveal the preferred root canal irrigants used by general dental practitioners in Serbia and to determine the prospective influence of continuing educational program delivered over the 3-year observation period. This was the first comprehensive survey of this nature carried out in Serbia, and with this report we wanted to present the baseline data for further investigation, and determine the trends and changes which could influence continuing education topics and general strategy.

However, there were some limitations to our study: despite popularity of web surveys, and their numerous advantages (rapid response, low cost and flexibility), these also carry significant sampling limitations: only a fraction of Serbian dentists willing to take part in a survey actively use IT technologies and there is always a possibility that repeated participation in the survey by the same individual might distort the results.

The preliminary findings from this study indicate that continuing educational programs might be a valuable vehicle for dissemination of alternative approaches amongst dental professionals in Serbia.

Conclusion

This study points to significant changes in the irrigation protocol applied in Serbian dental community. After 3 years of observation, NaOCl has became widely accepted as the irrigant of choice, chlorhexidine also gained popularity, whereas H_2O_2 lost its undue popularity from the past.

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Lip, oral cavity and pharyngeal cancers in the population of the city of Belgrade in the period 1999–2010

Karcinomi usana, usne duplje i ždrela kod stanovništva grada Beograda u periodu 1999–2010

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Abstract

Background/Aim. Cancers of the lip, oral cavity and pharynx (LOCP) are frequently grouped together mainly because they have similar risk factors. The incidence rate of these cancers varies worldwide depending on the geographic location. The aim of this study was to determine trends in age-standardized incidence rates of LOCP cancers in the Belgrade population during a 12-year period, from 1999 to 2010. **Methods.** From The Serbian Cancer Registry (The Registry), we extracted all cases of LOCP cancers registered in Belgrade from January 1, 1999 to December 31, 2010. Joinpoint regression analysis was used to define trends and annual percentage change (APC). **Results.** A total number of 2,025 (1,509 in men and 516 in women) LOCP cancers were reported to the Registry during the study period. The age standardized rate (ASR) for the entire period and for all LOCP cancers, was 6.24 *per* 100,000 persons (10.35 for men and 2.86 for women). ASR for lip cancers decreased ($p < 0.001$) during the study period with APC of -8.4%. The ASR for oral cavity and pharyngeal cancers increased ($p < 0.05$). **Conclusion**. Our results show a significantly decreasing trend of the incidence rate for lip cancers in the population of the city of Belgrade between 1999 and 2010. On the contrary, the incidence of oral cavity and pharyngeal cancers increased for both men and women.

Key words:
lip neoplasms; mouth neoplasms; pharyngeal neoplasms; incidence; risk factors; sex; age group.

Apstrakt

Uvod/Cilj. Karcinomi usana, usne duplje i ždrela često se grupišu zajedno zbog sličnih faktora rizika. Incidencija ovih karcinoma u svetu je različita i zavisi od geografske lokacije. Cilj rada bio je da se utvrdi promena incidencije karcinoma usana, usne duplje i ždrela kod stanovništva Beograda u periodu od 1999. do 2010. godine. **Metode.** Iz Registra za maligne bolesti Republike Srbije izdvojeni su svi registrovani slučajevi karcinomi usana, usne duplje i ždrela u Beogradu, od 1. januara 1999. godine do 31. decembra 2010. godine. Trend kretanja i godišnji procenat promene incidencije izračunat je regresionom analizom uz pomoć tačaka spajanja (*jointpoint regression analysis*). **Rezultati.** U periodu istraživanja ukupno je registrovano 2 025 slučajeva (1 509 kod muškaraca i 516 kod žena) karcinoma usana, usne duplje i ždrela. Standardizovana stopa incidencije za sve karcinome iznosila je 6,24 na 100 000 stanovnika godišnje (10,35 za muškarce i 2,86 za žene). Standardizovana stopa incidencije za karcinome usana se smanjenjivala tokom perioda ispitivanja sa godišnjim procentom promene od -8.4% ($p < 0.001$). Standardizovana stopa incidencije za karcinome usne duplje i ždrela povećavala se ($p < 0.05$). **Zaključak.** Naši rezultati pokazuju konstantno opadanje trenda incidencije karcinoma usana kod stanovništva grada Beogradu između 1999. i 2010. godine. Sa druge strane, incidencija karcinoma usne duplje i ždrela povećavala se kod oba pola.

Ključne reči:
usna, neoplazme; usta, neoplazme; farinks, neoplazme; incidencija; faktori rizika; pol; životno doba, grupe.

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Introduction

Cancers of the lip, oral cavity (OC) and pharynx (LOCP) are frequently grouped together [1] mainly because they have similar risk factors. The incidence rate of these cancers varies worldwide depending on the geographic location. The highest incidence rates of OC and pharyngeal cancers are found in south Asia, Pacific regions, Latin America and in parts of central and east Europe [2]. Oral and pharyngeal cancers are the sixth most common cancers in the world and the seventh in European Union [2,3]. Globally, cancers of the OC and pharynx, when viewed as a group, are the seventh most common type of carcinoma in Serbia in 2009 [4]. The major risk factors for LOCP cancers are use of tobacco, excessive alcohol consumption [5–8] and persistent infections with human papillomavirus (HPV) [9–12]. Other factors such as genetics, social inequality, nutritional factors and poor oral hygiene have also been reported [13–17]. The literature on the incidence rate of LOCP cancers in Serbia is scarce. In fact, to our knowledge, there is no published data that described the incidence rate of these cancers in Serbia. Registry-based studies are important because they provide valuable information for health policies planning and prevention in this growing health burden.

The aim of this study was to present and analyze trends in age-standardized incidence rates (ASR) of LOCP cancers in the Belgrade population during a 12-year period (1999–2010).

Methods

Type of study, data sources and study population

This retrospective descriptive epidemiological study gives the incidence rate of LOCP cancer during a 12-year study period.

Data were obtained from the Serbian Cancer Registry (The Registry), which covers the complete population of Serbia, excluding Kosovo. The Registry was established in 1970, but in the period from 1986 to 1998, the quality of data collection was rather scarce. After 1998, a new methodology was applied which substantially improved data quality and the Registry became a member of International Agency for Research on Cancer (IACR) and European Network of Cancer Registries (ENCR). Sources of data collection for the Registry are hospitals and outpatient's health institutions, oncology clinics, as well as dispensaries and institutes, pathology laboratories, death reports and health insurance funds. Cancer reporting is obligatory by law in Serbia and information on all potential new cases must be reported to the Registry. Our study was conducted in the city of Belgrade, which had a population of 1,568,754 persons in 1999 and 1,639,505 in 2010. Information on the Belgrade population size and migration in the past was obtained from the Statistical Office of the Republic of Serbia.

Coding and analysis

From The Registry, we extracted all cases of LOCP cancers registered in Belgrade from January 1, 1999 to December 31, 2010 according to the International Classification of Diseases Tenth revision (ICD-10) [1]. These cancers included: lip (ICD-10: C00), tongue (ICD-10: C01-C02), gum (ICD-10: C03), floor of the mouth (ICD-10: C04), palate (ICD-10: C05), cheek mucosa and other part of mouth (ICD-10: C06), tonsil (ICD-10: C09), oropharynx (ICD-10: C10), pyriform sinus (ICD-10: C12), hypopharynx (ICD-10: C13) and other ill-defined part of the lip, mouth and pharynx (ICD-10: C14). Cancers of nasopharynx (ICD-10: C11), nasal cavity (ICD-10: C30.0), skin of lip (ICD-10: C44.0) were excluded from this study. Tumors are grouped as lip cancers (ICD-10: C00), OC cancers (ICD-10: C01-C06) and pharyngeal cancers (ICD-10: C09-C10, C12-C14). We used the term "lip, oral cavity and pharyngeal cancers" to encompass all tumors. Tumors were classified according to the International Classifications of Diseases of Oncology, 3rd Edition (ICDO-3) [18]. Morphology codes for the selected cases included: 8000, 8010, 8020-1, 8032-3, 8050-2, 8070-6 and 8084. Only invasive cancers were included in the study (*ie* containing "/3" as the last digit in the morphology code).

For the purpose to avoid the effect of differences in population age structures and to allow comparison between our data and data from other areas we used the direct standardization method to the world standard population [19]. Firstly, we aggregated all new cases from the Registry per age group and divided these with the age-stratified population estimate for every year. After estimating age-specific rates, we applied these rates to the reference population – the world standard population. ASR were reported as the incidence *per* 100,000 persons yearly.

All patients were classified in five age groups: below 39, 40–49, 50–59, 60–69 and over 70 years of age. Trends and annual percentage change (APC) of the incidence rate with corresponding 95% confidence intervals (CI) were calculated by performing joinpoint regression analyses to identify the years in which a significant change in incidence rates occurred. For regression analyses, we used Joinpoint Regression Program version 4.1.0 (available at http: //surveillance.cancer.gov/joinpoint). The trend was considered to be significant increasing (positive change) or decreasing (negative change) when the *p*-value was below 0.05 ($p < 0.05$).

Results

A total number of 2,025 cases (1,509 in men and 516 in women) of LOCP cancers that fulfilled the criteria were reported to the Registry during the study period. In the same period, the Registry recorded 99,668 cases of all types of carcinoma (ICD-10: C00-C96), 50,575 in men and 49,093 in women. LOCP cancers comprised about 2.03% of all carcinomas recorded in population the Belgrade (2.98% in men and 1.05% in women). Table 1 presents distribution of all LOCP cancers in Belgrade, according to the site of the tumor, age and gender of the patients. The men represented 74.5% of all the persons and the women 25.5%. The men to women ratio was 2.9 : 1. The most common site in men and women was the OC cancers. Most cases were aged 40–69 (58.3%) and less than 4% below the age of 39.

A complete ASR (to the world standard population) of all cases according to gender and years of observation were shown in Table 2. ASR for the entire study period and for all

LOCP cancers, were 6.24 *per* 100,000 persons (10.35 for the men and 2.86 for the women). In the men ASR for LOCP cancers increased from 10.26 in 1999 to 13.40 *per* 100,000 persons in 2010 (APC, 0.9%; 95% CI: -3.1, 5) and in the women ASR remained almost stable (APC, -0.4%; 95% CI: -3.6, 2.9). Joinpoint regression analysis showed the decrease trend of ASR for LOCP cancers, for both gender combined, in the time period 1999–2007 with APC of -3.6% (95% CI: -7.9, 0.9) and increase trend in the last three years of the study period (APC, 17.7%; 95% CI: -4.6, 45.3) (Figure 1).

Table 1

Distribution of lip, oral cavity and pharyngeal cancer in Belgrade by gender and age, 1999–2010

Parameter	Primary site of cancer, n (%)			Total
	Lip	Oral cavity	Pharynx	
Gender				
men	280 (75.9)	692 (71.8)	537 (77.6)	1509 (74.5)
women	89 (21.1)	272 (28.2)	155 (22.4)	516 (25.5)
Age group (years)				
≤ 39	1 (0.3)	57 (5.9)	18 (2.6)	76 (3.7)
40–49	26 (7.1)	123 (12.8)	90 (13.0)	239 (11.8)
50–59	68 (18.4)	294 (30.5)	207 (29.9)	569 (28.1)
60–69	122 (33.0)	266 (27.6)	223 (32.2)	611 (30.2)
≥ 70	152 (41.2)	224 (23.2)	154 (22.3)	530 (26.2)
Total	369 (100)	964 (100)	692 (100)	2025 (100)

Table 2

Age-standardized incidence rate (*per* 100,000 person) of lip, oral cavity and pharyngeal cancers by sex and year of diagnosis

Year of diagnosis	Men		Women		Overall	
	WASR	(95 % CI)	WASR	(95 % CI)	WASR	(95 % CI)
1999	10.26	8.44–12.07	3.00	2.11–3.88	6.33	5.37–7.29
2000	11.33	9.41–13.25	2.92	2.06–3.78	6.75	5.76–7.74
2001	11.46	9.54–13.38	3.75	2.73–4.76	7.27	6.23–8.30
2002	11.90	9.94–13.87	2.19	1.47–2.92	6.64	5.66–7.62
2003	7.17	5.66 – 8.67	3.19	2.28–4.11	4.97	4.13–5.81
2004	8.43	6.78–10.07	2.38	1.56–3.21	5.12	4.25–5.99
2005	8.54	6.88–10.20	3.33	2.36–4.30	5.68	4.76–6.60
2006	9.38	7.66–11.09	2.32	1.56–3.07	5.48	7.24–5.17
2007	8.60	7.00–10.19	2.46	1.65–3.26	5.17	4.34–6.01
2008	8.97	7.32 –10.62	2.67	1.87–3.48	5.48	4.62–6.34
2009	14.17	12.04–16.29	3.30	2.38–4.23	8.21	7.12–9.29
2010	13.40	11.40–15.40	3.04	2.23–3.85	7.68	6.68–8.68
All years	10.35	9.83–10.87	2.86	2.61–3.10	6.24	5.97–6.51
APC*	0.9%, −3.1% to 5%		−0.4%, −3.6% to 2.9%		0.4%, −2.9 to 3.8%	

***Annual percentage change and 99% confidence interval; WASR – world age standardized rate; CI – confidence interval.**

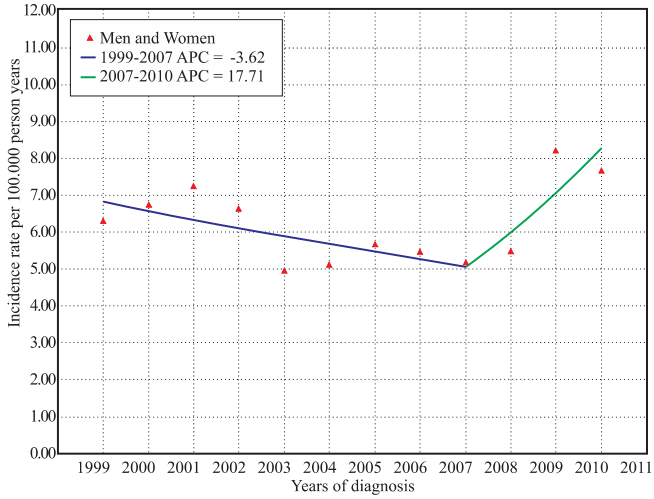

Fig. 1 – Joinpoint analyses of age standardized incidence rates (world standard population) of lip, oral cavity and pharyngeal cancer in Belgrade, 1999–2010, with annual percentage change (APC).

Considering lip cancers (ICD-10: C00) separately, there was a significantly decreasing trend ($p < 0.001$) of ASR, for both gender combined, throughout the whole study period (1999–2010) with APC of -8.4% (95% CI: -11.4, -5.3). In the males, ASR for lip cancer significantly decreased ($p < 0.001$) from 2.77 in 1999 to 1.27 *per* 100,000 persons in 2010 (APC, -7.7%; 95% CI: -10.2, 5.2) and in the females from 0.82 in 1999 to 0.30 in 2010 (APC -9.7%, 95% CI: -17.7, -1; $p < 0.05$) (Figure 2).

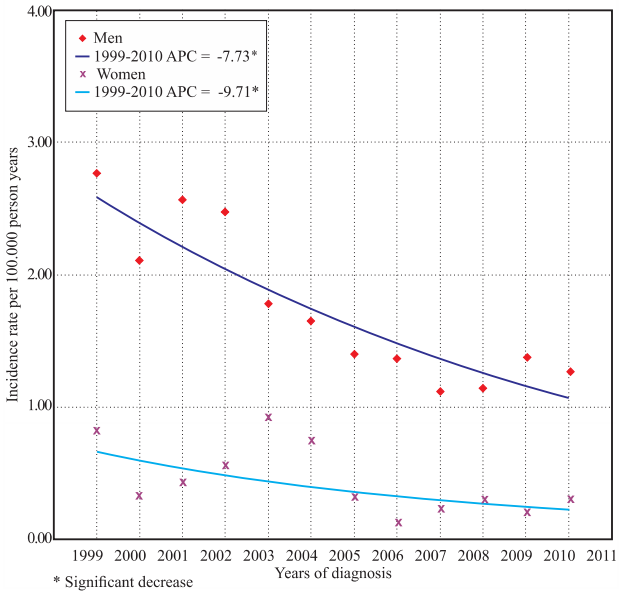

Fig. 2 – Joinpoint analyses of age standardized incidence rates (world standard population) of lip cancer in Belgrade, 1999–2010, with annual percentage change (APC).

When OC cancers (C01-C06) are considered separately there was almost a double increase of ASR in the men from 3.75 (95% CI: 2.64, 4.86) in 1999 to 7.23 (95% CI: 3.63, 6.06) *per* 100,000 persons in 2010 and in the women from 1.56 (95% CI: 2.64, 4.86) in 1999 to 1.62 (95% CI: 3.63, 6.06) in 2010. Jointpoint regression analysis showed that ASR for OC cancers increased significantly ($p < 0.05$) from 2004 to 2010 in the men (APC, 14.7%; 95% CI: -21.6, 29.9) and non-significant increase in the women from 2007 to 2010 (APC, 16.9%; 95% CI: -21.6, 29.9) (Figure 3).

When pharyngeal cancers (ICD-10: C09-C10, C12-C14) are considered separately from the other cancers there was a significantly increased trend ($p < 0.05$) of ASR in the period 2003–2010 with APC of 5.7% (95% CI: 0.5, 11.2). In the men, a low increase of ASR for pharyngeal cancers was observed from 1999–2010 (APC, 0.4%; 95% CI: -4.1, 5) while in the women the increase was significant ($p < 0.05$) with APC of 5.7% (95% CI: 0.2, 11.4) from 0.62 in 1999 to 1.12 in 2010 (Figure 4).

Discussion

LOCP were not common in our study before the age of 40 years (4% of all the cases) and more than half of the patients were between 40 and 69 years. During a 12-year study period, there was no sign of the increasing percentage of LOCP cancer cases in men or women under the age 40 years. This finding is mainly in accordance with the literature [20–22] although recent reports suggest the increased number of young patients [23, 24]. The literature from the most countries around the world presents at least about twice higher level of oral cancer in men than in women as we found in our study [11, 12, 25, 26]. This observation points to the fact that age and sex should be considered as one of the risk factors of developing LOCP cancers.

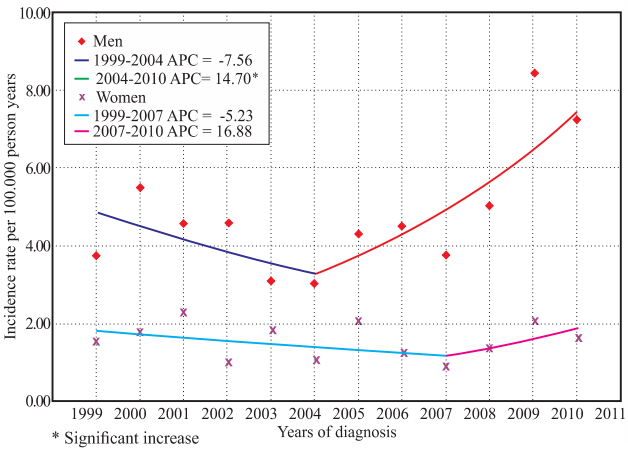

Fig. 3 – Joinpoint analyses of age standardized incidence rates (world standard population) of oral cancer in Belgrade, 1999–2010, with annual percentage change (APC).

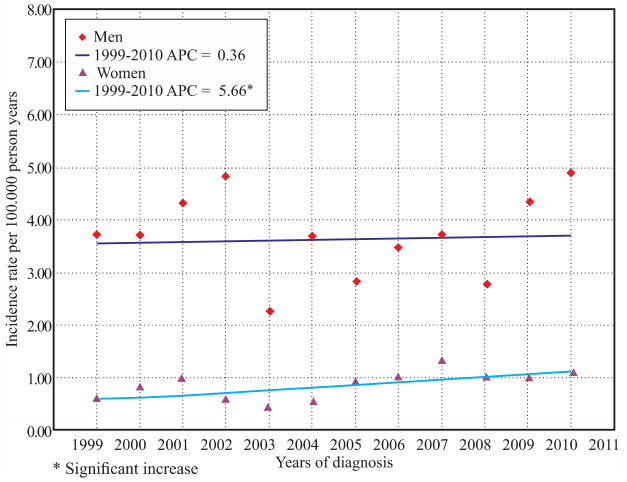

Fig. 4 – Joinpoint analyses of age standardized incidence rates (world standard population) of pharyngeal cancer in Belgrade, 1999–2010, with annual percentage change (APC).

We have shown a decline in the incidence rate for LOCP cancers, taken together, in most of the time of the study period. The main impact on this decrease had a significantly decreasing trend for lip cancers. The decreasing incidence rate of lip cancer has also been reported from Denmark [12], Portugal [27], Finland [28], Israel [25], and Australia [26]. Blomberg et al. [12] (2010) reported a significant decrease trend in age-standardized incidence rate in Danish male population from 3.36 in 1978 to 0.83 in 2007 and with APC of -5% .

Declines in lip cancers incidence rate may be attributed to a decrease in the number of smokers in Belgrade population. In Serbia, after 1999, several preventive and promotional anti-smoking activities have been initiated. The committee for Smoking Prevention was set up, World Health Organization (WHO) Framework Convention on Tobacco Control was signed and ratified, Tobacco Control Office was established and Tobacco Control Strategy for the Republic of Serbia was adopted [29]. The Ministry of the Health of Republic of Serbia in National Health Survey appraised reduction in smoking rate in Serbia by 6.9% after the year 2000 [30]. Tobacco smoking is a well-recognized risk factor of developing lip cancer [5–7]. Another cause of decreasing trend of lip cancer may be due to changes in the perception of patients for this, in contrast to oral and pharyngeal cancers, highly visible malignancy. This awareness could lead to increasing the removal lesion in the early stadium of the tumors (*eg* carcinoma *in situ*). In our study, only invasive cancers were included, which containing "/3" as the last digit of the morphology code.

In contrast to lip cancer ASR for carcinoma of the OC has doubled between 1999 and 2010 in Belgrade population. We observed a significantly increasing trend for OC cancers among men from 2004 to 2010 (APC, 14.7%, $p < 0.05$) and rapid, but not significant among women after the year 2007. Increasing trends of pharyngeal cancers were also showed from 1999 to 2010 (APC, 5.7%, $p < 0.05$). Our results confirm the rising incidence trends for oral and pharyngeal cancers reported in some European countries, such as Denmark [12], Norway [31], United Kingdom [20], Netherland [21], Portugal [27] and Germany [32]. Hwang et al. [33] (Canada) recently reported the increasing incidence rate of pharyngeal and oral cancers during the time period 1992–2007 with the highest incidence rate in Nova Scotia. Increasing incidence trends of OC and pharyngeal cancers in our study along with decreasing incidence trends of lip cancers and reduction in tobacco smoking rate suggests that different or additional etiological factors are involved in the development of OC and pharyngeal cancers. The major risk factors for cancers of the OC are tobacco use, excessive consumption of alcohol and infections with HPV [11, 12, 21, 34]. These factors could act separately or synergistically, together [8, 35]. The APC values of the incidence rate of OC cancer in our study are higher than those observed in the abovementioned developed western countries [12, 21, 27, 31]. These observed divergence in values of the incidence rates of OC cancer are most likely related to socioeconomic and demographic factors. Many studies show links between oral cancer incidence and socioeconomic status [14–16, 36]. Auluck et al. [13] (British Columbia, Canada) reported that socioeconomic status could be responsible for the increasing incidence trends of pharyngeal and oral cancers. In their study, the highest incidence rates in men, for both pharyngeal and oral cancers were observed in neighborhoods with the most deprived socioeconomic status. Most of the people in Serbia lack sufficient money to live at a standard considered comfortable or normal as in the above-mentioned developed countries. In such poor environments people are more prone to consume alcohol or smoke cigarettes to cope with difficulties or to avoid bad feeling. Although Serbia reduced the number of smokers after the year of 2000 still almost 40% of the male population and 30% of the female population are smokers [30]. According to WHO reports, consumption of alcohol *per capita* in Serbia, in the age older than 15 years, changed from 9.2 liters in 2003–2005 to 12.6 in 2008–2010 [37]. The same report shows 6.8 times higher prevalence of heavy episodic drinking in male than in female population. The changing pattern of alcohol consumption in Serbia and higher prevalence of heavy male drinkers along with a large percentage of male smokers are likely to be reasons for sex differences in the observed incidence rate of OC cancers in the population of Belgrade. Another possible explanation for the increased incidence rate is HPV infection as a consequence of changing sexual habits over the past years. Numerous authors agree that the increasing number of lifetime sexual partners and increasing numbers of oral sex partners are important risk factors for HPV infection and oral and pharyngeal carcinoma [9, 10, 21]. Hemminki et al. [38] showed a high incidence rate of tonsil and tongue cancers among husbands of cervical cancer patients.

To our knowledge, this study is the first registry-based study in Serbia that analyzed trends of LOCP cancers in more than one and a half million population, covered a long period (12 years). Our study has limitations similar to other registry-based studies. The Serbian Cancer Registry does not record risk behaviors such as smoking or alcohol consumption. Data on smoking and alcohol consumption presented in this paper are related to the whole country but not just to the population of Belgrade. The quality of the observed incidence rates may be influenced by the incompleteness of registration in the first years of the study period, but we believe such influences were small.

Conclusion

Our results show a significantly decreasing trend of the incidence rate for lip cancers in the population of the city of Belgrade between 1999 and 2010. On the contrary, the incidence rate of oral cavity and pharyngeal cancers considerably increased in both men and women. The increase in the incidence rate of oral cavity carcinoma in our study is greater than in some developed countries. By these findings, we would like to highlight the problem of a continuously increasing incidence rate of oral cavity and pharyngeal cancers to the health authority. Given the trend in recent years it will be an undoubted increase in the future since Serbia has no program for early detection of premalignant or malignant oral lesions. In face of these facts, serious national research, education and prevention program should be taken to ensure the reduction of this type of cancer in the future.

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Respiratory syncytial virus infection and bronchial hyperreactivity in children up to two years of age in correlation with atopy

Infekcija respiratornim sincicijalnim virusom i bronhijalna hiperreaktivnost kod dece uzrasta do dve godine u odnosu na atopiju

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Abstract

Background/Aim. Bronchiolitis in early childhood caused by respiratory syncytial virus (RSV) is considered to be important risk factor of the recurrent wheezing and asthma development. The aim of this study was to examine the frequency of RSV infection and atopy in children up to two years of age and to determine their correlation with bronchial hyperreactivity. **Methods.** The study included 175 children aged 5–24 months. The presence of RSV infection was identified by serum levels of IgA and IgG determined by ELISA. Bronchial hyperreactivity (BHR) has been defined as the existence of chronic bronchial disease and/or three or more previous suspected diagnosis of acute bronchial disease. Atopy was confirmed by detection of the specific serum IgE using quantitative multitest Phadiatop infant (cut off ≥ 0.35 kUA/L). **Results.** The children with atopy were more frequently infected with RSV (43.3%) than those without atopy (22.8%; $p = 0.02$). The higher frequency of RSV infection was found in children with BHR in comparison with those without it but only in the group who also had atopy (77.8% *vs* 28.6%, $p = 0.018$). In the female children, BHR and RSV infection were associated in 62.5% of cases, regardless the atopy. In the male children with atopy, RSV infection was associated with BHR in 83.3% of the cases, while in those without atopy, RSV infection with BHR was found in only 17.4% of the cases. **Conclusion.** Children up to two years of age with atopy are more frequently infected with RSV (43.3%) than non-atopic children. Every third child with atopy develops BHR and 77.8% of them also have RSV infection. Atopic children are at higher risk for development of BHR when infected with RSV also.

Key words:
respiratory syncytial viruses; bronchial diseases; hypersensitivity, immediate; comorbidity; child; serbia.

Apstrakt

Uvod/Cilj. Bronholitis u ranom detinjstvu izazvan respiratornim sincicijalnim virusom (RSV), smatra se značajnim faktorom rizika od pojave rekurentnog vizinga i astme. Cilj ovog rada bio je da se ispita učestalost RSV infekcije i atopije kod dece uzrasta do dve godine i da se ustanovi njihova korelacija sa bronhijalnom hiperreaktivnošću (BHR). **Metode.** U istraživanje je bilo uključeno 175 dece uzrasta od 5 do 24 meseca. Prisustvo RSV infekcije je ustanovljeno na osnovu serumske koncentracije anti-RSV IgA i IgG antitela određenih ELISA testom. Hiperreaktivnost bronhijalnog stabla (BHR) je definisana kao postojanje hronične bronhijalne bolesti i/ili tri i više prvih kurativnih pregleda pod dijagnozom akutne bronhijalne bolesti. Postojanje atopije utvrđeno je detekcijom serumskih specifičnih IgE antitela kvalitatitvnim multitestom *Phadiatop infant* (≥ 0,35 kUA/L). **Rezultati.** Deca sa atopijom češće su imala RSV infekciju (43,3%) nego deca bez atopije (22,8%; $p = 0,02$). Veća učestalost RSV infekcije kod dece sa BHR u odnosu na decu bez BHR postojala je samo u grupi atopičara (77,8% *vs* 28,6%; $p = 0,018$). Ženska deca imala su udruženost BHR i RSV infekcije u 62,5% slučajeva bez obzira na atopiju. Kod muške deca sa atopijom RSV infekcija bila je udružena sa BHR kod 83,3%, dok je kod muške dece bez atopije to bio slučaj kod samo 17,4% slučajeva. **Zaključak.** Deca uzrasta do dve godine sa atopijom češće su imala RSV infekciju (43,3%) nego deca bez atopije. Svako treće dete sa atopijom imalo je BHR, pri čemu je 77,8% imalo i RSV infekciju. Deca sa atopijom koja imaju RSV infekciju su u većem riziku od razvoja BHR.

Ključne reči:
respiratorni sincicijalni virusi; bronhusi, bolesti; hipersenzibilnost, rana; komorbiditet; deca; srbija.

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Introduction

Respiratory syncytial virus (RSV) is considered to be the most important causative agent of respiratory diseases in children in their earliest age [1, 2]. The incidence of RSV infection in early age in countries of European Union ranges from 5.4% to 40.8% [3–5]. In our population, positive RSV IgG was found in 24% of children within their first two years of life (in the age of 5–12 months – 12.9% and in the second year – 47%) [5].

RSV belongs to the genus of Pneumovirus and its genome is presented as a single-stranded RNA in the form of the negative chain. It is transmitted by aerosol or by the direct contact with the infected person [6]. Virus contagiosity degree is high and it has been confirmed that if only one RSV positive child comes to the kindergarten, 90% of healthy children will also be affected [7]. RSV infection most frequently remains in ciliary cells where the movements of cillia are disordered and infected cells peel off [7]. Epithelial cells damage initiates development of edema with the serum proteins excretion in the airways lumen. This results in respiratory obstruction and the initiation of immune response in the airways serosa. All these contribute to development of the respiratory disease symptoms and bronchial hyperreactivity [8].

RSV bronchiolitis in early childhood is considered to be important risk factor of the recurrent wheezing and asthma development [9], but a rather large number both of retrospective and prospective studies suggest that RSV infection itself could be determinant of bronchial hyperreactivity (BHR) [10, 11]. The previous study from Serbia reported that 20.3% of children with expressed bronchitis and/or bronchiolitis had anti-RSV IgG, while these antibodies were found in 33.3% of children diagnosed with asthma (J45) or chronic bronchial obstruction (J44.9) [5].

More than one half of all asthma cases develop before the third year of age and an early occurrence of asthma in the infant age is most frequently manifested by wheezing during viral respiratory infections [12, 13]. It has been confirmed that allergic sensibilization is an important risk factor for development of wheezing during and after RSV infection [14]. In children with atopy, the defect of respiratory epithelium due to the previous exposure to allergens, represents the risk factor for development of asthma after recurrent viral infections [15]. On the contrary, epithelium impaired by frequent infections may become the spot of more intensive absorption of aeroallergens, intensifying in this way the effect of allergens upon exacerbation of asthma [15]. It has been suggested that the second year of age is the risky period for the remodeling of airways, which is important as the pathologic basis of asthma [16]. Also, this age is the risky period for the synergic effect of both infection and atopy in asthma development [17]. On the other hand, some authors suggest that development of bronchial hyperreactivity is rather related to individual characteristics of children (such as male sex) than to RSV bronchiolitis [18].

In Serbia, there are no so far published data on RSV infection as determinant of BHR development in correlation with atopy. The aim of this study was: to examine the frequency of RSV infection and atopy in children up to two years of age and to determine the correlation of RSV infection and atopy with bronchial hyperreactivity.

Methods

The study included 175 children 5–24 months of age from the territory of the city of Kragujevac. Data on individual and sociodemograpfic characteristics were obtained from the questionnaire. The poll was conducted in the Outpatient Clinic in Kragujevac and in the Center for Allergic Diseases and Asthma Prevention of the Institute of Public Health in Kragujevac. Data on respiratory diseases were obtained from the data base of the Medical Center of Kragujevac.

The diagnosis of RSV infection was based on serum anti-RSV IgA and IgG levels above the cutoff value. Venous blood was taken from children in the morning; after 2 hours it was centrifuged and serum was stored at -75°C before analysis. Serum IgA and IgG antibodies were determined by the quantitative ELISA (SERION ELISA classic, Institute Virion/Serion GmbH, Würzburg, Germany). Serum levels of the specific anti-RSV antibodies were determined on the basis of optic values density, 4PL method (Single-point quantification using the 4PL method) by using the SERION software program (SERION easy base 4PL-Softwarey evaluate). Positive anti-RSV IgG result was defined as ≥ 15U/mL concentration and for IgA ≥ 10 U/mL. Finding of just one specific anti-RSV antibody (IgA or IgG class) was considered an evidence of RSV infection.

Lower respiratory tract diseases comprised acute and chronic bronchial diseases and pneumonia. The group of children with acute bronchial diseases included those with the diagnosis J20 – acute bronchitis (*Bronchitis acuta*) and J21 – acute bronchiolitis (*Bronchiolitis acuta*). The group of children with chronic bronchial diseases included those with the diagnosis J44.9 – chronic obstructive pulmonary disease (*Morbus pulmonis obstructivus chronicus alius*) and J45 – asthma (*Asthma bronchiale*). The group with pneumonia included children with the diagnoses J12–18, that is, viral, bacterial and non-classified pneumonias (*Pneumonia viralis, bacterialis and non specificatus*).

Bronchial hyperreactivity is defined as the presence of chronic bronchial disease (J44 or J45) and/or as three or more previous suspected diagnosis of acute bronchial disease (J20 or J21).

The presence of atopy was confirmed when the serum specific IgE was detected by the quantitative multitest Phadiatop infant (cut off ≥ 0.35 kUA/L). Allergens used as antigens in this test were proteins from the egg white, cow milk, peanuts, shrimps, cat's and dog's hair, mites, pollen of the silver birch, Timothy grass, ambrosia and nettle [19]. Phadiatop infant test was carried out *in vitro* by the fluorescent immunoassay using Immunocap-100 device (Phadia AB Upsala, Sweden). Children with atopy included those with positive Phadiatop infant test, namely, children with the serum level of specific IgE ≥ 0.35 kUA/L. Non-atopic children had serum specific IgE antibodies below 0.35 kUA/L level, that is, their Phadiatop infant test was negative.

Statistical analysis data was performed using the SPSS software package 20.0 (IBM SPSS Statistic for Windows, Amonk, NY, USA). For analysis of statistical differences in frequencies of the dependent variable in comparison with categorical variables, χ^2 test was used. The table of contin-

gence was used to determine differences in frequencies among variables with more than 2 categories (3 × 2, 4 × 2...).

Examination was carried out in accordance with ethical standards of the Helsinki Declaration from 1975, revised in 1983. The study was performed within the plan for allergic diseases prevention in children treated at the Institute of Public Health in Kragujevac and approved by the Ethical Board of the mentioned institute, as well as by the Faculty of Medical Sciences in Kragujevac. Biological samples (children's blood) were taken in the Medical Center under pediatric control and in accordance with their age and pediatric protocol for standard control of hematologic parameters for each child's blood count. The parents were informed about the purpose, aim and methods of this study and they gave informed consent for their children's inclusion into the study.

Results

The results on RSV infection and atopy in the different groups of children are presented in Table 1. A statistically significant difference in frequency of RSV infection was proven among the different age groups, where RSV was most frequent in the group of children 7–12 months old ($p = 0.000$). Also, the children born in autumn were more often infected with RSV ($p = 0.059$).

The children up to two years of age had RSV infection in 26.3% (46/175) and atopy in 17.1% (30/175). The children with atopy had more often RSV infection (43.3%; 13/17) in comparison with those without atopy (22.8%, 33/145; $p = 0.020$), (Figure 1).

No statistically significant difference in BHR frequency was found between the children with atopy (30%; 9/30) and those without atopy (19.3%, 28/145; $p = 0.192$). In the group of children with BHR a higher frequency of RSV infection (37.8%; 14/37) was observed in comparison with those without BHR where RSV infection was confirmed in 23.2% of the cases, (32/138; $p = 0.072$). A higher frequency of RSV infection in children with BHR in comparison with those without BHR, was found only in the group of children with atopy (77.8%, 7/9 *vs* 28.6%; 6/21; $p = 0.018$), (Figure 2).

Analysis of RSV infection and atopy according to age showed that the children with BHR tended to be more often

Table 1

Results of respiratory syncytial virus (RSV) infection and the presence of atopy in different groups of children

Group	Total n* = 175 [n* by category (%)]	RSV infection (%) (n* RSV pos./ n* in category)	*p*	Atopy (%) (n* with atopy /n* by category)	*p*
Age (months)					
5–6	59 (33.7)	20.3 (12/59)	0.000	11.9 (7/59)	0.163
7–12	74 (42.3)	14.9 (11/74)		16.2 (12/74)	
13–24	42 (24.0)	54.8 (23/42)		26.2 (11/42)	
Sex					
male	101 (57.7)	27.7 (28/101)	0.614	18.8 (19/101)	0.494
female	74 (42.3)	24.3 (18/74)		14.9 (11/74)	
Natural feeding					
non breastfed	114 (65.1)	28.1 (32/114)	0.463	15.8 (18/114)	0.516
breastfed	61 (34.1)	23 (14/61)		19.7 (12/61)	
Season of birth					
spring	39 (22.3)	38.5 (15/39)	0.059	25.6 (10/39)	0.162
summer	40 (22.9)	32.5 (13/40)		12.5 (5/40)	
autmn	59 (33.7)	22 (13/59)		20.3 (12/59)	
winter	37 (21.1)	13.5 (5/37)		8.1 (3/37)	

*** The number of patients.**

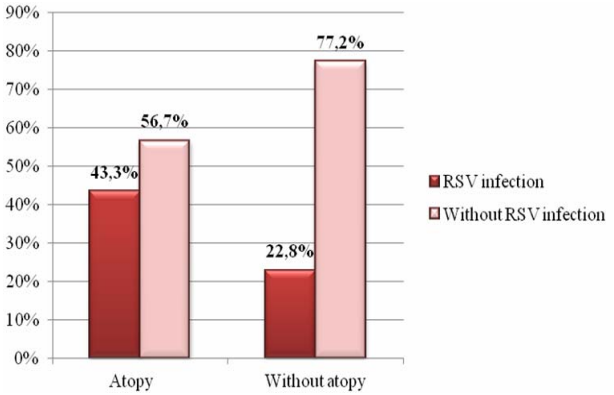

Fig. 1 – Frequency of atopy in children with or without respiratory syncytial virus (RSV) infection.

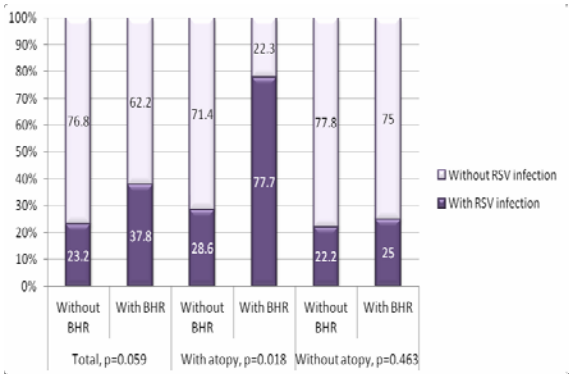

Fig. 2 – Frequency of respiratory syncytial virus (RSV) infection in children with and without bronchial hyperreactivity (BHR), regarding the presence or absence of atopy.

non-atopic in older groups. On the other hand, children with atopy tended to develop BHR more often as age advanced. In the non-atopic children, the frequency of RSV infection as a causative agent of BHR was 30–33% of those older than 7 months, while in the children with atopy and BHR, RSV infection was present in 85.7% of children in the second year of age (Figures 3 and 4).

Analysis of the children with BHR (n = 37) confirmed no statistically significant difference in the presence of atopy between male (20.7%, 6/29) and female sex (37.5%, 3/8; $p = 0.292$). The presence of BHR together with RSV infection was found in 62.5% of female children and was equally frequent in girls with and without atopy. In male children with atopy, RSV infection was associated with BHR in 83.3%, but in male children without atopy BHR was associated with RSV infection in only 17.4% (Figure 5).

Analysis of the children with BHR also showed that 78.4% (29/37) were not breastfed. In the non-breastfed children, detected RSV infection frequently developed in BHR (37.5%) in comparison with RSV non-infected children (20.7%; $p = 0.056$. In the breastfed children this association was not observed ($p = 0.594$).

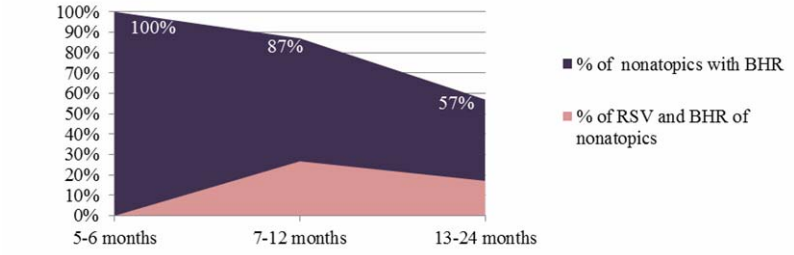

Fig. 3 – Percentage of non-atopic children with bronchial hyperreactivity (BHR) in comparison those with BHR and with syncytial virus (RSV) infection, regarding the age of children.

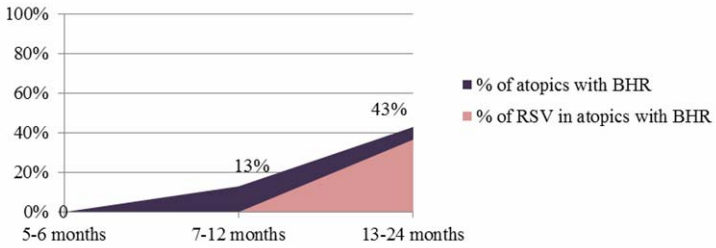

Fig. 4 – Frequency of syncytial virus (RSV) infection in atopics with bronchial hyperreactivity (BHR) regarding the age of children.

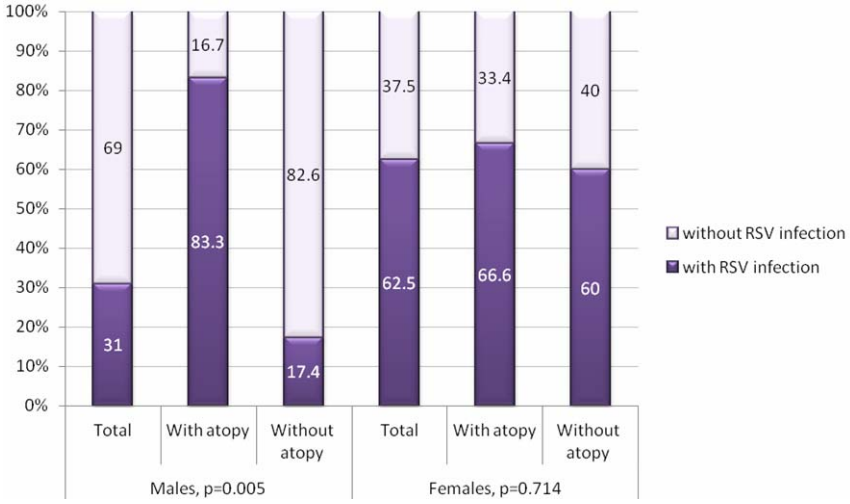

Fig. 5 – Frequency of respiratory syncytial virus (RSV) infection in male and female children with bronchial hyperreactivity (BHR) regarding the presence or absence of atopy.

In the non-breastfed children with atopy, the association of BHR and RSV infection was found in 75% (6/8), while in the non-breastfed children without atopy, this association was discovered in 28.6% (6/21; $p = 0.033$).

The children with atopy born in spring and summer had BHR in 53.3% (8/15), and those born in autumn and winter had BHR in only 6.7% (1/15; $p = 0.007$). In the children without atopy, the difference in BHR frequency between seasons of birth was not observed ($p = 0.487$). The children born in spring or summer who had atopy and who developed BHR, were also positive for RSV infection in 75% (6/8) of the cases.

Discussion

Bronchial hyperreactivity, defined as frequent and excessive bronchial narrowing, may be a consequence of genetic factors associated with intensified bronchial reactivity during inflammation, anatomy – small bronchial lumen, disorder of bronchial smooth muscles associated with altered function of the autonomous nerve system, damaged bronchial epithelium [20]. BHR, that was clinically defined as the presence of chronic bronchial disease and/or 3 or more of any lower respiratory disease, was found in 21.1% of the children up to two years of age. In this study, the groups identified to be at risk for BHR development were the children older than 7 months, male, non-breastfed and positive for RSV infection.

It is known that RSV infection can cause BHR in the critical moment of the pulmonary immune system development in genetically susceptible babies [21, 22], where age has great importance for innate and adoptive immune response against RSV infection [23]. The differentiation of progenitor cells into epithelial alveolar cells that produce immunomodulating factors (surfactant and clara cell protein**)** plays an important role during alveolar phase of the postnatal lung development within the first several years of life. The main target for viral infection in the lower respiratory tract are ciliary cells of bronchioles and pneumocytes type-1 in alveoli [24].

This study confirms that the presence of BHR at the age of 7–12 months is often associated with RSV infection, especially in children without atopy. It is possible that dysfunction of progenitor cells that occurs during RSV infection is responsible for the reduced presence of immunosuppressive factors important for development of chronic inflammation.

In this study, the children without RSV infection most frequently developed BHR at the age of 13–24 months. In those with BHR developed in older age, wheezing and BHR might be the result of rhinovirus (RV) infection, since it was known that children with RV infection were usually older and with the personal and family history of asthma [25, 26]. A study [27], including 198 children at great risk for atopy, confirms that RSV and RV are causative agents of all respiratory diseases. These diseases were associated with persistent wheezing in the first year of life, and in some of the following years developed into asthma.

It has been suggested that children with atopy had different susceptibility to viral infections in comparison with those without atopy during early childhood, where atopic children are far more susceptible [27]. In this research, children with atopy were more often infected with RSV (43.3%), than those without atopy (22.8%).

There is a dilemma whether genetic predisposition for atopy creates *per se* susceptibility for asthma after viral infection, or for development of asthma it is necessary to have active expression of the atopic phenotype with sensibilization to environmental allergens in early childhood. In the international study of asthma and allergies in childhood (ISSAC study) various wheezing phenotypes within the "asthma syndrome" are defined. One group includes children who, after being repeatedly exposed to infection in their early childhood, develop "infective asthma" and the other includes children with "atopic asthma" [27, 28]. However, the present study defined the unique phenotype of BHR in the age up to two years in which RSV infection and atopy were associated. Namely, every third child with atopy had BHR and 77.8% also had RSV infection. In comparison, in the group of children without atopy, BHR was diagnosed in every fifth child, and association with RSV infection was in only 25%. Some recent studies support standpoint, that viral infections of the lower respiratory tract represent the marker of atopic predisposition [29]. Prospective studies including high risk cohorts of infants whose mothers had asthma, suggested that serious bronchiolitis was a result of already present respiratory susceptibility to viral infection [29, 30]. Thomsen et al. [31] studied 8,280 pairs of twins and concluded that RSV probably represented genetic predisposition for asthma. RSV infection followed by BHR is characteristic for the first three years of life and is attributed to the virus tropism for developing pulmonary tissue [7]. In this study, occurrence of BHR in children with atopy was characteristic for the second year of life in which RSV became the most important causative agent of BHR (85.7%). It is possible that the dynamics of pulmonary development in children with atopy, in the second year of life, enables synergistic effect of RSV infection and atopy in BHR development.

In the first year of life, BHR was dominantly observed in children without atopy where RSV infection was discovered in 1/3 of the cases. The frequency of RSV infection in this group of children remained the same in the second year of life. It is possible that unknown defect in pulmonary development may attribute to early occurrence of BHR after RSV infection in children without atopy.

In the Tucson Children's Respiratory Study [32] male gender was marked as the risk factor for RSV infection of the lower respiratory tract and Hibbert et al. [33] found that in boys, lower respiratory tract is less developed than in girls. Small lumen of airways is described as a contributing factor in expression of lower respiratory tract diseases in viral infections [7]. In the present study, boys with atopy and expressed BHR had RSV infection in more than 80% of cases, but BHR in male children without atopy was not associated with RSV infection. BHR developed after RSV infection in boys only in case of the present atopy, which implied the importance of genetic factors associated with atopy. On the other hand, it is known that small respiratory lumen may be

the cause of BHR development in male children without atopy and that it could be in the form of transitory wheezing in the infant period not associated with later asthma development [12]. In girls, BHR was in 62.5% of cases associated with RSV infection, but equally in both groups, with and without atopy. It is possible that female gender is a determinant of BHR associated with RSV infection, regardless of the presence of atopy.

More expressed Th2 immune response is also associated with BHR after RSV infection [7]. Firstly, RSV infection favors Th2 immune response because it blocks the production of interferon (IFN) from the plasmocytoid cells and reduces interleukin (IL-12) production [7, 34]. Then, during the infant period, immune response is underdeveloped and physiologic Th2 immune response is present. Also, Th2 immune response is the dominant cellular response in the developed bronchus-associated lymphoid tissue (BALT) [35]. There is a question whether respiratory defect caused by immune response during viral infection is the result of Th2 immune response or a consequence of reduced mechanism of immunosuppression. Children with atopy, beside being genetically prone for development of Th2 response, also have reduced function of the immune response regulation through transforming growth factor bera 1 (TGFβ1) and IL-10. Somewhat insufficient regulation of the immune response with lower TGFβ1 is particularly present in the initial phase of infection [36], which may be the reason for chronic inflammation in children with atopy. In this study, non-breastfed children most often had BHR associated with RSV infection in comparison with breastfed children. Mother's milk is not only the source of nutritive elements for infants, but it also has potent protective effect, owing to present immunoglobulins and immunomodulating effect, owing to immunosuppressive cytokines such as TGFβ1 and IL-10 [37].

The results obtained in animal models clarify interaction between viral infection and exposition to inhalation allergens: infection can create a pro-allergic milieu and if an experimental animal was exposed to inhalation of these allergens for certain period of time, allergen-specific respiratory inflammation could be developed. Also, previous epidemiological data support direct causative role of RSV infection in development of sensibilizationn in the early phases of asthma [34]. The present study confirmed that children with atopy and born either in spring or summer often have RSV infection associated with BHR (more than 50%). This was not the case with children born in autumn or winter, so it is possible that there is a synergy between RSV infection and exposure to pollen resulting in BHR development.

It is known that the correlation between respiratory infections and asthma/persistent wheezing is complicated and seemingly based upon interaction of the host factors (such as age and the stage of development of innate and adoptive immune response at the moment of infection) and infectious agents. Resistance to viral infection and atopic sensibilization depends on the number of immune mechanisms that are strongly regulated in early life, particularly among children with high risk of atopy [38]. Additional studies on asthma pathogenesis are necessary, especially those that will include the following characteristics: primary cause of defect in the respiratory barrier of children with atopy; RSV tropism for respiratory epithelium during lung development; allergens as reactive molecules having chemical and enzymatic effect upon respiratory epithelium; disorders of the immunoregulatory mechanisms in BALT of children; disbalance of tissue factors during infection such as M2 muscarine receptors on smooth muscles in respiratory tract.

Conclusion

The results of this study suggest that the presence of bronchial hyperreactivity associated with respiratory syncytial virus infection can be early risk marker for asthma development, in all female children and in male children with atopy within the first two years of life. Therefore, early asthma prevention should include routine detection of respiratory syncytial virus infection and atopy in all children in primary health care. Also, very helpful would be education of parents on how to reduce children's exposure to infection and inhalation of allergens during critical periods of early life.

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Medico-legal expertise of pain in dental trauma

Veštačenje bola u traumatologiji zuba

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Introduction

Dental practice has been facing highly challenging medico-legal environment and problems related to legal expertise in everyday practice. Selaković et al. [1] have pointed out numerous issues arising in this field and offered potential strategies for overcoming such problems. The legitimacy in dental practice, responsibility and importance of relevant documents are topics addressed by numerous authors [2–5]. The best response to this problem is the appropriate education of dental practitioners in this field. The literature currently available to our dental practitioners encompasses mainly the field of forensic medicine [6, 7]. Forensic dentistry as a separate field of forensic medicine has not been paid adequate attention. Basic knowledge in forensic dentistry is accessible from rather sparse textbooks available in our country [8–11], whereas subspecialization reference books [12], [13] focused on specific topics of forensic dentistry are rare even at the global level. "Forensic dentistry, legal and medical aspects" is the only textbook [14] currently available in our country that comprehensively addresses the problems in this field. For understanding key issues of legal medicine, the book „Medico-legal expertise of non-material damage" is of great importance [15], and it is the recommended and mandatory reading for each medical and dental practitioner. The lack of up-to-date knowledge in the field of forensic medicine and dentistry is clearly reflected in the fact that only few articles addressing this topic have been published recently [16–19].

This article is an attempt to illustrate an original methodological approach to medico-legal expertise of total pain experienced after trauma of multiple teeth and surrounding tissues. The basic methodology was developed in the 90's of the last century [20, 21]. Identification and correction of certain shortcomings resulted in the first revision and modification of the original methodology some 8 years ago [15], and the second revision was performed three years ago [14].

The majority of dentists are not familiar with expert evaluation of non-material damage (civil liability) including reduction of life activities, total suffered pain, mental suffering for facial disfigurement, and these terms are mostly considered abstract classifications. It is mainly due to the fact that this segment of education is not properly addressed throughout the study curriculum, neither undergraduate nor postgraduate (specialization). Expert evaluation of the severity of injury (criminal liability) is also quite unknown to dentists. Medico-legal expertise of disability (invalidity), i.e. working inability involves salary loss and is considered material damage. In the majority of cases, disability is associated with the reduction of life activities; however, the reduction of life activity does not necessarily implicate disability. The rate (severity) of injury does not correspond with the rate of reduction of life activities.

Such an environment characterized by the lack of basic knowledge is not supportive for novel scientific and methodological approaches. The greatest support to this field came from the university teaching staff in the field of law and lawyers. This methodology does not offer the estimation of total experienced pain (subjective attitude), but its evaluation (objective attitude). The main objection of medical profession to this methodology is shifting of philosophical concept of pain into the field of mathematics. However, legal profession emphasizes this shift as the best improvement of the methodology. Nevertheless, application of this methodology enables reaching of identical conclusions irrespective of the person performing the expertise. It offers better quality

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of legal expertise in dental medicine, as well as improved competence and reputation of dentists involved in medico-legal expertise. Application of this methodology will exclude the necessity of additional "super-expertise" or unnecessary confrontation of experts in the future.

Medico-legal importance of pain

Ilić et al. [18] made a review of the book "*Libellus de dentibus*" ("Book on Teeth") by Bartolomeus Eustachio, the first book dedicated to dental medicine and teeth. In the first chapter, Eustachio attempted to elucidate the sensitivity of hard dental tissue, whereas in the fourth chapter he comprehensively addressed the innervation and vascularisation of the upper and lower jaws. This is the first step in emphasizing the relation between teeth and dental pain.

The assessment of the total physical pain experienced is one of the basic requests for medico-legal expertise in a civil litigation. The central issue of the civil procedure is the complainant, who is expected to be adequately compensated for suffered material and non-material damage.

The assessment of the total physical pain experienced is one of the basic requests for medico-legal expertise in a civil litigation. Objectification of pain intensity is considered major medical problem in dentistry practice. Pain intensity scale guides may partly simplify this task. The main problem occurs on medico-legal expertise of total pain experienced from the moment of injury until complete recovery. Dental polytrauma (several teeth injury) is the secondary problem that may include a variety of injuries and the health status of teeth at the moment of injury. Such situations assign to the expert witness a role of an "estimator" of the total pain that the patient has experienced, emphasizing the subjective factor.

The aim of this paper was to introduce a uniform procedure that simplifies medical expertise of pain, and results in the identical conclusions irrespective of the person performing the expertise. The ultimate goal is to design the methodology applicable for assessing the total pain in dental trauma, featuring the objective factor.

Methodology for assessing the total pain in dental trauma

The methodology for assessing total pain after tooth and jaw trauma was developed in the mid-90s of the XX century by Selaković [20, 21], while certain modifications were introduced during past several years [14, 15]. This methodological approach accentuates all relevant factors with high level of objectivity, and with maximum avoidance of subjective attitude towards the problem. This methodology enables reviewing and verification of each medico-legal expertise. The ultimate goal of this attempt is to develop a uniform procedure that simplifies the procedure and results in the identical conclusions irrespective of the person performing the expertise.

Physical pain as the consequence of the afflicted injury, and establishing criteria for compensation for non-material damage is a very complex problem. Diversity of organs and tissues, age and health status of a patient are only some of the factors indicating the difficulties in determining common criteria related to pain assessment. On the other hand, the medico-legal expertise of polytrauma and pain located at several sites and of different intensity is particularly intricate. Such cases mostly implicate the dominant highest-intensity pain, whereas other parameters are of somewhat less importance. Nevertheless, each individual pain can neither be ignored nor regarded as an isolated injury. Our opinion is that in case of dental polytrauma the injury and highest-intensity pain are to be considered important factors, whereas other parameters should be taken into consideration with some reservations.

Systematization of all common criteria and factors relevant to pain is an essential issue, disregarding its localization in the body. Each branch of medicine implicates particular injury categorization systems based on different criteria. Medico-legal expertise of physical pain as a form of non-material damage requires an accurate injury categorization in view of experienced physical pain and applied medical treatment. Thereby, the "International statistical classification of diseases and related health problems" [22] should be taken as the "outset" document.

Expertise of physical pain in dental trauma – starting points for pain classification

The first instance in the process of medico-legal expertise of physical pain in dental trauma is defining and predicting the preliminary medical factors. Taking these factors into consideration is the standpoint for designing the intensity scale of experienced and anticipated pain.

Physical pain is subjective sensation resulting from a somatic injury and disturbance of body integrity due to physical injury or disease.

Pain intensity evaluation and its objectivization are accomplished using the variety of scales. The most widely used is a 10-point scale, whereas 5-, 4- and 3-point scales are less common. To the purpose of medico-legal expertise a 5-point scale is the most appropriate, which impeccably reflects pain intensity in the pulp, periodontium, in the bone and soft tissues. It enables an accurate pain classification, without redundant details. The scale is as follows:

Intensity grade 1 – low-intensity pain lasting as long as the stimulation itself;

Intensity grade 2 – higher-intensity pain lasting longer then the stimulation itself;

Intensity grade 3 – high-intensity; pain responsive to analgesics, immobilization and resting and provoked by moving, speaking, eating;

Intensity grade 4 – particularly severe and enduring pain, irresponsive to analgesics;

Intensity grade 5 – the worst possible pain, long-lasting pain resulting in the state of shock.

Duration of pain is an inevitable factor, since pain can occur as instant and transient or can persist until complete recovery of the patient. Four different points in time are defined pertaining to the occurrence and persistence of pain, i.e. pain at the moment of injury, pain persistent until cure, pain in the course of medical procedure, pain during healing

and recovery period. At each of these points in time, pain can be rated according to 1–5 points at the pain scale.

Surgical classification of injuries and therapy procedures

A range of classification models for categorization of tooth injuries proved inconsistent, limited or overextensive and too complex, hence inadequate for physical pain assessment. Categorization of tooth injuries that encompasses pain intensity at the moment of injury and in the course of appropriate therapy procedure enables an objective assessment of pain. Such a categorization, relying on the "International statistical classification of diseases and related health problems" [22] are displayed in Tables 1 and 2. These categorization models considerably simplify the expertise, enabling reproducibility of the procedure using the same methodology approach in the control or super expertise. This method is enough feasible and comfortable for the expert, and yet enhances credibility of dentistry and dentists among the clients, i.e. legal entities.

Auxiliary factors

Auxiliary factors deserve particular consideration as an inevitable part of this methodology, thus their precise identification and classification is of particular importance.

Health status of teeth (organs) before injury

Assessment of pain intensity is to the great extent determined by the tooth condition (status), i.e. healthy, defective or healed tooth, sprouting tooth or parodontopathic tooth. Within the scope of tooth trauma, a parodontopathic tooth is defined as "tooth in which the height of the crown and uncovered root portion is greater than the root portion in the jawbone, irrespective of tooth-mobility". This element is important in view of lever-principle, lever arm and fulcrum. Namely, such tooth is more easily luxated or broken than the healthy one. In that respect, expertise of injury involving tooth crown breakage associated with an uncovered pulp chamber requires additional differential diagnosis confirming whether the tooth was previously healthy (vital), devitalized (endodontically-cured or diseased), parodontopathic, with a certain degree of mobility. All this necessarily suggests that the identical injury does not produce pain of the same intensity.

Multiple tooth injuries – dental polytrauma

In multiple injuries the pain of highest intensity at the moment of injury, until dental surgery procedure, in the course of procedure and recovery (healing) is considered dominant. The intensity of pain at each stage is rated [1–5]. Lower-intensity pain in other teeth is of secondary importance; however, it cannot be completely ignored. Following the rating of all individual injuries, the highest pain intensity is considered dominant, whereas other pains are graded with rate 1. Pain cannot be regarded as simple mathematical calculation. Pain of highest intensity is always dominant and most distressing for a patient. Localization of different types of pain in dental polytrauma is, however, far too limited area to enable assessment of all pain types in the same manner.

Secondary procedures

In most cases a patient completely recovers after treatment and healing, and continues with daily and professional activities. However, additional dental and medical procedures are necessary, which mostly causes pain. Such procedures include fixed prosthetics (crown grinding), apictomy, removal of ligature wires, miniplates or scars correction. This methodology implicates evaluation of such pain category with grade 1, irrespective of the number of subsequent procedures, because they are considered logical finishing point of the therapy.

Classification and assessment of pain intensity

A variety of approaches in this field results from the complex pathology and fairly large number of different injuries occurring in this region. Each classification reflects a particular aspect or aspects of this problem. The abundant literature offers comprehensive classifications of injuries of teeth, alveolar crest and surrounding soft tissues. It is of particular importance in assessing pain intensity that is determinative factor in appraising the pecuniary compensation (nonmaterial damage). The entire procedure involves two classification instances: classification of tooth and surrounding tissue injuries with a rating scale for individual pain intensity at the moment of injury until dental surgery procedure (Table 1); classification of therapy procedure – pain in the course of dental procedure and wound healing (Table 2).

These two classifications depict the injury in the course of time. The procedure differentiates the actual experienced pain and previous health status of the tooth. Namely, pain intensity and corresponding therapy procedure are not necessarily identical, even in case of the same type of injury. Injury classification itself encompasses 13 different situations, distributed into 3 sub-groups: isolated tooth injuries, combined tooth injuries and injuries of surrounding soft tissues.

Table 1 shows pain intensity at the moment of injury and until dental surgery procedure. The first column contains the injury code. The first digit is 1 (one) that represents pain mark at the moment of injury until medical processing. The second digit is the code for particular injury – the classification differs between 13 distinct injuries. The second column contains injury classification and diagnose in Latin. The third column is divided into four sub-columns for the first 10 injuries, each one for a specific health status of the tooth immediately before the injury. Under the Table is given an explanation on initial codes for each sub-column. Injuries designated with 11–13 refer to injuries of surrounding tissues. Numerical marks within these columns describe pain intensity of the injury itself. The first digit indicates pain intensity at the moment of injury, the second one the pain intensity until medical processing, whereas the third one represents the

Table 1

Classification of tooth and surrounding tissue injuries with the scale of individual pain intensities at the moment of injury until dental surgery

Code	Type of injury classification Injury-shaded fields	Pain intensity Tooth diseases and conditions H	P	DH	DI
	Isolated tooth injuries				
1.1.	*Fractura enameli et dentini coronae dentis traumatica*	1 + 0 = 1	1 + 0 = 1	0 + 0 = 0	0 + 0 = 0
1.2.	*Fractura coronae dentis completa traumatica*	2 + 1 = 3	2 + 1 = 3	1 + 0 = 1	1 + 0 = 1
1.3.	*Fractura radicis dentis traumatica*	2 + 1 = 3	1 + 1 = 2	1 + 1 = 2	1 + 1 = 2
1.4.	*Luxatio dentis traumatica*	2 + 1 = 3	1 + 1 = 2	2 + 1 = 3	2 + 1 = 3
1.5.	*Intrusio dentis traumatica*	3 + 1 = 4	3 + 1 = 4	3 + 1 = 4	3 + 1 = 4
1.6.	*Extractio dentis traumatica*	2 + 1 = 3	1 + 0 = 1	2 + 1 = 3	2 + 1 = 3
	Combined tooth injuries				
1.7.	*Luxatio dentis traumatica cum fractura enameli et dentini coronae dentis*	2 + 1 = 3	1 + 1 = 2	2 + 1 = 3	1 + 1 = 2
1.8.	*Luxatio dentis traumatica cum fractura coronae dentis completa*	3 + 2 = 5	2 + 2 = 4	2 + 1 = 3	1 + 1 = 2
1.9.	*Luxatio dentis traumatica cum fractura radicis*	3 + 2 = 5	2 + 2 = 4	2 + 1 = 3	1 + 1 = 2
1.10.	*Fractura dentis comminutiva*	4 + 3 = 7	3 + 3 = 6	4 + 3 = 7	3 + 3 = 6
	Injuries of surrounding tissues				
1.11.	*Fractura processus alveolaris*				3 + 2 = 5
1.12.	*Fractura mandibulae (maxillae)*				4 + 3 = 7
1.13.	*Vulnus lacerocontusum* (*cutis, labii oris, gingivae, linguae*)				2 + 1= 3

H – healthy, P – parodontopathic, DH – devitalized healthy, DI – devitalized infected.

Table 2

Classification of therapy procedures and table of individual pain intensity (5-point scale)

Code	Procedure – description of healing stage	PIP	PIH	TIP
2.1.	Direct pulp-capping – No pain during healing stage, secondary procedure involve dental filling	1	0	1
2.2.	*Exstirpatio pulpae vitalis* – No pain during healing stage, secondary procedure involve dental filling or crown onlay/inlay	1	0	1
2.3.	*Exstirpatio pulpae vitalis* – Ortodontic root extraction and crown onlay/inlay	1	1	2
2.4.	Apicotomia – Sutures present during healing stage, secondary procedure involve dental filling or crown onlay/inlay	1	1	2
2.5.	*Exstractio dentis* – No pain during healing stage, secondary procedure involve prosthetic denture	1	0	1
2.6.	*Exstractio chirurgica* – Sutures present during healing stage, secondary procedure involve prosthetic denture	1	1	2
2.7.	*Reinplantatio dentis* – splint at later phase	1	1	2
2.8.	*Repositio cum fixatio dentis* – splint at later phase	1	1	2
2.9.	*Repositio cum fixatio peocessus alveolaris* – splint at later phase	1	2	3
2.10.	*Repsitio cum immobilisatio bimaxillaris* – Inability of mouth opening, problems at eating	2	2	4
2.11.	Wound treatment – Sutures present during healing stage, secondary procedure may include scar correction	1	1	2
2.12	*Exstractio chirurgica sequestri* (corpori alieni) – Sutures or iodine gauze present during healing stage	2	1	3

PIP –Pain intensity during procedure; PIH – Pain intensity during healing; TIP –Total intensity of experienced pains.

sum thereof. Hence, the code 1.3.1 in a report refers to the breakage of healthy tooth root, and the intensity of experienced pain is rated 3.

Table 2 contains the classification of therapy procedures associated with pain intensity scale. Selection of therapy procedure is determined by the character of injury, period elapsed from the moment of injury, skills of a dentist and staff, available equipment and facilities, age of the patient, level of oral hygiene, cooperativeness of the patient during therapy and the patients' needs with respect to particular oral health standard. Some procedures are repeated from practical reasons, with the aim to determine the continuity of pain intensity in the course of healing, as well as differences resulting from the need for secondary procedures. The assessment of pain intensity during the procedure is always associated with administration of local anesthetics, which are (by ethical reasons) indispensable under such circumstances.

Based on the obtained overall assessment of all experienced pains, the following pain scale can be designed, encompassing the 5 basic pain categories with the range 1–25: Category I – Mild pain – intensity rate 1–5; Category II –

Substantial pain – intensity rate 6–10; Category III – Severe pain – intensity rate – 11–15; Category IV – Extremely severe pain – intensity rate 16–20; Category V – Excruciating pain – intensity rate 21–25.

In the context of general medical traumatology, tooth injuries cannot reach the rate beyond 15, i.e. could be classified into categories I to III. Only associated with the jaw fracture they could be assigned to category IV.

Note: This methodology is currently applied only in dentistry, yet the possibility of its wider application is evident. In that respect, the category V is introduced for evaluation of overall pain experienced by general polytrauma of the body.

The procedure encompasses several stages: identifying the number of injured teeth; diagnosing the health status of the tooth at the moment of injury; selecting therapy procedures for each particular tooth; identifying prospective secondary procedures; entering codes into the "Record Sheet", as well as all numerical indicators; assessment and categorization of all experienced pain.

On the basis of specialist reports, record protocols and sheets, injury-record sheets, radiographs and diagnostic procedures, actual status is determined. All the parameters are entered into the "Record Sheet on Intensity of Overall Pain Experienced at the Trauma of Teeth and Surrounding Tissue". It is essential to rank all individual injuries according to the intensity of overall experienced pain. The injury with highest overall intensity is dominant, i.e. it is accepted as a whole. Other individual injuries are presented through their total intensity in the next to the last column of the Record Sheet. This methodology regards all other pains as minimal, thus their rating at the pain scale is 1. The final overall rating of the total experienced pain is calculated by summarizing pain intensity rates for all individual injuries ranked and assessed using the aforementioned pattern.

Some examples from everyday practice

Example 1 (Table 3)

The medico-legal expertise of total pain experienced in tooth trauma can be established based on comprehensive medical records. The relevant medical records and documentation [19] give a detailed overview of maxillary tuberosity fracture arising as a complication of tooth extraction. Medical error and medical malpractice lawsuit is determined, which is considered criminal liability. In a consequent civil procedure, compensation for non-material damage, i.e. total pain suffering damage is requested. Medical factors considered in the evaluation of total pain suffered revealed the following: The patient referred to the dentist with pain in tooth 17 that was infected and indicated for extraction. During procedure performed in local anesthesia, tuberosity fracture occurred (1.11.), along with traumatic luxation of two teeth (1.4.4. and 1.4.1.), laceration and contusion of gingiva (1.13.). The injury was not recovered after 2 days. Under local anesthesia, the tuberosity was removed (2.12.), both teeth extracted (2.6.) along with the management of oroantral communication (2.11.). The procedure was well documented, containing all medical elements for further expertise. The dentist is not responsible for pain preceding patient's first visit. At the moment of injury, pain was not of maximum intensity because of local anesthesia. Augmentation or pterygoid implant is a possible solution.

According to the intensity of pain, the most intense pain is associated with tuberosity fracture. The overall pain suffered is rated 12, which is assigned into pain category III.

Example 2 (Table 4)

A 46-year-old woman fell down by an abrupt braking of a bus and suffered fracture of a lower jaw body as well as the bite wound in the lower lip, extrusion of two parodontopathic and one healthy tooth, and tearing of the surrounding gingiva (a total of 6 individual injuries). The wounds in the mouth and skin were sutured, and lower jaw immobilized. Secondary intervention included bridge mounting. Table 4 shows that this patient experienced category IV pain – extremely severe pain, rated 17 at the intensity rate scale. Without jaw fracture, the total intensity of pain cannot exceed rate 15 of the pain scale.

Example 3 (Table 5)

Multiple injuries in the head and chest are made by two close-range gunshots. The victim falls to her knees because of the hit in the head with the gun's stock (downward force).

Table 3

Example 1 – Filled-out Record Sheet total intensity of experienced pain

Name of the patient	
Address	
Identification number	
Date	

No	Injury localization	Injury code (total intensity)	Therapy code (total intensity)	Total pain intensity	Rate
1.	Maxillary tuberosity	1.11. (5)	2.12. (3)	8	8
2.	Tooth 17	1.4.4. (4)	2.6. (2)	6	1
3.	Tooth 18	1.4.1. (4)	2.6. (2)	6	1
4.	Gingiva	1.13. (3)	2.11. (2)	5	1
Secondary procedure - description:		Augmentatio, inplantatio			1
Overall rate of experienced pain					12

Table 4

Example 2 – Filled-out Record Sheet total intensity of experienced pain

Name of the patient _____
Address _____
Identification number _____
Date

No	Injury localization	Injury code (total intensity)	Therapy code (total intensity)	Total pain intensity	Rate
1.	Jaw fracture	1.12. (7)	2.10. (4)	11	11
2.	Lower lip	1.13. (3)	2.11. (2)	5	1
3.	Gingiva	1.13. (3)	2.11. (2)	5	1
4.	Tooth 33	1.6.1. (3)	/	3	1
5.	Tooth 32	1.6.2. (1)	/	1	1
6.	Tooth 31	1.6.2. (1)	/	1	1
Secondary procedure - description:		Dental bridge			1
Overall rate of experienced pain					17

Table 5

Example 3 – Filled-out Record Sheet total intensity of experienced pain

Name of the patient _____
Address _____
Identification number _____
Date

No	Injury localization	Injury code (total intensity)	Therapy code (total intensity)	Total pain intensity	Rate
1.	Chest	5 + 4 = 9	2 + 3 = 5	14	14
2.	Pneumothorax	4 + 3 = 7	2 + 2 = 4	11	1
3.	Jaw fracture	1.12. 7	2.10. 4	11	1
4.	Tooth 14	1.10. 7	2.12. 3	10	1
5.	Tooth 13	1.10. 7	2.12. 3	10	1
6.	Tooth 12	1.10. 7	2.12. 3	10	1
7.	Fractura proc. alveolaris	1.11. 5	2.12. 3	8	1
8.	VLC linguae	1.13. 3	2.11. 2	5	1
9.	Scalp	1.13. 3	2.11. 2	5	1
Secondary procedure - description:		Dental bridge			1
Overall rate of experienced pain					23

VLC – *Vulnus lacerocontusum.*

At this position of the victim, the defendant was on her right side and shot the first bullet from the distance of 50 cm, which caused entry wound in the right nasolabial sulcus. Along the bullet pathway, destruction of the alveolar ridge (1.11.) and 3 teeth 14, 13 and 12 (1.10.1.) was apparent. The penetrating missile hit the lower jaw on the lingual side. The bullet retarded by striking the juncture between the alveolar crest base and the body of the lower jaw in the region 34–36, causing fracture of the lower jaw (1.12.). After recoiling, the bullet and its fragments remained in the left sublingual space causing laceration and contusion of the tongue and mucosa of the floor of oral cavity (1.13.). The victim fell facedown and the defendant fired the second shot causing entry wound in the region of the scapula. Along its track, the bullet penetrated the upper part of the right lung causing pneumothorax. The clavicle region is the exit site of the bullet. The injuries apparent in the maxillofacial region and associated suffered pain are classified into Category IV. Maxillofacial pain even exceeding grade [21] without chest injury would not be accepted. Potential secondary procedures associated with pain include augmentation of upper jaw ridge and placement of three implants. In this case, the pain can be assigned to the Category V, i.e. excruciating pain.

If there had not been any chest injuries, the most intense and durable pain would have been the lower jaw injury (grade 11) with total suffered pain being grade 18. Thus, the head injury would be classified as total pain of the Category IV.

Because of gunshot wound in the chest, the most intensive pain was reported in this region. The victim experienced pain of highest intensity (grade 5 pain resulting in the state of shock and consciousness lost) at the moment of bullet passing through her chest. Until medical treatment, she experienced pain graded 4 (enduring pain irresponsive to analgesics or immobilization). During medical treatment (before, during and after general anesthesia) she suffered pain graded 2. Throughout the postoperative recovery stage, she reported grade 3 pain. All this resulted in assessment of the intensity of total suffered pain to be 14. The total pain suffered is graded 23, classif it to Category V (excruciating pain).

In this case, 20 years after developing this methodology and on the basis of a 30-year-long experience in oral surgery,

the author allowed himself to perform medico-legal evaluation of pain that is not the subject of his medico-legal license.

Conclusion

The described methodology enables the uniform approach in pain assessment. Methodologies applied so far encompassed more subjective approach. In repeated expertise, discrepancies were frequent and anticipated due to an inconsistent procedure protocol. The described approach provides an appropriate protocol for obtaining identical results in repeated expertise. This approach is somewhat arguable from the point of view of medicine and dentistry, yet it is highly feasible in legal practice. It provides clear legal qualification excluding any doubts with respect to the competence of medico-legal expertise.

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CASE REPORT

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Successful primary percutaneous coronary interventions in a patient with two consecutive ST-segment elevation myocardial infarctions and dual left anterior descending artery (type IV)

Uspešne primarne perkutane intervencije kod bolesnika sa dva uzastopna infarkta miokarda sa elevacijom ST-segmenta i dvostrukom prednjom descedentnom arterijom (tip IV)

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Abstract

Introduction. Dual left anterior descending (LAD) artery is a very rare inherited anomaly. It can be incidentally revealed during primary percutaneous coronary intervention (pPCI) and may produce difficulties in detecting and treating the culprit lesion. **Case report.** We presented a 52-year-old male patient with ST-segment elevation myocardial infarction (STEMI) of inferior wall, in whom dual LAD anomaly was revealed during pPCI: a short LAD artery originated from the left main coronary artery and a long LAD artery originated from the proximal part of the right coronary artery (RCA). A bare metal stent was successfully implanted in the place of the culprit lesion in RCA and ST-segment resolution was achieved in ECG. After two hours, the patient was referred again to the catheter lab due to new STEMI of anteroseptal wall. Another bare metal stent was implanted in new infarction related artery, this time it was proximal part of the short LAD. **Conclusion.** Careful and correct interpretation of ECG is very helpful in detection and treatment of the culprit lesion in cases with dual LAD.

Key words:
coronary vessels; congenital abnormalities; myocardial infarction; stents; reoperation; electrocardiography.

Apstrakt

Uvod. Dvostruka prednja descedentna arterija (PDA) je veoma retka urođena anomalija i može biti otkrivena slučajno tokom primarne perkutane koronarne intervencije (pPKI) što može stvoriti poteškoće u određivanju i lečenju infarktne lezije. **Prikaz bolesnika.** Prikazali smo muškarca, starog 52 godine, sa infarktom miokarda sa ST-elevacijom (STEMI) donjeg zida, kod koga je tokom pPKI otkrivena dvostruka PDA. Kratka PDA je imala ishodište iz levog glavnog koronarnog stabla, a dugačka PDA iz proksimalnog dela desne koronarne arterije (DKA). Na mestu infarktne lezije u DKA uspešno je implantiran metalni stent i postignuta rezolucija ST-segmenta u EKG-u. Nakon dva sata, bolesnik je vraćen u angiosalu zbog novog STEMI anteroseptalne lokalizacije. Drugi metalni stent implantiran je na mestu nove infarktne lezije, ovog puta u proksimalnom delu kratke PDA. **Zaključak.** Pravilna interpretacija EKG-a od velike je pomoći u detekciji i lečenju infarktne lezije kod retke anomalne dvostruke PDA.

Ključne reči:
aa. coronariae; anomalije; infarkt miokarda; stentovi; reoperacija; elektrokardiografija.

Introduction

The incidence of the dual left anterior descending (LAD) artery is uncommon and ranges from 0.01% to 0.03%. In 1983, Spindola et al. [1] published a classification of this very rare inherited coronary artery anomaly. This classification included four types of dual LAD. The first three types implied a variety of both short and long LAD arteries originating from the left coronary sinus or the left main coronary artery, and the fourth type implied that long LAD artery originating from the proximal part of the righ coronary artery (RCA). In 2010, Manchanda et al. [2] described the fifth type of dual LAD, where the

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short LAD artery originates independently from the left coronary sinus, and the long LAD artery originates from the right coronary sinus. In 2012 a new, sixth type of dual LAD was proposed by Maroney and Klein [3], where a unique route of long LAD artery was discovered. It arouse from proximal RCA going underneath the right ventricular outflow tract to the anterior interventricular groove. Dual LAD anomaly can be a rare cause of ischemic heart disease or even sudden cardiac death, especially when the long LAD artery goes between large cardiac vessels, like aorta and pulmonary trunk, which can lead to artery compression during the high blood flow through those big arteries [4, 5].

We presented a patient with type IV, dual LAD anomaly discovered during primary percutaneous coronary intervention (pPCI), who also had two consecutive STEMIs in the span of two hours with culprit lesions in two different coronary arteries: the RCA and short LAD artery.

Case report

A 52-year-old male patient with chest pain and diaphoresis was admitted to the Emergency Department of the Military Medical Academy in Belgrade, Serbia. Pain had the character of tightening in the chest, spreading to the shoulders and upper arms and there was a time lapse from pain onset to admission of only one hour. The patient stated similar pain in the past. He was also treated for hypertension and diabetes mellitus type 2. He smoked 25 pack year of cigarettes.

His blood pressure was 120/80 mmHg and heart rate 67 beats *per* minute. Other physical findings on admission were unremarkable except for a pale, dewy skin.

Baseline ECG showed ST-segment elevation in the leads for the inferior wall, and reciprocal ST-segment depression in the D1 and AVL leads (Figure 1).

As *per* treatment protocol, before pPCI, the patient received oral administration of aspirin 300 mg, clopidogrel 600 mg and parenteral administration of unfractionated heparin 80 U *per* kilogram of body weight.

On coronary angiography, a short LAD artery was seen, arising from the left coronary sinus and exhausting in the middle segment, after the separation of a large septal trunk. In its proximal part, a significant, tubular-type, stenosis of about 80% of vessel lumen was seen (Figure 2).

The RCA had subtotal stenosis in the proximal segment just after the separation of a long artery which bended toward apical lateral region of the heart. That anomalous artery, which arouse from the proximal part of the RCA appeared to be a long LAD artery in a very rare coronary artery anomaly called dual LAD. It had also a tubular stenosis of about 40–50% in its proximal part (Figure 3). According to ECG changes, the ST-segment elevation in the leads for the inferior wall, lesion of the RCA was recognized as the culprit lesion and after several balloon predilatations, a bare metal stent (dimension 3.5 × 15 mm) was implanted. The final coronarography effect was referred as excellent (TIMI 3 flow) (Figure 3).

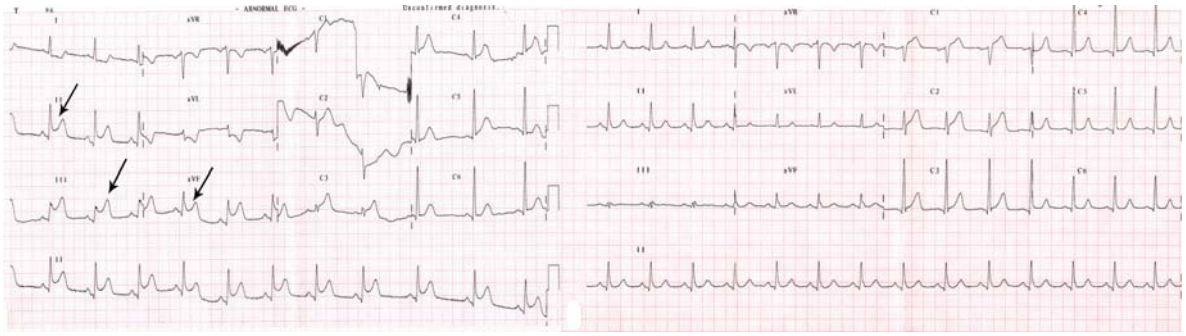

Fig. 1 – Left: ECG on admission: ST-segment elevation in the leads for the inferior wall (arrows); Right: Complete ST-segment resolution after performing primary PCI with stent implantation in the RCA as infarction related artery. ECG – electrocardiogram; PCI – percutaneous coronary intervention; RCA – right coronary artery.

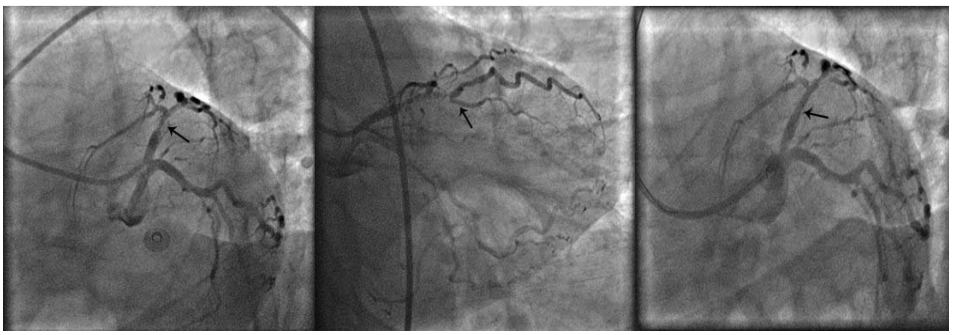

Fig. 2 – Left and middle: coronary angiogram, "spider" and caudal view, showing the short LAD artery (arrow) with a significant stenosis in its proximal part. Right: short LAD artery after stent implantation (arrow). LAD – left anterior descending.

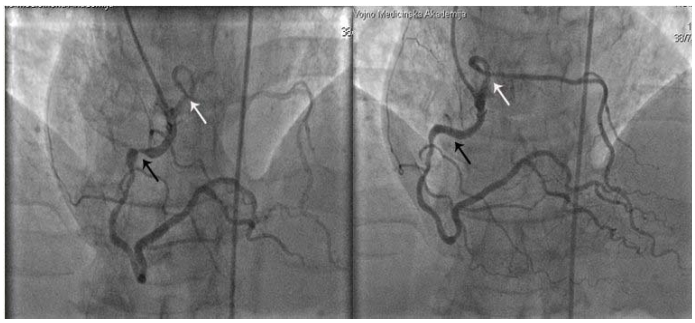

Fig. 3 – Left: coronary angiogram, cranial view, with the RCA and significant stenosis as culprit lesion (black arrow) and the anomalous long LAD artery with mild proximal stenosis (white arrow). Right: RCA after stent implantation in culprit lesion (black arrow) and the long LAD artery (white arrow).
RCA – right coronary artery; LAD – left anterior descending.

Soon after pPCI, the patient was without chest pain, and ECG showed complete ST-segment resolution (Figure 1).

Two hours after the intervention, while recovering in intensive care unit, the patient had chest pain again, with identical propagation and diaphoresis. ECG revealed ST-segment elevation in AVL and V1 to V3 (Figure 4).

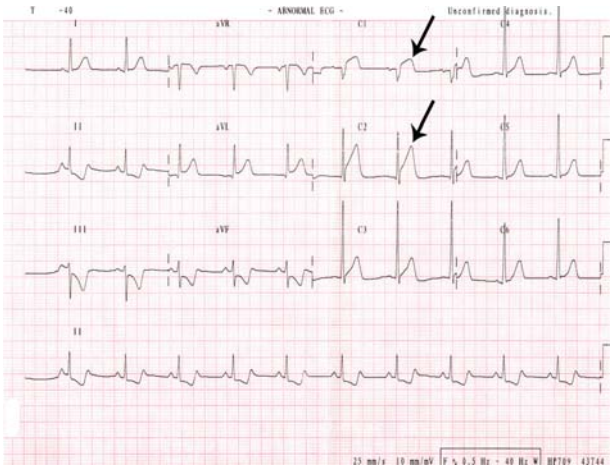

Fig. 4 – ECG two hours after the primary PCI on the RCA, ST-segment elevation in the leads for the anteroseptal wall (arrows) suggesting new culprit lesion in the short LAD artery.
ECG – electrocardiogram; PCI – percutaneous coronary intervention; RCA – right coronary artery; LAD – left anterior descending.

The patient was returned to the catheter lab and another pPCI was performed. Thrombus morphologic lesion was noticed in the place of the previous stenosis in the proximal segment of the short LAD artery originating from the left coronary sinus, so we implanted another bare metal stent in this new culprit lesion. An excellent coronarography effect was achieved with TIMI 3 flow. A control angiography of recently stented RCA was performed and TIMI 3 flow remained.

After this intervention, chest pain disappeared, and complete ST-segment resolution was observed on ECG.

In further course of hospitalization, there were no complications and cardiac specific enzymes activity completely returned to normal values. Echocardiography at discharge, seven days after admission, showed mild hypokinesis of the middle and basal part of the septum with left ventricular ejection fraction of 50%. The patient was discharged home in good condition and referred to further follow-up. One year after pPCI, the patient underwent dobutamine stress echo examination which was negative for ischemic heart disease. A few months later, he complained of mild chest discomfort, but on examination in emergency unit, serial ECGs were without changes, serum cardiac specific enzymes activities were in normal value range, arterial blood pressure was 130/80 mmHg and the rest of physical examination was unremarkable. Having in mind his current health state and the previous medical history we decided not to expose him to invasive coronary angiography, but to emergency multi-detector computed tomography (MDCT) coronary angiography. It revealed that both of implanted stents were without signs of occlusion or restenosis, the lesion in the long LAD artery was estimated as unchanged, and culprit lesion was not seen. Using this imaging we recognized the dual LAD to be of type IV because the long LAD artery did not have trajectory under, but above the right ventricular outflow tract (Figure 5).

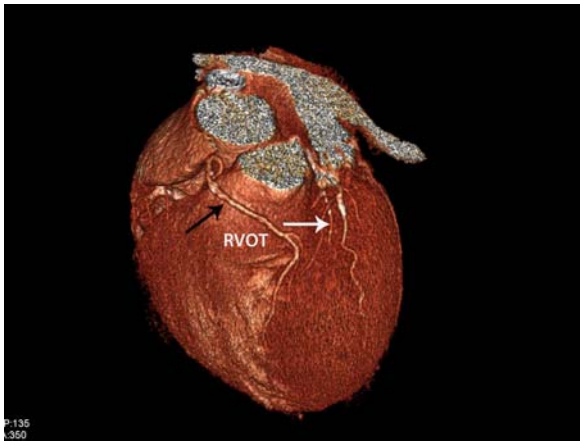

Fig. 5 – MDCT coronary angiography performed one year later. Long LAD artery originates from the proximal RCA and goes above RVOT toward anterior interventricular groove (black arrow), suggesting type IV dual LAD. Short LAD artery is also seen (white arrow).
MDCT –multidetector computed tomography; LAD – left anterior descending; RCA – right coronary artery; RVOT – right ventricular outflow tract.

Discussion

Acute coronary syndrome in patients with anomalous coronary arteries has been reported as specific and sometimes

difficult to treat when performing PCI [6, 7]. In the presented patient with STEMI of the inferior wall a very rare coronary artery anomaly, called dual LAD, was incidentally revealed during the primary PCI. He also had multivessel coronary disease which could have make treatment decision difficult, especially if a patient had more diffuse ECG changes or myocardial infarction of another localization. A short LAD artery could sometimes look as acutely blocked infarction related artery and trying to open it may lead to perforation. Additionally, early separation of a long LAD artery from the RCA also might be missed on coronarography due to catheter malposition, or due to the separate source of the anomalous artery from the right or left coronary sinus [8]. The presented patient had inferior wall myocardial infarction, which was clear due to ST-segment elevation on ECG in the leads for inferior wall. Although the RCA was not occluded and had TIMI 3 flow, significant proximal lesion was recognized as culprit lesion. After two hours, the patient developed another STEMI, but this time, ST-segment elevation was in the leads for anteroseptal region, which guided us to the short LAD artery, with its proximal lesion, as infarction related artery and not the long LAD artery which also had borderline stenosis. This suggests that ECG guided primary PCI could be of enormous help in revealing a culprit lesion in STEMI patients with anomalous coronary arteries, especially in patients with multivessel coronary disease. What was a rupture trigger for the second culprit lesion remains unclear, but it is understandable that plaque in the short LAD artery was highly unstable and complex interplay of variables in and outside the plaque could cause rupture with subsequent thrombosis. Acute myocardial infarction is a state of increased inflammatory and hemodynamic activities which can cause further plaque destabilization, arterial spastic reaction, intraplaque hemorrhage and plaque disruption [9, 10]. It is possible that selective injection of contrast fluid, during PCI, can cause microscopic foci of endothelial loss or even erosions on the unstable plaque surface which can lead to thrombotic cascade. An interesting question could be asked in this case: What if anteroseptal STEMI had appeared first in our patient and anomalous long LAD artery had not been seen on coronarography due to separate ostium in the right coronary sinus or due to catheter malposition in the RCA? Because the short LAD artery diminished after giving large septal trunk, thinking of occlusion and trying to open it could have had dangerous consequences and at least it would have consume a precious time.

Furthermore, very rare anomalies of coronary arteries may be a problem if urgent cardiac surgery is required [11].

This case was interesting to us due to several reasons: incidental revelation of one of the rarest coronary anomalies; development of two consecutive STEMIs of two separate regions (first inferior, and second was anteroseptal) within two hours; correct interpretation of culprit lesions sites among several hemodynamically significant stenosis of the coronary arteries including the anomalous LAD artery, and successfully performed PCI with excellent recovery of the patient.

Conclusion

Dual LAD artery is very rare anomaly which might be incidentally revealed during primary PCI. In these patients, if multivessel coronary disease exists, recognizing culprit lesion is more complicated which can be amended by careful interpretation of ECG changes together with understanding the anomalous arteries' trajectories.

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CASE REPORT

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Prenatal diagnosis of lissencephaly: A case report

Prenatalna dijagnoza lizencefalije

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Abstract

Introduction. Lissencephaly ("smooth brain") forms a major group of brain malformations due to abnormal neuronal migration. It can cause severe intellectual and motor disability and epilepsy in children. The prenatal diagnosis of this malformation is rare. **Case report.** We presented a case of the prenatal diagnosis of lissencephaly. A 30-year old pregnant woman was reffered to the hospital at the week 35 of gestation for magnetic resonance imaging (MRI) after an ultrasound examination demonstrated fetal cerebral ventriculomegaly. Fetal MRI of the brain showed "smooth", agyrya cortex. The female infant was born at term with birth weight of 2,500 g and Apgar score 8, showing global developmental delay. Postnatal ultrasound and MRI confirmed classical lissencephaly. She is now 8 years old and has spastic quadriparesis, mental retardation and epilepsy. **Conclusion.** Confirmation of the ultrasound diagnosis with MRI is desirable for the prenatal diagnosis of lissencephaly.

Key words:
lissencephaly; fetal monitoring; ultrasonography; magnetic resonance imaging; mental retardation.

Apstrakt

Uvod. Lizencefalija („gladak mozak") predstavlja važnu grupu malformacija mozga koja nastaje zbog poremećaja neuronske migracije. Može uzrokovati teško zaostajanje u intelektualnom i motornom razvoju i epilepsiju kod dece. Prenatalna dijagnostika ovog poremećaja je retka. **Prikaz bolesnika.** Prikazali smo jedan slučaj prenatalno dijagnostikovane lizencefalije. Trudnica, stara 30 godina, upućena je u bolnicu u 35. nedelji gestacije radi magnetne rezonancije (MR) posle ultrazvučnog pregleda koji je ukazao na fetalnu moždanu ventrikulomegaliju. Fetalni MR pregled mozga pokazao je glatku koru, sa izostankom razvoja vijuga. Dete ženskog pola rođeno je u terminu, telesne mase 2 500 g i Apgar skora 8, a pokazalo je usporen rani razvoj. Postnatalni ultrazvučni i MR pregled mozga potvrdili su dijagnozu lizencefalije. Devojčica sada ima 8 godina i kliničku sliku spastične kvadripareze, mentalne retardacije i epilepsije. **Zaključak.** Potvrda ultrazvučne dijagnoze putem MR pregleda značajna je za prenatalnu dijagnostiku lizencefalije.

Ključne reči:
lizencefalija; fetus, praćenje; ultrazvuk; magnetna rezonanca, snimanje; mentalna zaostalost.

Introduction

Malformations of cortical development are significant causes of delay in psychomotor development and epilepsy in children [1–3]. Lyssencephaly ("smooth brain") forms a major group of brain malformations due to widespread abnormal migration [1, 2, 4]. Other two major categories of malformations are cobblestone complex malformations (also known as type 2 lissencephaly) and all types of heterotopia. With magnetic resonance imaging (MRI), these disorders can be identified in life [5].

Classical lissencephaly (OMIM # 607432), formely designed as type 1, is a severe neurological malformation characterized by a lack of sulcation of the cortical plate, that produces a smooth brain surface, cortical thickening with four primitive layers and ventriculomegaly [3, 6, 7]. The brain has no gyri (agyria) or very low gyri (pachygyria) or there is a related disorder known as subcortical band heterotopia (SBH) [3, 7, 8]. Due to contribution of computed tomography (CT) and MRI, this spectrum of gyral abnormalities was graded in the following way: grade 1, complete agyria; grade 2, diffuse agyria with few sulci in anterior regions; grade 3, anterior pachygyria (few, broad gyri) and posterior agyria; grade 4, pachygyria more prominent in the posterior brain regions than in anterior; grade 5, pachygyria posteriorly with SBH and grade 6, SBH only [9]. Lissencephaly due to

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mutations of LIS1 at 17 p.13.3 is highly specific for more severe changes in posterior brain regions ($p >$ a gradient), while lissencephaly due to mutations of XLIS at X q 22.3-q23 often have more severe gyral abnormalities in the anterior brain regions (a > p gradient) [10]. TUBA1A usually show posterior-predominant lissencephaly similar to LIS1 [11].

Studies have identified two major genes responsible for classical lissencephaly: LIS1 (named *PAFAH1B1*) gene at 17 p13.3 and the XLIS (DCX) gene at Xq 22.3-q23 [12–14]. Both proteins are important for normal neuronal migrational processes. Approximately 76% of patients with classical lissencephaly show mutations in these two genes [15]. Recently, mutations of TUBA1A gene at the 12q12-q14 is detected in several cases with lissencephaly. TUBA1A belongs to the alpha-tubulin protein family which is needed for correct cell movements. Mutation of TUBA1A are responsible for 1–4% of cases [16, 17]. Other types of lissencephaly caused by mutation of: RELN, VLDLR and ARX have been decribed [10, 18]. These types of lissencephaly are less common and known as "variant lissencephaly". It is important that the morphology of lissencephaly caused by mutations of those three genes differs from that caused by *LIS1*, *DCX* and *TUBA1A* mutations. Lissencephaly with cerebellar hypoplasia (LCH) results from mutations of two genes: the reelin (*RELN*) gene and very low-density lipoprotein receptor gene (*VLDLR*). X-linked lissencephaly with abnormal genitalia and agenesis of the corpus callosum (XLAG) has been associated with ARX gene.

Children with classical lissencephaly usually have hypotonia at birth, but spasticity develop later in infancy. Clinical manifestations include seizures, spastic quadriplegia and profound mental retardation. The onset of seizures is usually between 6–12 months. Infantile spasms followed by hypsarrhytmia are seen in the majority of children and they respond at first to corticotropin or other antiepileptic drugs. Unfortunately, almost all children will go on to have frequent seizures and severe psychomotor retardation. *LIS1* gene which cause classical lissencephaly is connected with two clinical disorders. The first one is the isolated lissencephaly sequence (ILS) which is characterized by lacks of typical facial appearance and the second is Miller-Dieker syndrome (MDS) (OMIM #247000), where typical facial features and other congenital defects exist [19, 20].

The prenatal diagnosis of this malformation is rare. MRI imaging should be useful in screening for malformation of cortical development such as lissencephaly. The aim of this case report was to characterise the delivery and postnatal neurodevelopmental outcome of the fetus reffered for MRI following suspicion on ultrasound of ventriculomegaly.

Case report

We presented a case of the prenatal diagnosis of the lissencephaly. A 30-year-old pregnant woman was reffered to the hospital at the week 35 of gestation for MRI after an ultrasound examination demonstrated fetal ventriculomegaly, defined as ventricular size (measured at the atrium of the lateral ventricle) more than 10 mm. At the week 35 of gestation, the fetal MRI showed that the gyral pattern was smoother than the expected third-trimester configuration, suggesting lissencephaly (Figure 1). Fetal blood sampling by cordocentesis revealed a normal karyotype of 46, XX and screening for infections (toxoplasma, rubella, cytomegalovirus and herpes simplex virus) confirmed normal results. There was no consanguinity or family history of neurological disorders. The mother had three older sons and no history of spontaneous abortion. She did not have diabetes mellitus and denied any exposure to teratogenic agents or infectious diseases during pregnancy. The pregnancy was normal until 35 weeks gestation when ventriculomegaly was first noted on prenatal ultrasound. Then, the prenatal suspicion of lissencephaly was made, the parents were counseled accordingly, and they elected to continue the pregnancy.

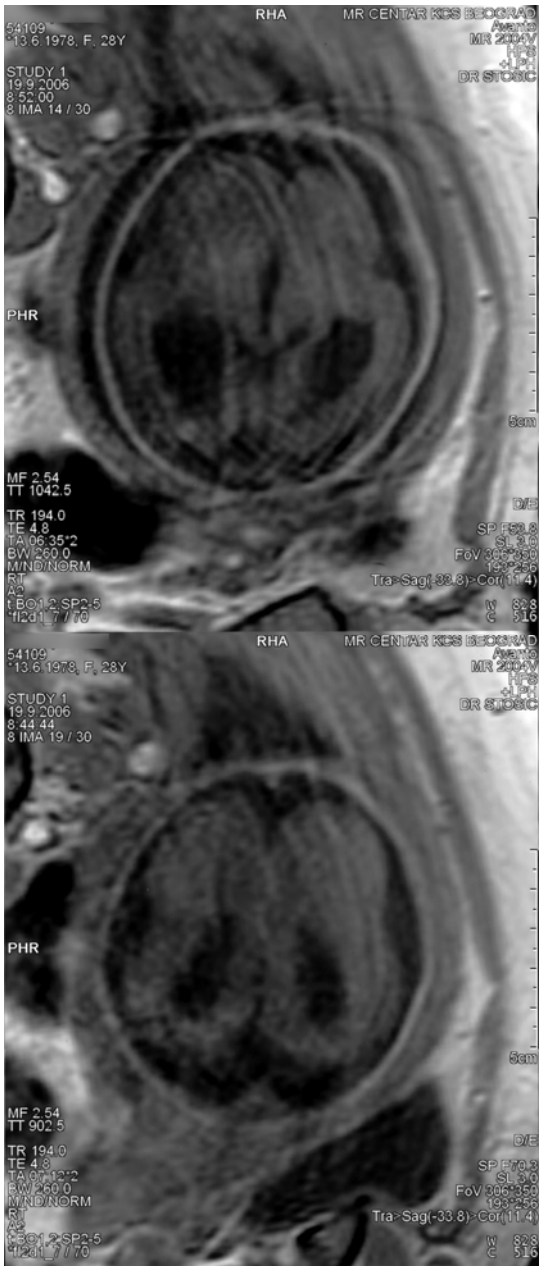

Fig. 1 – Fetal magnetic resonance imaging of the brain shows that the gyral pattern is smoother than the expected third-trimester configuration, suggesting lissencephaly.

The female infant was born at the week 38 of gestation with the body weight of 2,500 g and 5-minutes Apgar score was 8. Abnormal fetal movement had not been noted during fetal ultrasonography. There was no complications during the vaginal vertex delivery which followed. Upon examination, mild generalised hypotonia and poor growth were noted, but apart from that, physical condition was unremarkable. Postnatal ultrasound of the brain showed characteristic findings for lissencephaly. There was the absence of gyration underneath the supperior sagittal sinus and a pseudo-liver pattern of echoreflections in the parenchyma between pia matter and ventricle caused by subcortical heterotopic neurons. The interhemispheric fissure was not flanked by branching sulci and the lateral fissure did not show a horizontal Y, but had been reduced to a slit, the point of which courses caudally downwards. This was a consequence of the absense of opercularization with a widely patient sylvian fossa that points caudally. Discrepant dilatation of the occipital horns, colpocephaly, was present and agenesis of the corpus callosum, too. MRI of the brain showed that the surface of the brain was flat due to the lack of sulcation, and that the sylvian fissures were shallow and vertically oriented; therefore, the brain had a figure-of-eight shape in axial section. The cortex was markedly thickened, and a hyperintense band corresponding to the sparse cell zone was clearly visible with asymmetric and mildly dilated lateral ventricles. There was also marked callosal hypoplasia and the diagnosis of lissencephaly was made. Colpocephaly, the completely "smooth" (agyrya) cortex and the open insula, were seen on axial MR planes (Figures 2 and 3). Cytogenetic analysis of blood lymphocytes revealed a 46, XX karyotype. Mutation analysis of the LIS1 gene was not performed because the parents refused.

First seizures were noted at the age of two months, and the baby was admitted to our hospital. Infantile spasms were continuosly observed and electroencephalography (EEG) showed hypsarrhythmia; thus, the diagnosis of West syndrome was made (Figure 4). Neurological examination was unremarkable except for hypotonia. Facial appearance, cranial nerves, and deep tendon reflexes were normal. Spasms disappeared after adrenocorticotropic hormone (ACTH) and vigabatrin therapy. Focal seizures appeared at the age of two years and were intractable, not responding to various antiepileptic drugs. Hypotonia was replaced by hypertonia and opisthotonic posturing. Deep tendon reflexes were exaggerated, and the Babinski sign and ankle clonus were elicited bilaterally. The growth was poor associated with microcephaly. She was not aware of her surroundings. Visual tracking was not adequate for the age, in the presence of intermittent ocular deviation with nystagmus. Later, focal seizures predominated and interictal EEG showed generalized spike and wave discharges (Figure 5). They were resistant to different medications (lamotrigine, valproate, topiramate, levetiracetam). The weakness progressed to paralysis and intelectual retardation was severe. Fundoduplication was performed at the age of four years due to persistent symptoms of gastroesophageal disease.

The girl is now aged 8 years, and her general condition is relatively stable. She remained with severe psychomotor delay, developing head control at the age of 4 years and not rolling until the age 5 years. She did not show any progression in psychomotor development and displayed spastic quadriplegia, mental retardation, intractable seizures and microcephaly.

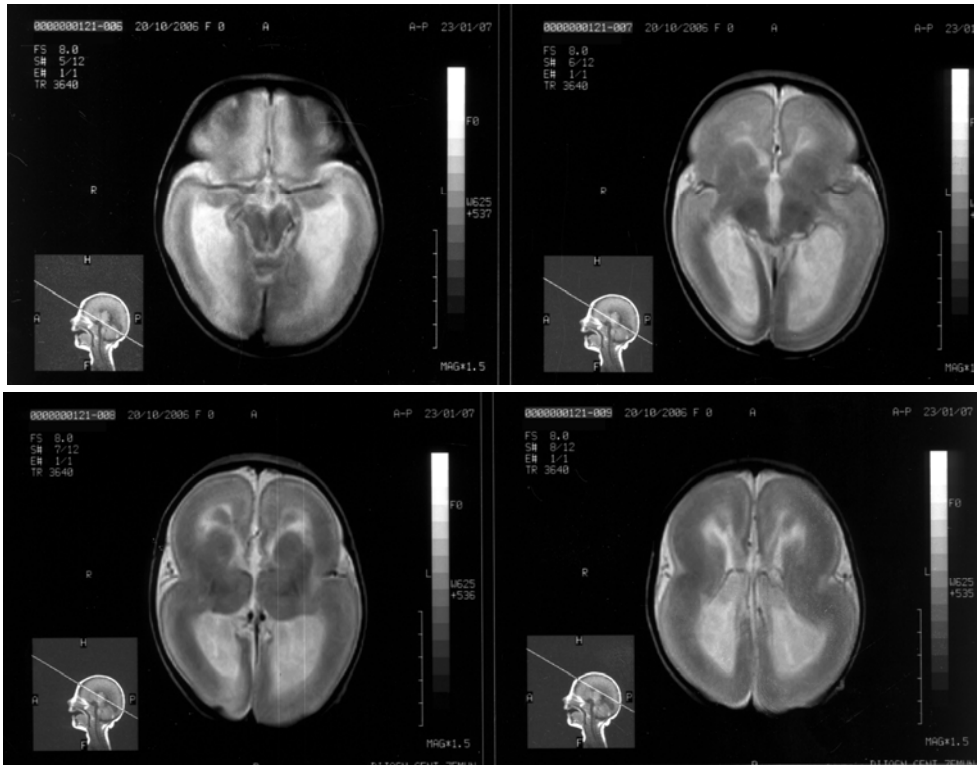

Fig. 2 – Axial magnetic resonance images at the age of two months shows colpocephaly, the absence of gyration and an open insula - features typical for lissencephaly.

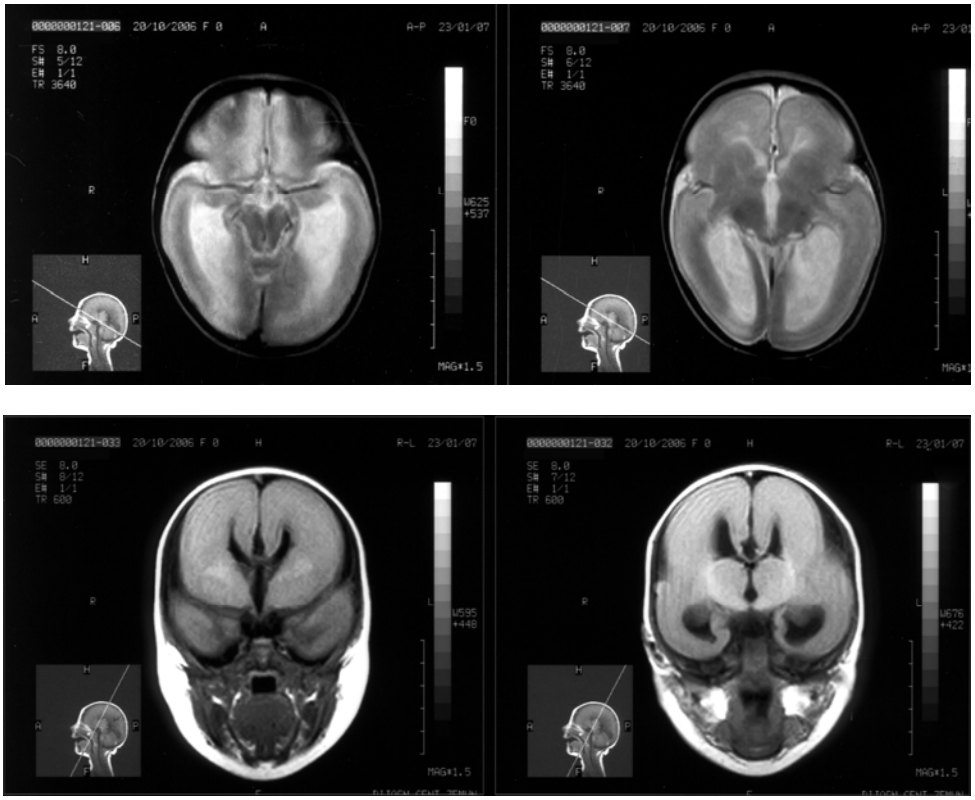

Fig. 3 – Coronal magnetic resonance planes show that the cortical surface is flat in classical lissencephaly.

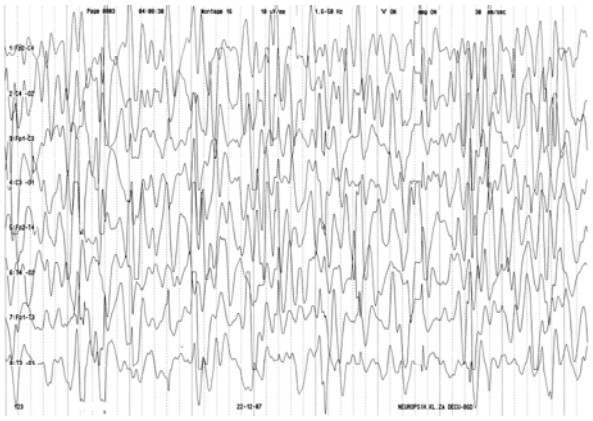

Fig. 4 – Electroencephalography shows hypsarrhythmia.

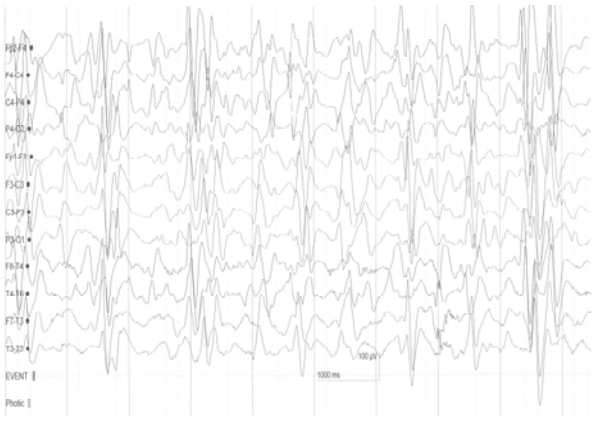

Fig. 5 – Electroencephalography shows generalized spike and wave discharges.

Discussion

Gene mutations, extrinsic factors, maternal metabolic disturbances and specific syndromes are associated with malformations of cortical development. Apart from genetic factors which are responsible for lissencephaly, different environmental factors can cause lissencephalic-like syndromes, such as teratogens (trauma, hypoxia, toxins, drugs, radiation), infections (fetal cytomegalovirus infection) and maternal diabetes mellitus and phenylketonuria [21, 22]. Genetic testing when there is a chromosome abnormality or gene mutations in the affected family, or ultrasound and MRI findings by detecting different structural defects, are diagnostic tools for prenatal diagnosis of lissencephaly [23–25]. The diagnosis is not easy when it is an isolated case as the one reported.

We reported the ultrasound and MRI prenatal diagnosis and postnatal confirmation of classical lissencephaly associated with severe intellectual and motor disability and intractable epilepsy. Clinical course of this child was significant for continued seizures and global developmental delay. Seizures were not responding to various antiepileptic drugs, confirming results of other studies about no effective treatment [26]. Many patients require better care because of feeding problems and infectious complications, and in that cases, children do reach early adulthood [27]. It is similar with our reported case.

Gyryfication is perhaps the most important change that occurs in the fetal brain during gestation [28]. In very preterm babies born around the week 22–23 of gestation the brain surface is smooth with very few sulci and gyri. Gyrification is progressing rapidly between 25 and 30 weeks [29]. Only after the 30th gestational week will gyration be developed sufficiently to allow the diagnosis of lissencephaly [30]. Discrepant dilatation of the occipital horns, colpocephaly and the absence of opercularization of insula are nearly always present ultrasound findings which should raise the suspicion on lissencephaly. When the disorder occurs in connection with other malformations such as cardiac defects, genital abnormalities and characteristic facies, the Miller-Dieker syndrome may be present. In many cases there is a deletion of the short arm of chromosome 17 and genetic testing is important to diagnose it [31].

The important characteristic of classical lissencephaly is the similar pathological and radiological pattern even when different genetic causes are responsible for disease [16]. The new data show that location of the mutation can not predict the severity of the clinical presentation in the LIS1–related lissencephaly directly [32, 33]. On the other hand, the severity of the mutation on the LIS1 protein confirm good relationship with radiological phenotype and lissencephaly grading [34, 35]. The results suggest that genetic causes of lissencephaly could account for the type of neuroimiging changes. Here, we reported a non-moleculary confirmed case, but we showed the importance of fetal ultrasound and MRI for understanding of normal brain development and providing practical help to families of affected patients in the form of prognosis and counselling.

Depending on the severity, malformation of the cortex can cause a range of outcomes including death in infancy, psychomotor retardation and seizures [36]. Prognosis is usually poor and related to the degree of smoothness, but early diagnosis could allow better care for the patient. The phenotype could be characterised by severe neurological abnormalities, like in the presented case [36–38].

Fetal MRI can depict smooth brain surface, but only in the third trimester of pregnancy [27, 28, 39, 40]. The presented case confirms that fetal MRI may identify additional important finding apart from ventriculomegaly, which could alter patient counselling [41]. Although prenatal diagnosis of ventriculomegaly is now easy and much more frequent finding in routine ultrasound examination, ventriculomegaly could be associated with different neurological outcomes. In the presented case, MRI was helpful to carefully identify lissencephaly *in utero*. We conclude that the ventriculomegaly detected with ultrasound is important clinical indication for fetal MRI. As the genetics of congenital malformations becomes more complex, MRI in combination with ultrasound can provide important information on specific brain phenotypes.

Conclusion

Confirmation of the ultrasound diagnosis with MRI is desirable for the prenatal diagnosis of lissencephaly. A combination of these two technique *in utero* is an important diagnostic tool in the combination with genetic testing for lissencephaly.

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CASE REPORT

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Successful treatment with cladribine of Erdheim-Chester disease with orbital and central nervous system involvement developing after treatment of Langerhans cell histiocytosis

Uspešna primena kladribina u lečenje Edhajm-Česterove bolesti sa zahvatanjem orbita i centralnog nervnog sistema nastale nakon lečenja Langerhansove histiocitoze

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Abstract

Introduction. Erdheim-Chester disease (ECD) is a rare, systemic form of non-Langerhans cell histiocytosis of the juvenile xanthogranuloma family with characteristic bilateral symmetrical long bone osteosclerosis, associated with xanthogranulomatous extraskeletal organ involvement. In ECD, central nervous system (CNS) and orbital lesions are frequent, and more than half of ECD patients carry the V600E mutation of the proto-oncogene BRAF. The synchronous or metachronous development of ECD and Langerhans cell histiocytosis (LCH) in the same patients is rare, and the possible connection between them is still obscure. Cladribine is a purine substrate analogue that is toxic to lymphocytes and monocytes with good hematoencephalic penetration. **Case report.** We presented a 23-year-old man successfully treated with cladribine due to BRAF V600E-mutation-negative ECD with bilateral orbital and CNS involvement. ECD developed metachronously, 6 years after chemotherapy for multisystem LCH with complete disease remission and remaining central diabetes insipidus. During ECD treatment, the patient received 5 single-agent chemotherapy courses of cladribine (5 mg/m^2 for 5 consecutive days every 4 weeks), with a reduction in dose to 4 mg/m^2 in a fifth course, delayed due to severe neutropenia and thoracic dermatomal herpes zoster infection following the fourth course. Radiologic signs of systemic and CNS disease started to resolve 3 months after the end of chemotherapy, and CNS lesions completely resolved within 2 years after the treatment. After 12-year follow-up, there was no recurrence or appearance of new systemic or CNS xanthogranulomatous lesions or second malignancies. **Conclusion.** In accordance with our findings and recommendations provided by other authors, cladribine can be considered an effective alternative treatment for ECD, especially with CNS involvement and BRAF V600E-mutation-negative status, when interferon-α as the first-line therapy fails.

Key words:
erdheim-chester disease; histiocytosis, non-langerhans cells; orbital pseudotumor; central nervous system; brain stem; cerebellum; proto-oncogene proteins b-raf; cladribine; magnetic resonance imaging.

Apstrakt

Uvod. Edhajm-Česterova bolest (EČB) je redak sistemski oblik ne-Langerhansove histiocitoze iz porodice juvenilnih ksantogranuloma, koga karakteriše simetrična bilateralna osteoskleroza dugih kostiju sa ksantogranulomskom infiltracijom različitih organa. Kod EČB često su prisutne lezije centralnog nervnog sistema (CNS) i orbita, a više od polovine bolesnika sa ovom bolesti ima V600E mutaciju protoonkogena BRAF. Sinhroni ili metahroni razvoj EČB i Langerhansove histiocitoze (LH) kod istog bolesnika je redak, a moguća veza između njih još uvek je nejasna. Kladribin je purinski analog koji deluje toksično na limfocite i monocite sa dobrom hematoencefalnom penetracijom. **Prikaz bolesnika.** Prikazali smo muškarca, starog 23 godine, koji je uspešno lečen kladribinom zbog EČB sa obostranim zahvatanjem orbita i CNS. Kod prikazanog bolesnika BRAF V600E mutacija nije bila prisutna, a EČB je nastala metahrono, šest godina nakon hemioterapijskog lečenja multisistemske LH, sa kompletnom remisijom bolesti i zaostajanjem centralnog insipidnog dijabetesa. Tokom lečenja EČB, bolesnik

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je dobio pet ciklusa monohemioterapije kladribinom (5 mg/m² tokom pet dana, svake 4 nedelje), uz redukciju doze na 4 mg/m² u petom odloženom ciklusu zbog izražene neutropenije i pojave herpes-zoster infekcije po okončanju četvrtog ciklusa. Rezolucija radiološki viđenih sistemskih i CNS lezija počela je nakon tri meseca, uz potpuni nestanak lezija u CNS unutar dve godine od završetka hemioterapije. Bolesnik je periodično praćen tokom 12 godina, pri čemu nije uočena pojava relapsa bolesti kao ni sekundarnog malignitеta. **Zaključak.** U odnosu na naše prikazano iskustvo i preporuke drugih autora, kladribin se može razmatrati kao efikasna alternativna terapija za EČB, naročito kod bolesnika sa lezijama u CNS i odsustvom BRAF V600E mutacije, kada je primena interferona α kao leka prvog izbora bila neuspešna.

Ključne reči:
erdheim-chesterova bolest; histiocitoza, non-langerhans ćelije; pseudotumor orbite; centralni nervni sistem; moždano stablo; mozak, mali; proteini, protoonkogeni, b-raf; kladribin; magnetna rezonanca, snimanje.

Introduction

Erdheim-Chester disease (ECD) is a rare, non-inherited and orphan disease of unknown origin [1, 2]. It is systemic form of non-Langerhans cell histiocytosis (non-LCH) of the juvenile xanthogranuloma (JXG) family, with characteristic bilateral symmetrical long bone osteosclerosis associated with extraskeletal organ involvement in more than 50% of cases [1–3]. Central nervous system (CNS) and orbital lesions in ECD are frequent [1–5], and more than half of ECD tested patients carry the V600E mutation of the proto-oncogene BRAF [1, 2]. ECD differs from Langerhans cell histiocytosis (LCH) clinically and in the histological, immunohistochemical and ultrastructural characteristics of the respective histiocytes [3, 6]. The synchronous or metachronous development of ECD and LCH in the same patients is rare [1, 4–7]. The choice of cladribine (2-chlorodeoxyadenosine) for ECD treatment before 2005, or the beginning of the "interferon-α (IFN-α) era" [1], was based on its successful use in the treatment of adult and pediatric multisystem LCH [8, 9]. We reported a rare case of young man successfully treated with cladribine due to metachronous BRAF V600E-mutation-negative ECD involving the orbits and CNS after chemotherapy treated LCH, with 12-year follow-up.

Case report

A 23-year-old Caucasian male presented in August 1999 with a 6-month history of progressive severe exophthalmos and vision loss of the left eye, caused by a large orbital, retrobulbar, intraconal soft tissue mass, revealed by orbital computed tomography (CT). He also complained of occasional knees and ankles pain. He had the 6-year history of central diabetes insipidus (CDI) which had remained after chemotherapy (methotrexate, vinblastine, prednisolone) for multisystem LCH during the period 1991–1993, with complete remission of the disease. The patient underwent left transcranial upper orbitotomy with complete extirpation of the intraconal tumor, together with the optic nerve which was intimately and completely enveloped and partly infiltrated by the tumor. Histopathology showed xanthogranulomatous orbital pseudotumor. Six months later the patient noticed proptosis of the right eye. The full range of eye movements was preserved with good visual acuity. Orbital CT showed a well defined, posteromedial, retrobulbar, intraconal, orbital soft tissue mass which had displaced the right globe forward and outward. In Novmber 2000, the patient underwent debulking of the right orbital lesion. The histopathology was the same as previously reported. Despite corticosteroid treatment, the right exophthalmos worsened in the next 6 months with limitation of eye movements and decreased visual acuity. The patient also noted progressive gait incoordination and the loss of balance, legs weakness with pain, dizziness and fatigue. A contrast-enhanced head magnetic resonance imaging (MRI) in May 2001 showed enlargement of the right orbital tumor, recurrence of the left orbital tumor and multiple nodular lesions in the pons, middle cerebellar peduncles and midbrain (Figure 1). A tehnecium^{99} (^{99}Tc) bone scintigraphy showed increased uptake in the proximal and distal ends of the long bones of the legs, the hands and facial bones (Figure 2). Echocardiography showed pericardial thickening with effusion and right atrial collapse. Thoracic and abdominal CT revealed mediastinal and retroperitoneal fibrosis with "hairy perirenal infiltration". Laboratory investigation disclosed an inflammatory syndrome with hypoalbuminemia. Liver biopsy was negative. Re-examination of both orbital tumor specimens confirmed xanthogranulomatosis with Touton multinucleate giant cells and lipid-laden foamy histiocytes positive for CD68, CD14 and HLA-DR, and negative for CD1a and S100 immunoreactivity, surrounded by fibrosis (Figure 3). Based on the above radiologic findings and histopathology, a diagnosis of ECD was reached. Intravenous single-agent chemotherapy by cladribine was started in August 2001, at a dose of 5 mg/m^2 for 5 consecutive days every 4 weeks. In total, 5 courses were given with reduction of cladribine dose to 4 mg/m^2 in the delayed fifth course, due to severe neutropenia and thoracic dermatomal herpes zoster infection following the fourth course. Radiologic signs of systemic and CNS disease started to resolve 3 months after the end of chemotherapy, and CNS lesions completely resolved within 2 years of treatment. The progression of cerebellar symptoms was arrested but there was no regression of neurological deficit. The orbital lesions partially regressed and scarred. CDI was controlled with desmopressin. Yearly follow-ups showed no recurrences or appearances of new systemic or CNS xanthogranulomatous lesions (Figure 4) or second malignancies 12 years after cladribine treatment. The patient is still alive. Subsequently, the BRAF V600E-mutation status in both paraffin-embedded orbital tumor specimens was determined by allele-specific real-time polymerase chain reaction (detection limit less than 1%), and presence of the V600E mutation were not detected.

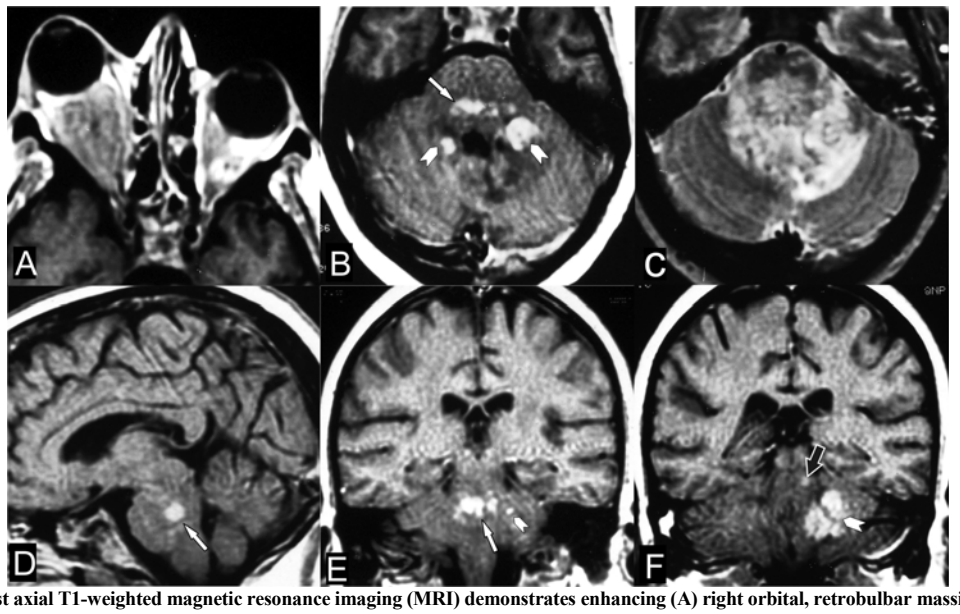

Fig. 1– Post-contrast axial T1-weighted magnetic resonance imaging (MRI) demonstrates enhancing (A) right orbital, retrobulbar massive infiltration with prominent exophtalmos and recurrence of the left retrobulbar mass, as well as (B) nodular enhancing lesions in the pontine tegmentum (white arrow) and the middle cerebellar peduncles (white arrowheads) with perifocal edema. Axial T2-weighted MRI (C) shows bright asymmetric hyperintense signal in the pontine tegmentum and the middle cerebellar peduncles predominantly on the left side. Post-contrast sagittal (D) and coronal (E, F) T1-weighted MRI demonstrates nodular enhancing lesions in the pontine tegmentum (white arrows), left middle cerebellar peduncle (white arrowheads) and ipsilateral midbrain tegmentum (black arrow).

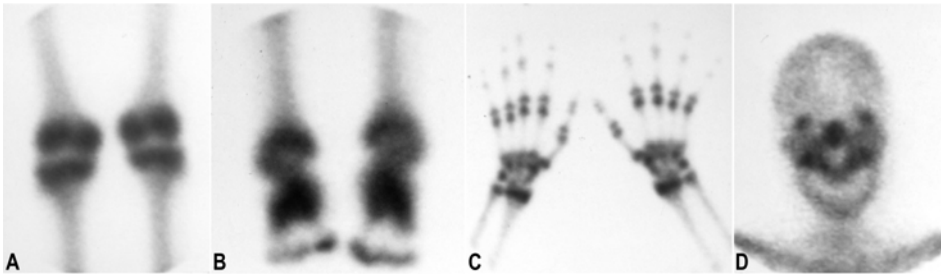

Fig. 2 – ^{99}Technetium bone scintigraphy shows symmetric and abnormally increased technetium uptake in (A) the distal ends of the femurs and the proximal ends of the tibiae, (B) the distal ends of the tibiae and the tarsal bones bilaterally, (C) the distal ends of the ulnae and the radii as well as bilaterally in some of the carpal bones, the proximal and distal ends of the metacarpal bones and the proximal ends of the proximal phalanges, and in (D) the facial bones.

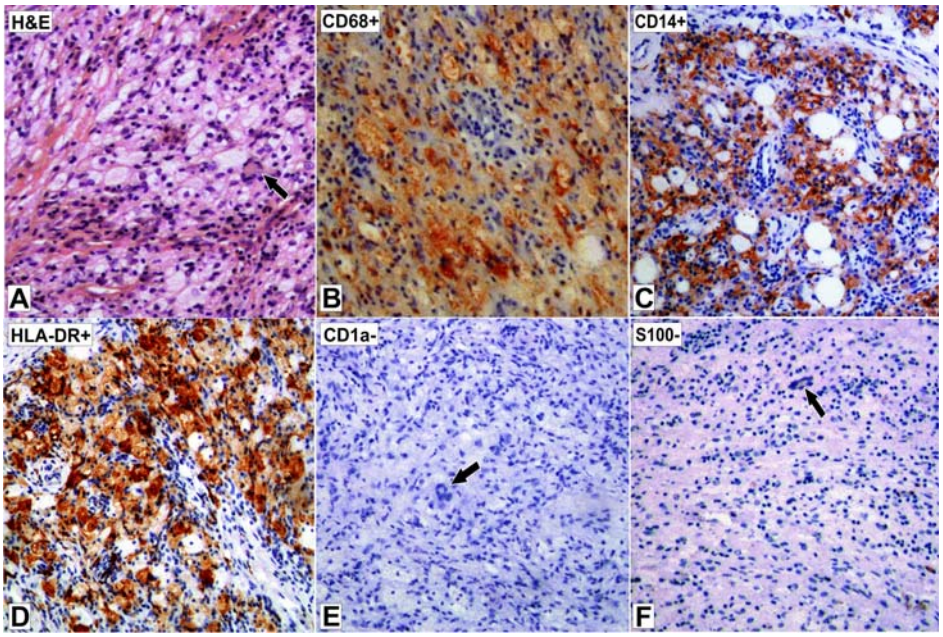

Fig. 3 – Photomicrographs of the right orbital retrobulbar surgical specimen shows (A) clusters of foamy histiocytes intermixed with mature lymphocytes and individual Touton multinucleate giant cell (arrow) nested in fibrosis (hematoxylin and eosin staining). Immunohistochemistry of the same specimen shows foamy histiocytes positive for (B) CD68, (C) CD14 and (D) HLA-DR, and negative for (E) CD1a and (F) S100 immunoreactivity (immunohistochemical staining with DAB as a chromogen contrasted with hematoxylin). Touton multinucleate giant cell (arrow) is also negative for (E) CD1a and (F) S100 immunoreactivity. The original magnification of all images (A-F) is ×100.

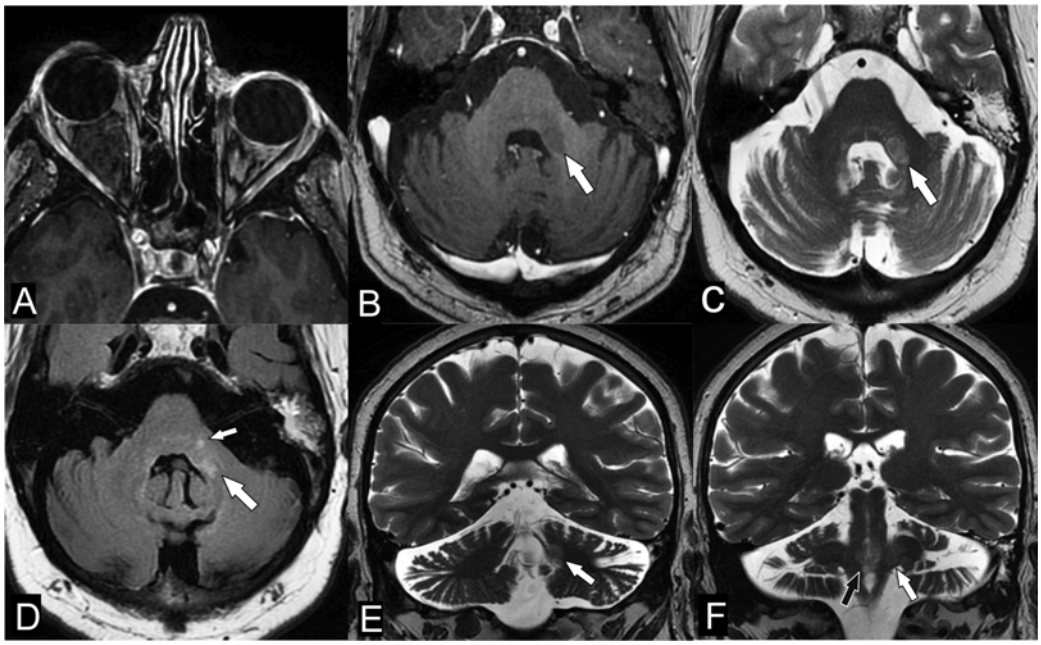

Fig. 4 – Post-contrast axial T1-weighted magnetic resonance imaging (MRI) after 12 years follow-up demonstrates residual, no enhancing (A) right orbital, retrobilbar, intraconal scar tissue with significantly less pronounced exophtalmos and the absence of left retrobulbar infiltration, as well as (B) a hypointense gliotic scar in the left middle cerebellar peduncle (white arrow). Axial T2-weighted (C), T2-weighted fluid attenuated inversion recovery (D) and coronal T2-weighted (E, F) MRI demonstrates a slightly hyperintense gliotic scar in the left middle cerebellar peduncle (white arrows) and in the pontine tegmentum (black arrow).

Discussion

ECD is a multisystemic disease whose extent and distribution determines the clinical course [1, 2]. Widely accepted diagnostic criteria of ECD are the following [1–6]: typical histological findings – xanthogranulomatosis with CD68-positive and CD1a-negative immunostaining; characteristic skeletal abnormalities – symmetric and abnormally intense labeling of the distal ends of the long bones of the legs revealed by ^{99}Tc bone scintigraphy. Our patient fulfilled both criteria. Painful skeletal lesions in our patient were accompanied by mediastinal, retroperitoneal, pericardial, orbital and CNS involvement.

CNS involvement in ECD is a strong prognostic factor and an independent predictor of death [1, 10]. Infiltrative nodular lesions in the brainstem, cerebellum and especially in the middle cerebellar peduncles have frequently been reported [4–6], as was the case in our patient, resulting in cerebellar and pyramidal syndromes with severe handicaps of ECD patients [1, 5]. The typical MRI features of infiltrative ECD CNS lesions are bright hyperintensity on T2-weighted sequence and intense homogeneous gadolinium enhancement on T1-weighted sequence without mass effect [4, 5], as we observed in our patient, with prolonged gadolinium retention of the lesions even after several days [5]. Other described forms of CNS involvement in ECD were meningioma-like lesions, pituitary stalk infiltration and intracranial periarterial infiltrations [4, 5]. Surgical resection is only reasonable in ECD patients with circumscribed intracranial meningioma-like lesions and resulting focal neurological deficits. As an initial diagnostic method, intracranial biopsy procedures should be performed only when diagnosis of the systemic disease is not possible [11]. Orbital involvement in ECD is often bilateral and intraconal [1, 2, 4], and frequently associated with osteosclerosis of the facial bones [2, 4] as in the case of our patient. Surgical debulking of orbital lesion is required as in our case, when retrobulbar infiltration is massive and refractory to conventional therapy [1].

ECD mainly affects middle-aged adults with male predominance [1, 2]. In this case, the patient was a young man who developed ECD following chemotherapy treated LCH with residual CDI. Reportedly, 11% of ECD patients have associated LCH [1, 5]. Several theories have been proposed to explain synchronous coexistence or metachronous development of ECD and LCH [2, 6, 7], but still without conclusive answers. Theoretically, an abnormality in the common CD34+ progenitor cell could be responsible for both diseases, depending on changes in the cellular cytokine microenvironment at different points in time. Additionally, metachronous ECD following chemotherapy treated LCH could represent an evolution, maturation or conversion of LCH to non-LCH (ECD), with differentiation switch of the respective histiocytes due to a change in patient's immune response inducted by therapeutic interventions [7].

ECD is a systemic form of non-LCH of the JXG family, composed of histiocytic cells exhibiting monocite/macrophage differentiation [3, 6], and with increased monocyte activation in the peripheral blood [12]. Cladribine is an antimetabolite that is toxic to lymphocytes and monocytes, with good hematoencephalic penetration [8, 13]. Tissue histiocytes and circulating monocytes have common progenitor cell origin [3]. These facts, together with the reports that described encouraging response to cladribine in adult and pediatric patient with LCH [8, 9], made the use of cladribine in ECD with CNS involvement a rational therapeutic choice. This was the

main reason to choose cladribine as a first-line therapy for our patient at the time of his treatment (August 2001 – January 2002), before beginning of the "IFN-α era" [1]. Despite residual neurological deficit, we considered the cladribine treatment to have been successful, providing good and long-lasting remission to our patient 12 years after ending the treatment. Myra et al. [12] also reported a significant recovery and maintained clinical improvement in a patient with ECD with orbital involvement 2 years following treatment with cladribine.

As for adverse effects, cladribine may be associated with dose-dependent myelosuppression, and neurological toxicity [2]. Also, cladribine is a powerfully immunosuppressive purine substrate analogue, its incorporation into DNA may be mutagenic with the risk of secondary malignancies [8]. Our patient had a severe neutropenia and thoracic dermatomal herpes zoster infection which occurred after the penultimate course of cladribine and resulted in a dose reduction and delay of the last cladribine course, with no secondary malignancies during the 12-year follow-up. It is possible that an alternative 3-day dosing schedule (5–6.5 mg/m^2/day for 3 days, every 3–4 weeks) [9] would be less myelotoxic with the same efficiency as the one used.

Currently, IFN-α is the most extensively studied agent in the treatment of ECD and serves as the first line of treatment [1, 2, 14]. Furthermore, treatment with IFN-α was identified as an independent predictor of survival [10]. Since more than half of ECD tested patients carry BRAF V600E mutation, targeted therapy with vemurafenib, an inhibitor of V600E-mutated BRAF, represents promising treatment option in multisystemic and refractory cases of BRAF V600E-mutation-associated ECD, particularly if life-threatening [1, 14]. Cladribine is advocated as an alternative treatment for ECD [2, 14] and may be beneficial for treating CNS ECD lesions that are not responsive to IFN-α [1], especially in BRAF V600E-mutation-negative ECD patients, which is in accordance with our experience in this case.

Conclusion

Erdheim-Chester disese is a rare and orphan multisystemic disease whose management and treatment is complex. Central nervous system involvement in Erdheim-Chester disese is associated with severe morbidity and mortality. Since there is still no definitive cure, the goals of Erdheim-Chester disese treatment should be prolonging life and maximizing its quality. Presented case of Erdheim-Chester disese with orbital and central nervous system involvement, with good and long-lasting response to cladribine just confirms this.

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CASE REPORT

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Failure of intravenous lipid emulsion in treatment of cardiotoxicity caused by mixed overdose including dihydropyridine calcium channel blockers

Nedelotvornost intravenske emulzije masti u lečenju kardiotoksičnosti izazvane predoziranjem kombinacije lekova uključujući dihidropiridinske blokatore kalcijumovih kanala

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Abstract

Introduction. Calcium channel blockers and beta-blockers are among the most frequently ingested cardiovascular drugs in self-poisoning causing significant mortality. Intravenous lipid emulsion (ILE) is reported as a potentially novel antidote for treatment of acute poisoning caused by some of these drugs. **Case report.** We presented two cases of poisoning with these drugs. The case 1, a 24-year-old woman ingested amplodipine, metformin and gliclazide for self-poisoning. She presented with tachycardia and hypotension. Laboratory analyses revealed hyperglycaemia and metabolic acidosis. Despite the treatment which included fluid resuscitation, vasopressors, intravenous calcium, glucagon and ILE, circulatory shock occurred. The patient died 10 hours after admission due to cardiac arrest refractory to cardiopulmonary resuscitation. The case 2, a 41-year old man, was found in a coma with empty packages of nifedipine, metoprolol and diazepam tablets. On admission vital signs included Glasgow Coma Scale (GCS) of 3, weak palpable pulses, undetectable blood pressure, and irregular breathing with oxygen saturation of 60%. An electrocardiography showed AV block (Mobitz II) with ventricular rate of 44/min with progression to third degree of AV block. In attempt to increase heart rate and blood pressure the following agents were administered: atropine boluses, normal saline with dopamine, glucagon, calcium chloride and ILE. Temporary transvenous pacemaker was placed, electrical capture was recorded, but without improvement in haemodynamics. Three hours after admission cardiac arrest happened and cardiopulmonary resuscitation was unsuccessful. **Conclusion**. Intravenous lipid emulsion may be ineffective in acute poisonings with amlodipine, nifedipine or metoprolol.

Key words:
poisoning; suicide; calcium channel blokers; adrenergic beta-antagonists; heart arrest; fat emulsions, intravenous.

Apstrakt

Uvod. Antagonisti kalcijumovih kanala i beta blokatori predstavljaju najčešće upotrebljavane kardiološke lekove prilikom samotrovanja koji prouzrokuju značajnu smrtnost. Intravenske emulzije masti (IEM) u poslednje vreme koriste se kao protivotrov u lečenju akutnih trovanja prouzrokovanih ovim lekovima. **Prikaz bolesnika.** Prikazali smo dva bolesnika otrovana ovim lekovima. Bolesnica, stara 24 godine, popila je tablete amplodipina, metformina i gliklazida u cilju samotrovanja. Klinička slika na prijemu uključivala je tahikardiju i hipotenziju, a laboratorijske analize hiperglikemiju i metaboličku acidozu. I pored terapije koja se sastojala od intravenske primene tečnosti, vazopresora, kalcijuma, glukagona i IEM, razvio se cirkulatorni šok. Deset časova nakon prijema došlo je do srčanog zastoja refraktornog na mere kardiopulmonalne resuscitacije. Drugi bolesnik, star 41 godinu, nađen je u komi sa ispražnjenim pakovanjima nifedipina, metoprolola i diazepama. Na prijemu, vrednost Glazgovske koma skale (GKS) iznosila je 3, postojao je slab palpabilan puls, krvni pritisak bio je nemerljiv, a disanje nepravilno sa zasićenjem kiseonikom od 60%. Na elektrokardiogramu registrovan je AV blok tipa Mobitz II sa komorskom frekvencijom od 44/min koji progredirao u AV blok III stepena. U terapiji su primenjivani bolusi atropina, fiziološki rastvor sa dopaminom, glukagon, kalcjium hlorid i ILE. Plasiran je privremeni pejsmejker čija se električna aktivnost registrovala, ali nije došlo do popravljanja hemodinamskih parametara. Tri časa nakon prijema došlo je do srčanog zastoja refrakternog na terapiju. **Zaključak**. IEM mogu biti neefikasne u lečenju akutnih trovanja amlodipinom, nifedipinom i metoprololom.

Ključne reči:
trovanje; samoubistvo; kalcijum, blokatori; adrenergički beta blokatori; srce, zastoj; masne emulzije, intravenske.

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Introduction

Calcium channel blockers (CCBs) and beta blockers are among the most frequently ingested cardiovascular drugs in self-poisoning [1]. Toxicity of CCBs is primarily a consequence of negative cardiac conduction and contractility properties, along with peripheral vasodilatory effects and is manifested as hypotension, bradycardia, heart block, and circulatory shock. Dihydropyridines (like nifedipine and amlodipine) tend to produce sinus tachycardia instead of bradycardia with fewer conduction disturbances than diltiazem and especially verapamil which tend to produce the most pronounced effects. Nevertheless, in severe overdoses these differences are less evident and all classes of CCBs are potentially fatal [2, 3].

Standard therapeutic modalities used in the management of CCB overdose include gastrointestinal decontamination, fluid resuscitation, vasopressor agents, atropine, intravenous calcium, glucagon, hyperinsulinemic euglycemia, and in some cases sodium bicarbonate or cardiac pacing [4]. Despite all these treatments, self-poisonings with CCBs continue to cause a significant mortality [1]. In recent years, intravenous lipid emulsion (ILE) is reported as a potentially novel antidote for overdoses of lipophilic substances including CBBs [5, 6]. Published case reports of successful treatment in critically ill patients may suggest that ILE is more efficient and more powerful than it really is. Therefore, after some positive experience with ILE in treatment of cardiotoxicity in acute drug poisoning [7–9], we reported two cases of poisoning with fatal outcome despite ILE administration.

Case report

Case 1. A 24-year-old woman was brought to a local emergency department, approximately 2 hours after self-poisoning. The patient was able to provide history. She ingested 150 mg (30 tablets) of amplodipine, 10 g (20 tablets) of metformin, and 2.4 g (30 tablets) of gliclazide. There was no significant prior medical history. She was hypotensive with blood pressure of 80/40 mmHg. Gastric lavage was performed, intravenous glucose and dopamine were started and the patient was transferred to the hospital. She received 20 mL of calcium gluconate 10% during transportation.

On admission, 5 hours after drugs ingestion, the patient was alert, blood pressure was 70/30 mmHg despite dopamine infusion, and other vital signs were normal. A 12-lead ECG showed sinus rhythm, with a rate of 112/min and incomplete right bundle branch block. Laboratory results on presentation were normal except for glucose of 20 mmol/L. Arterial glood gases (ABG) showed pH 7.27, pCO_2 28 mmHg, pO_2 61 mmHg, lactates 4.9 mmol/L, acid-base excess (ABE) 13.1 mmol/L. Toxicological analysis of blood revealed metformin 6.32 mg/L, gliclazide 3.95 mg/L, and amlodipine 0.05 mg/L (HPLC-UV method).

The patient was admitted to the intensive care unit (ICU). She received additional intravenous fluids with glucagone (total dose of 15 mg) and calcium chloride (total dose of 2 g). Because she ingested hypoglycemic drugs, 10% glucose was administered in continuous infusion and small dose of insulin was tapered to maintain glucose at upper normal level. Dopamine dose was gradually increased up to 40 μg/kg/min, but in a few hours blood pressure decreased to non measurable values. Lipid emulsion (Intralipid® 20%) was started 3 hours post-admission. The patient received 100 mL bolus followed by 400 mL in the next 20 minutes. It resulted in transient (during approximately 30 minutes) increase of blood pressure reaching maximum of 90/50 mmHg. Additional infusion of 500 ml 20% Intralipid®was given, but there was no significant improvement. All the time cardiac monitoring recorded sinus tachycardia with AV block grade I and ST depression of 3–4 mm. The patient remained hypotensive and without urine output for the next 10 hours when cardiac arrest refractory to cardiopulmonary resuscitation happened.

Case 2. A 41-year-old man was found by family members in a coma. They found empty packages of nifedipine, metoprolol and diazepam tablets which he was using in his hypertension therapy.

On admission the patient was in a coma (Glasgow coma score was 3), with weak palpable pulses, undetectable blood pressure, irregular breathing with respiratory rate of about 8 breaths *per* minute and oxygen saturation of 60%. An ECG showed second degree AV block (Mobitz II) with ventricular rate of 44/min.

The results of blood chemistry revealed glucose level of 16 mmol/L, while levels of electrolytes, blood urea nitrogen (BUN), creatinine, transaminases and creatine kinase were normal. Toxicological analysis of blood proved nifedipine in concentration of 0.62 mg/L, metoprolol 0.57 mg/L, and diazepam 1.04 mg/L (HPLC-UV method).

The patient was promptly intubated without medication and placed on mechanical ventilation. In attempt to increase heart rate and blood pressure the following agents were administered: atropine (in boluses), normal saline with dopamine, glucagon (12 mg, given as 3 mg boluses), calcium chloride (1g), intravenous lipid emulsion (500 ml of Intralipid® 20% solution). Despite these, the patient's systolic blood pressure remained in the range of 50–70 mmHg, and ECG monitoring showed worsening with third degree AV block. Temporary transvenous pacemaker was placed. Though electrical capture was recorded, there was no improvement in blood pressure. Three hours after admission cardiac arrest happened and cardiopulmonary resuscitation was unsuccessful.

Discussion

Intravenous lipid emulsion is thought to act through several mechanisms including shifting of lipophilic drugs from tissue into circulation, providing energy for heart muscle from lipid acids, or enabling calcium influx into myocardium [10]. The first mentioned, so-called lipid sink mechanism is supported by experimental studies showing that ILE is effective in cases of certain lipophilic substances, especially local anesthetics [11]. However, ILE was not always superior to standard treatment protocols in attenuating toxicity of some

other drugs, including CBBs [12]. Though there is an emerging number of case reports suggesting benefit of lipid emulsion use in poisoned patients [6], in order to gain more experience with different agents, we find it important also to present cases in which ILE was not effective.

The first case we reported was fatal overdose with amlodipine and oral antidiabetics (metformin and gliclazide). Refractory hypotension due to peripheral vasodilatation and tachycardia as a reflex cardiac response may be attributed largely to amlodipine [13], though metformin may contribute to these effects [14]. The initial hyperglycemia despite hypoglycemics co-ingestion, is also suggestive of CBBs suppression of insulin release. Metabolic acidosis with border-line elevated lactate may be a consequence of reduced peripheral perfusion but metformin also may add to it [15].

The patient did not respond to standard therapy. Because of gliclazide co-ingestion and the risk of prolonged hypoglycaemia [16], hyperinsulinemic euglycemia treatment protocol was not tried. Due to the lipophilic nature of amlodipine, lipid emulsion rescue therapy was administered. Though benefits of ILE in critical patients ingested amlodipine has been presented in several reports [17–20] the only noticeable effect in our patient was transient, unsustainable increase of blood pressure.

The second case we presented was a fatal poisoning with combination of calcium channel blocker (nifedipine), beta blocker (metoprolol) and benzodiazepine (diazepam). In this case, cardiotoxicity with third-degree AV block and circulatory shock may be attributed to synergic action of metoprolol and nifedipine. ILE may be not effective with less lipophilic beta blockers such as metoprolol compared with propranolol [8, 21–23]. However, in case of metoprolol-induced cardiac arrest refractory to standard treatment, combination of high-dose insulin, lipid emulsion and venoarterial extracorporeal membrane oxygenation was successful rescue therapy [24]. On the other side, ILE was not effective in reversal of metoprolol-induced hypotension in rabbit model [25]. The other cardiotoxic drug ingested by our patient, nifedipine, is relatively lipophilic and some benefit may be expected from ILE though experimental study in rats showed no significant difference in the haemodyanamic parameters and mortality between groups receiving ILE or placebo [26]. In the presented case, there was no response at all, neither to medications, including ILE, nor to cardiac pacing.

Conclusion

Reports on the beneficial effect of lipid emulsion in poisoning with different agents have accumulated in recent years. Though intravenous lipid emulsion may be life-saving treatment of poisonings with certain calcum channel blockers or beta blockers, like verapamil and propranolol, it may be ineffective in some other circumstances, including toxicity of amlodipine, nifedipine and metoprolol.

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LETTER TO THE EDITOR

A comment on the article: *Rabrenović M, Trešnjić S, Rabrenović V, Čikiriz N, Mašić S, Matunović R.* Neurotoxic effects of oxygen in hyperbaric environment. Vojnosanit Pregl 2015; 72(9): 827–30.

Komentar na članak: Rabrenović M, Trešnjić S, Rabrenović V, Čikiriz N, Mašić S, Matunović R. Neurotoksični efekat kiseonika u hiperbaričnim uslovima. Vojnosanit Pregl 2015; 72(9): 827–30.

To the Editor:

Personally, I felt obligated to write this comment on the alleged case report published by the respected and well-known Serbian Military Medical and Pharmaceutical Journal:

1. In the introduction, the authors write: "… if not controlled, inhalation of oxygen under increased pressure in condition of hyperbaric oxygen therapy can lead to serious damage (to human health?) and even death".

As specialists of underwater and hyperbaric medicine in my 35 years of practice I met no case in personal experience, nor data in the literature that oxygen used as a medicine in hyperbaric chambers has led to serious health damage or death in human.

2. Therapeutic partial pressures of O_2 allow comfortable use, but the duration of stay in hyperbaric chambers is strictly limited. To demonstrate toxic effects of oxygen it is necessary that the partial pressure of oxygen is above 3 ATA (atmospheres absolute) and that the length of exposure is prolonged to several hours. The toxic effect on primarily the lung tissue, and after that on the CNS has been noticed. Toxic events on the CNS are called "oxygen epilepsy" as is clinically manifested as seizures without further or permanent consequences. In the test for oxygen sensitivity for divers partial pressure of 2.8 ATA and exposure time of 60 min is implemented. This test is or was implemented only in military or police divers who dive with closed circuit diving equipment, where the breathing medium is 100% oxygen. Military divers exercises done at the pressure of 2.0 ATA can last for several hours.

In divers career the test is done once in the early stage of career. The use of this test is questionable because a diver can experience oxgen epilepsy even if the oxygen hypersensitivity is not detected by the test. The question is why? Because increased sensitivity to oxygen usually develops as a cause of some other medical problems in the background (infection of bacterial or viral commonly, fever or other medical problem that is not detected in the process of selection, or possible, because individual predisposition which is rarely detected by EEG). That's why the test is being used less and less, and my opinion is that it should be removed from service completely.

3. Epileptic seizures induced by oxygen leads to a powerful tonic-clonic convulsions and seizures, and each seizure brings higher possibility of injuries, muscle aches, increase muscle creatine kinase, ... but biochemical changes that indicate damage of parenchymal organs are not present.

What is a possible background hypersensitivity to oxygen? I repeat that there is no late or permanent consequence, because, theoretically, the seizure is caused by rapid metabolic consumption of enzymes and decrease of GABA (gama-amino butyric acid), that causes metabolic block in the CNS and epileptic seizures.

4. In the process of selection for Special Forces divers, individuals who are sensitive to oxygen are immediately eliminated from the group that dives using 100% oxogen. In these cases it is necessary to pay special attention to the application of hyperbaric oxygenation or completely exclude application of HBO therapy at pressures greater than 1.5 ATA (5 m deep, 100% oxygen), which is normally recommended for the safe treatment of CNS.

It is considered to be a medical error if a person with oxygen hypersensitivity is treated with oxygen under high pressure.

Especially when it comes as a medical treatment recommendation as it is by the authors of this article!

Recommendations provided by the doctors of Military Medical Academy and published in Military Medical and Pharmaceutical Journal of Serbia has a special significance because of the importance of Military Medical Academy and Military Medical and Pharmaceutical Journal of Serbia in our medical practice.

Prim. Dr Med. Miodrag Živković,
undersea medicine specialist, physiologist
HBO, Medical Centar, Belgrade, Serbia

Authors' reply:

In our practice, we had several cases of patient reaction to 100% oxygen in hyperbaric conditions, which were manifested

as oxygen epilepsy, normally without any sequelae, except that the therapy was interrupted. In this particular case, it was associated with skin manifestations, pain in the joints and muscles, as well as suffusion of the eye, which is why it drew our attention as the literature does not list these changes as a companion to the neurotoxic effects of hyperbaric oxygen conditions. Therefore, we decided to present this case, thinking it will be interesting and unusual.

We are glad that it drew the attention of a honorable colleague, so we would like to provide additional explanations and our observations.

Our conclusion that uncontrolled inhalation of oxygen within hyperbaric therapy can lead to organ damage, including coma and death, is supported by the harmful effects of high partial pressure of oxygen above 3 ATA. We wanted to draw attention to the need for caution and not as something that happens every day. This is the reason that today's modern hyperbaric chambers are programmed so that if the human factor fails, computer automatically cuts off oxygen in the event of uncotrolled increase or exceeding programed depth.

Oxygen sensitivity tests are performed to this day both in our, as well as in other militaries, but with different oxygen inhallation time at the aforementioned pressure and depth.

In our case presentation, we were not dealing with demage to the CNS, but rather with the neurotoxic effects of oxygen which does not lead to any effects on the CNS, except that as a result, the person did not pass the selection process.

As is visible in photographs, the individual had changes in the neck and chest in the form of erythematous stains, as well as suffusion in the eye and pain in joints and muscles, which lasted several hours and was therefore treated in a hyperbaric chamber. Treatment profile included safe depth where he breathed 100% oxygen intermittently with air, and resulted in the changes disipating within 30 minutes.

We found the biochemical results interesting, and thus we listed them, because they are all improved after that single treatment.

We wanted to present this case in order to describe our experience to other colleagues who deal with hyperbaric oxygen treatment.

On behalf of the authors:
Prim. Dr. Milorad Rabrenović
Center of Hyperbaric Medicine,
Military Medical Academy, Belgrade, Serbia

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

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

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Primeri referenci:

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Tabele

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

Ilustracije

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

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

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